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Center for Drug Evaluation
and Research (CDER)

Reviewer Guidance

Validation of Chromatographic Methods

November 1994
CMC 3

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REVIEWER GUIDANCE[1]

VALIDATION OF CHROMATOGRAPHIC METHODS

I. INTRODUCTION

The purpose of this technical review guide is to present the issues to consider when evaluating chromatographic test methods from a regulatory perspective. The document discusses the points to note and weaknesses of chromatography so that CDER reviewers can ensure that the method's performance claims are properly evaluated, and that sufficient information is available for the field chemist to assess the method. Analytical terms, as defined by the International Conference of Harmonization (ICH), 1993, have been incorporated in this guide.

Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids. The components monitored include chiral or achiral drug, process impurities, residual solvents, excipients such as preservatives, degradation products, extractables and leachables from container and closure or manufacturing process, pesticide in drug product from plant origin, and metabolites.

The objective of a test method is to generate reliable and accurate data regardless of whether it is for acceptance, release, stability or pharmacokinetics study. Data are generated for the qualitative and quantitative testing during development and post-approval of the drug products. The testing includes the acceptance of raw materials, release of the drug substances and products, in-process testing for quality assurance, and establishment of the expiration dating period.

Validation of a method is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose. Data thus

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[1]This guidance has been prepared by the Analytical Methods Technical Committee of the Chemistry Manufacturing Controls Coordinating Committee (CMC CC) of the Center for Drug Evaluation and Research at the Food and Drug Administration. Although this guidance does not create or confer any rights for or on any person and does not operate to bind FDA or the industry, it does represent the agency's current thinking on the validation of chromatographic methods. For additional copies of this guidance, contact the Division of Communications Management, HFD-210, CDER, FDA, 5600 Fishers Lane, Rockville, MD 20857 (Phone: 301-594-1012). Send one self-addressed adhesive label to assist the offices in processing your request. An electronic version of this guidance is also available via Internet the World Wide Web (WWW) (connect to the FDA Home Page at WWW.FDA.GOV/CDER and go to the "Regulatory Guidance" section).

generated become part of the methods validation package submitted to CDER.

Methods validation should not be a one-time situation to fulfil Agency filing requirements, but the methods should be validated and also designed by the developer or user to ensure ruggedness or robustness. Methods should be reproducible when used by other analysts, on other equivalent equipment, on other days or locations, and throughout the life of the drug product. Data that are generated for acceptance, release, stability, or pharmacokinetics will only be trustworthy if the methods used to generate the data are reliable. The process of validation and method design also should be early in the development cycle before important data are generated. Validation should be on-going in the form of re-validation with method changes.

II. TYPES OF CHROMATOGRAPHY

Chromatography is a technique by which the components in a sample, carried by the liquid or gaseous phase, are resolved by sorption-desorption steps on the stationary phase.

A. High Performance Liquid Chromatography (HPLC)

HPL chromatographic separation is based on interaction and differential partition of the sample between the mobile liquid phase and the stationary phase. The commonly used chromatographic methods can be roughly divided into the following groups, not necessarily in order of importance:

1. Chiral
2. Ion--exchange
3. Ion--pair/affinity
4. Normal phase
5. Reversed phase
6. Size exclusion

1. Chiral Chromatography

Separation of the enantiomers can be achieved on chiral stationary phases by formation of diastereomers via derivatizing agents or mobile phase additives on achiral stationary phases. When used as an impurity test method, the sensitivity is enhanced if the enantiomeric impurity elutes before the enantiomeric drug.

2. Ion-exchange Chromatography

Separation is based on the charge-bearing functional groups, anion exchange for sample negative ion (X^-), or cation exchange for sample positive ion (X^+). Gradient elution by pH is common.

3. Ion-pair/Affinity Chromatography

Separation is based on a chemical interaction specific to the target species. The more popular reversed phase mode uses a buffer and an added counter-ion of opposite charge to the sample with separation being influenced by pH, ionic strength, temperature, concentration of and type of organic co-solvent(s). Affinity chromatography, common for macromolecules, employs a ligand (biologically active molecule bonded covalently to the solid matrix) which interacts with its homologous antigen (analyte) as a reversible complex that can be eluted by changing buffer conditions.

4. Normal Phase Chromatography

Normal phase chromatography is a chromatographic technique that uses organic solvents for the mobile phase and a polar stationary phase. Here, the less polar components elute faster than the more polar components.

5. Reversed Phase Chromatography

The test method most commonly submitted to CDER is the reversed phase HPLC method. UV detection is the most common detection technique.

Reversed phase chromatography, a bonded phase chromatographic technique, uses water as the base solvent. Separation based on solvent strength and selectivity also may be affected by column temperature and pH. In general, the more polar components elute faster than the less polar components.

UV detection can be used with all chromatographic techniques. The concern for this type of detector is the loss of sensitivity with lamp aging, and varying sensitivity at the low level depending on design and/or manufacturer. A point to note is that observations on the HPL chromatograms, by UV detection in combination with reversed-phase HPLC, may not be a true indication of the facts for

the following reasons:

- Compounds much more polar than the compound of interest may be masked (elute together) in the solvent front/void volume.

- Compounds very less polar than the analyte may elute either late during the chromatographic run or are retained in the column.

- Compounds with lower UV extinction coefficients or different wavelength maxima may not be detectable at the low level relative to the visibility of the analyte since only one wavelength is normally monitored.

6. Size Exclusion Chromatography

   Also known as gel permeation or filtration, separation is based on the molecular size or hydrodynamic volume of the components. Molecules that are too large for the pores of the porous packing material on the column elute first, small molecules that enter the pores elute last, and the elution rates of the rest depend on their relative sizes.

B. Gas Chromatography (GC)

Gas chromatography is based on the volatilized sample transported by the carrier gas as the moving phase through the stationary phase of the column where separation takes place by the sorption/desorption process.

Samples for gas chromatographic analysis are normally low molecular weight compounds that are volatile and stable at high temperature. In this respect, residual solvents in drug substances and drug products are suitable for gas chromatographic analysis. Chemical derivatives can also be formed to achieve volatility and thermal stability.

Common detectors are flame ionization (FID) for carbon-containing compounds, electron capture (ECD) for halogenated compounds, flame photometric (FPD) for compounds containing sulphur or phosphorous and nitrogen-phosphorous (NPD) for compounds containing nitrogen or phosphorous. Chiral separation also can be achieved by gas chromatography. Separation by the packed column is rapidly being replaced by the capillary column that provides improved resolution and analysis speed. The location of the analyte on the gas chromatogram is

described by retention time (R_t) which is similar to HPLC.

C. Thin-Layer Chromatography (TLC)

Thin-layer chromatography is the simplest of the more common chromatographic techniques. Separation is based on migration of the sample spotted on a coated (stationary phase) plate with one edge dipped in a mixture of solvents (mobile phase). The whole system is contained in an enclosed tank.

Detection techniques include fluorescence, UV and sprays (universal and specific) for compounds that are not naturally colored. The location of the analyte on the TLC plate is described by the R_f value which is the ratio of the migration distance of the compound of interest to the mobile phase front.

Of the three techniques, gas, liquid and thin-layer, TLC is the most universal test method as all components are present on the plate and with appropriate detection techniques, all components can be observed. However, it normally is not as accurate or sensitive as HPLC. TLC has a higher analytical variation than HPLC, although one sees the "whole picture" when appropriate detection schemes are selected.

III. REFERENCE STANDARDS

A reference standard is a highly purified compound that is well characterized. Chromatographic methods rely heavily on a reference standard to provide accurate data. Therefore the quality and purity of the reference standard is very important. Two types of reference standards, chemical and nuclidic, exist. With the latter, the radio-label purity should also be considered as well as the chemical purity.

As described in the Guideline for Submitting Samples and Analytical Data for Methods Validation, the two categories of chemical reference standards are as follows:

- USP/NF reference standard that does not need characterization, and
- non-compendial standard that should be of the highest purity that can be obtained by reasonable effort and should be thoroughly characterized to assure its identity, strength, quality and purity.

The points to note are:

- Most USP/NF reference standards do not state the purity of the compound.

- The purity correction factor for non-USP reference standards is recommended to be included in the calculation of the test method.

- In addition to structurally-related impurities from the synthesis process, other process impurities like heavy metals, residual solvents, moisture (bound and unbound), pesticides for products of plant origin, and degradation products can also contribute to the lack of purity in the reference standard.

- The drying of the reference standard before use, if stated in the method, will eliminate residual solvent(s), unbound moisture and sometimes bound moisture (depending on the drying conditions). The drying step is always included for hygroscopic compounds. On the other hand, drying can result in the loss of a hydrate or cause degradation in heat-sensitive compounds.

Chromatographic test methods use either external or internal standards for quantitation.

A. An external standard method is used when the standard is analyzed on a separate chromatogram from the sample. Quantitation is based on a comparison of the peak area/height (HPLC or GC) or spot intensity (TLC) of the sample to that of a reference standard of the analyte of interest.

The external standard method is more appropriate for samples as follows:

1. Sample with a single target concentration and narrow concentration range, e.g., acceptance and release tests.

2. Simple sample preparation procedure.

3. Increased baseline time for detection of potential extraneous peaks, e.g., impurities test.

B. With an internal standard method, compound of known purity that does not cause interference in the analysis is added to the sample mixture. Quantitation is based on the response ratio of compound of interest to the internal standard vs the response ratio of a similar preparation of the reference standard (HPLC or GC). This technique is rarely used for TLC methods.

The internal standard method is more appropriate for samples as follows:

1. Complex sample preparation procedures, e.g., multiple extractions.

2. Low concentration sample (sensitivity being an issue), e.g., pharmacokinetics studies.

3. Wide range of concentrations expected in the sample for analysis, e.g., pharmacokinetics studies.

Although CDER does not specify whether the method must use an internal or external standard for quantitation, it is commonly observed that HPLC methods for release and stability and TLC methods use external standards; and methods for biological fluids and GC methods use internal standards.

The working concentration is the target concentration of the compound of interest as described in the method. Keeping the concentrations of the sample and the standard close to each other for the external standard method improves the accuracy of the method.

Recommendations:

1. Include the purity correction factor, if known, of the reference standard in the calculation.

2. State the working concentrations of the standard and sample in the method.

IV. PARAMETERS FOR VALIDATION OF HPL CHROMATOGRAPHIC METHODS FOR DRUG SUBSTANCE AND DRUG PRODUCT

Though many types of HPL chromatographic techniques are available, the most commonly submitted method, the reversed-phase HPLC with UV detection, is selected to illustrate the parameters for validation. The criteria for the validation of this technique can be extrapolated to other detection methods and chromatographic techniques. For acceptance, release or stability testing, accuracy should be optimized since the need to show deviation from the actual or true value is of the greatest concern.

A. Accuracy

Accuracy is the measure of how close the experimental value is to the true value.

Accuracy studies for drug substance and drug product are recommended to be performed at the 80, 100 and 120% levels of label claim as stated in the Guideline for Submitting Samples and Analytical Data for Methods Validation.

For the drug product, this is performed frequently by the addition of known amounts of drug by weight or volume (dissolved in diluent) to the placebo formulation working in the linear range of detection of the analyte. This would be a true recovery for liquid formulations. For formulations such as tablet, suppository, transdermal patch, this could mean evaluating potential interaction of the active drug with the excipients in the diluent. From a practical standpoint, it is difficult to manufacture a single unit with known amount of active drug to evaluate recovery. This test evaluates the specificity of the method in the presence of the excipients under the chromatographic conditions used for the analysis of the drug product. It will pick up recovery problems that could be encountered during the sample preparation and the chromatographic procedures. However, it does not count the effect of the manufacturing process.

At each recommended level studied, replicate samples are evaluated. The RSD of the replicates will provide the analysis variation or how precise the test method is. The mean of the replicates, expressed as % label claim, indicates how accurate the test method is.

Recommendations:

Recovery data, at least in triplicate, at each level (80, 100 and 120% of label claim) is recommended. The mean is an estimate of accuracy and the RSD is an estimate of sample analysis precision.

B. Detection Limit and Quantitation Limit

These limits are normally applied to related substances in the drug substance or drug product. Specifications on these limits are submitted with the regulatory impurities method relating to release and stability of both drug substance and drug product.
Detection limit is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated

experimental conditions. Quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

With UV detectors, it is difficult to assure the detection precision of low level compounds due to potential gradual loss of sensitivity of detector lamps with age, or noise level variation by detector manufacturer. At low levels, assurance is needed that the detection and quantitation limits are achievable with the test method each time. With no reference standard for a given impurity or means to assure detectability, extraneous peak(s) could "disappear/appear." A crude method to evaluate the feasibility of the extraneous peak detection is to use the percentage claimed for detection limit from the area counts of the analyte. For example, detection limit claim of 0.01% for the analyte integrated area count of 50,000 will give an area count of 5 that is not detectable.

Though USP expresses detection limit and quantitation limit in terms of 2 or 3, and 10 times noise level respectively, this concept is not very practical. Noise level on a detector during the method development phase may be different when samples are assayed on different detectors, etc. The use of standard(s) in the test method at the quantitation limit level (proposed by the applicant) is assurance that the impurity can be observed and quantitated.

Detector sensitivity can vary with the model number and/or manufacturer as illustrated in Table 1 for the analysis of a compound by two commercial detectors. The data should not be taken as the expected ratio of sensitivity of the two detectors. It is not known if other parameters which can also play a part, e.g., age of lamp, column, were considered when setting these limits.

Table 1. Comparison of Detector Sensitivity Limits in Two Commercial Detectors.

	Detector 1	Detector 2
Quantitation Limit	0.21%	0.07%
Detection Limit	0.16%	0.05%

One also should be cautious that baseline noise is not interpreted as extraneous peaks. Undulations may be observed at the void volume if the diluent for the sample is different from the solvents (proportion and type) used in the mobile phase.

If a reference standard for the compound of interest is available, a standard close to the quantitation limit or the specification could be used. For monitoring peak(s) with no reference standard for the impurity, a diluted reference standard of the drug substance is recommended. The method should then check that the high and low concentrations are operating in the linear range of detection of the drug substance. Otherwise the information that is expressed as % area or height of the drug substance peak from the same HPL chromatogram will be biased. It should also be noted that the extraneous peak using area count does not consider the detection response which depends on the UV extinction coefficient or absorptivity of the compound.

Recommendations:

1. Analysis repeatability and injection repeatability data at the quantitation limit.

2. Use of an additional reference standard at the quantitation limit level in the test method.

C. Linearity

The linear range of detectability that obeys Beer's Law is dependent on the compound analyzed and detector used. The working sample concentration and samples tested for accuracy should be in the linear range.

Figures 1 and 2 illustrate the behavior of UV response vs. concentration of a (a) linear and (b) non-linear relationship. A point to note is that when monitoring impurity peaks expressed as percent area of the parent drug substance, the impurity observed may not be a true reflection of the theoretical amount if the non-linear section of the concentration curve is employed. In addition, the actual amount will be obtained only if the extinction coefficient or absorptivity values are the same for both impurity and parent compound. Impurity reference standards are often needed.

Figure 1. Concentrations vs. Peak Areas of Standards to Illustrate Linearity.

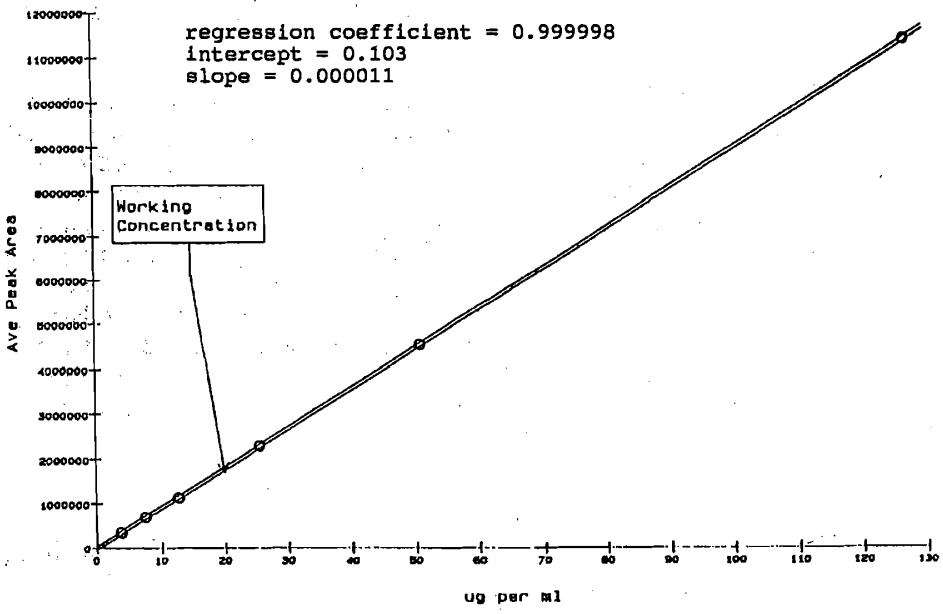
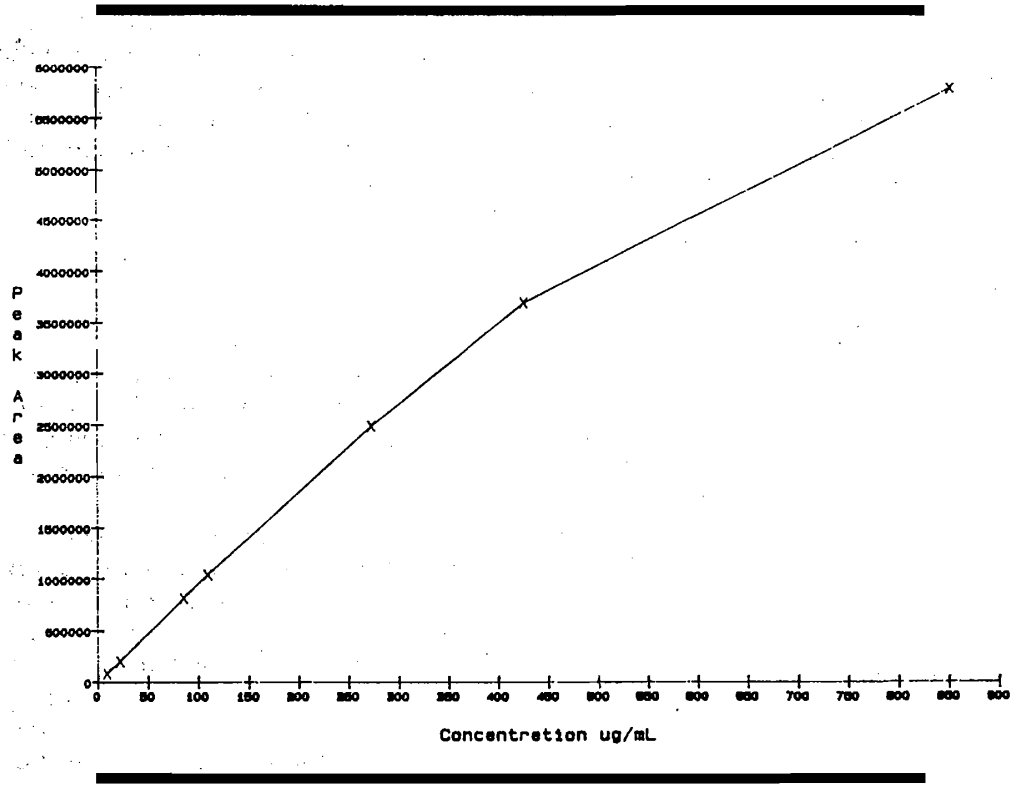


Figure 2. Concentrations vs. Peak Areas of Standards Outside the Linear Range.



Recommendations:

The linearity range for examination depends on the purpose of the test method. For example, the recommended range for an assay method for content would be NLT ± 20% and the range for an assay/impurities combination method based on area % (for impurities) would be +20% of target concentration down to the limit of quantitation of the drug substance or impurity. Under most circumstances, regression coefficient (r) is ≥ 0.999. Intercept and slope should be indicated.

D. Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility. Ruggedness as defined in USP XXII <1225>, 1990 incorporates the concepts described under the terms "intermediate precision", "reproducibility" and "robustness" of this guide.

1. Repeatability

   a. Injection Repeatability

      Sensitivity is the ability to detect small changes in the concentration of the analyte in the sample. Sensitivity can be partially controlled by monitoring the specification for injection reproducibility (system suitability testing).

      The sensitivity or precision as measured by multiple injections of a homogeneous sample (prepared solution) indicates the performance of the HPLC instrument under the chromatographic conditions and day tested. The information is provided as part of the validation data and as a system suitability test. The specification, as the coefficient of variation in % or relative standard deviation (RSD), set here will determine the variation limit of the analysis. The tighter the value, the more precise or sensitive to variation one can expect the results. This assumes that the chromatograph does not malfunction after the system suitability testing has been performed. Keep in mind, however, that it does not consider variations due to the drug product manufacturing and laboratory sample preparation procedures. As an illustration for injection and R_t variation, Table 2 provides representative data collected when a leak developed in the chromatographic system during sampling. The set of four duplicate samples were injected sequentially. Variations in peak area and drift of retention times are noted. Sets of typical data from a well-behaved system for comparison are shown in Table 3.

Table 2. Representative Injection Repeatability Data for an HPL Chromatographic System that Developed a Leak During Sampling.

Sample	R_t	Peak Area	ΔR_t	Δ Peak Area
A1 A2	5.62 5.66	2155699 2120466	0.04	35233
B1 B2	5.87 6.13	2205659 2288355	0.26	82696
C1 C2	6.21 6.48	2227066 2265279	0.27	38213
D1 D2	6.73 6.99	2581888 2602016	0.26	20128

Table 3. Representative Injection Repeatability Data for Select Formulations from a Normally Functional HPL Chromatographic System.

Dosage Form	n	Mean ± SD	RSD
Inhalation Solution	10	1993162 ± 5029	0.25%
Solution for Inhalation	10	1722253 ± 6288	0.37%
Capsule	10	1744320 ± 3133	0.18%

Recommendations:

As part of methods validation, a minimum of 10 injections with an RSD of ≤1% is recommended. With the methods for release and stability studies, an RSD of ≤ 1% RSD for precision of the system suitability tests for at least five injections ($n \geq 5$) for the active drug either in drug substance or drug product is desirable. For low level impurities, higher variations may be acceptable.

b. Analysis Repeatability

Determination, expressed as the RSD, consists of multiple measurements of a sample by the same analyst under the same analytical conditions. For practical purpose, it is often combined with accuracy and carried out as a single study. See section IV.A under Accuracy.

2. Intermediate Precision

Intermediate precision was previously known as part of ruggedness. The attribute evaluates the reliability of the method in a different environment other than that used during development of the method. The objective is to ensure that the method will provide the same results when similar samples are analyzed once the method development phase is over.

Depending on time and resources, the method can be tested on multiple days, analysts, instruments, etc.

Intermediate precision in the test method can be partly assured by good system suitability specifications. Thus, it is important to set tight, but realistic, system suitability specifications.

Recommendations:

As a minimum, data generated as described under section IV.A Accuracy, for two separate occasions, is recommended to indicate the intermediate precision of the test method.

3. Reproducibility

As defined by ICH, reproducibility expresses the precision between laboratories as in collaborative studies. Multiple laboratories are desirable but not always attainable because of the size of the firm.

Recommendations:

It is not normally expected if intermediate precision is accomplished.

E. Range

Range is the interval between the high and low levels of analyte studied. See also sections IV.A and C under Accuracy and Linearity respectively.

The ranges recommended in sections IV.A and C under Accuracy and Linearity can be applied to other analytes, e.g., preservatives.

F. Recovery

Recovery is expressed as the amount/weight of the compound of interest analyzed as a percentage to the theoretical amount present in the medium.

Full recovery should be obtained for the compound(s) of interest. During the sample preparation procedure, the compound of interest is recovered from excipients in the formulation matrix ranging from a simple aqueous solution to complex cream formulation, and from potential adhesion to container/closure components, e.g., glass vial, metered valve. In general, a simpler sample preparation procedure will result in a lower variation of recovery. Data collection for recovery are discussed in section IV.A under Accuracy.

G. Robustness

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. Robustness can be partly assured by good system suitability specifications. Thus, it is important to set tight, but realistic, system suitability specifications.

Testing varying some or all conditions, e.g., age of columns, column type, column temperature, pH of buffer in mobile phase, reagents, is normally performed.

Recommendations:

Data obtained from studies for robustness, though not usually submitted, are recommended to be included as part of method validation.

H. Sample Solution Stability

Solution stability of the drug substance or drug product after preparation according to the test method should be evaluated according to the test method. Most laboratories utilize autosamplers with overnight runs and the sample will be in solution for hours in the laboratory environment before the test procedure is completed. This is of concern especially for drugs that can undergo degradation by hydrolysis, photolysis or adhesion to glassware.

Recommendations:

Data to support the sample solution stability under normal laboratory conditions for the duration of the test procedure, e.g., twenty-four hours, should be generated. In exceptional cases where multiple days are needed for sample preparation or solution storage, an appropriate stability time should be selected.

I. Specificity/selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. A representative HPL chromatogram or profile should be generated and submitted to show that the extraneous peaks either by addition of known compounds or samples from stress testing are baseline resolved from the parent analyte. Examples of the extraneous peaks are as follows:

- For the drug substance or raw material, the related substances to consider are process impurities (which include isomeric impurities) from the synthesis process, residual pesticides, solvents, and other extraneous components from extracts of natural origin.

- For the drug product, the related substances may be impurities present in the active drug, degradation products, interaction of the active drug with excipients, extraneous components, e.g., residual solvents from the excipients or manufacturing process, leachables or extractables from the container and closure system or from the manufacturing process.

Submission of data from stress testing of the drug substance using acid and base hydrolysis, temperature, photolysis and oxidation according to the Guideline for Submitting Samples and Analytical Data for Methods Validation is recommended. Representative HPL chromatograms are recommended for stressed and non-stressed samples that include test methods for impurities, preservatives, etc. and placebo sample. With the impurities test method, the HPL chromatogram should indicate the presence of impurities at the level of detection/quantitation claimed. The chromatograms should be legible, labeled, and the time or time scale and attenuation should be indicated.

Points to note are as follows:

1. The parent peak may be expanded, e.g., by increasing the concentration, attenuation change, so that extraneous peaks can be observed at a reasonable size to evaluate stability-indicating capability. See comments in section IV.B under Limits of Detection/Quantitation.

2. The baseline should be on-scale as off-scale baseline (observed as a flat straight line) can hide minor peaks.

Peak purity can be determined by the photo-diode array detector. Low level extraneous components present under the compound of interest, however, may not interfere or influence the UV spectrum of the analyte.

Figures 3 and 4 illustrate the combination of UV spectroscopy and HPL chromatography by photo-diode array detection using (a) 3-dimensional plot and (b) conventional HPL chromatogram. The analyte elutes at 4.7 minutes. It should be noted that the quality of the UV spectra for the low level components is poor.

Figure 3. **A Representative 3-Dimensional Plot of the HPL Chromatogram with the UV Spectra.**

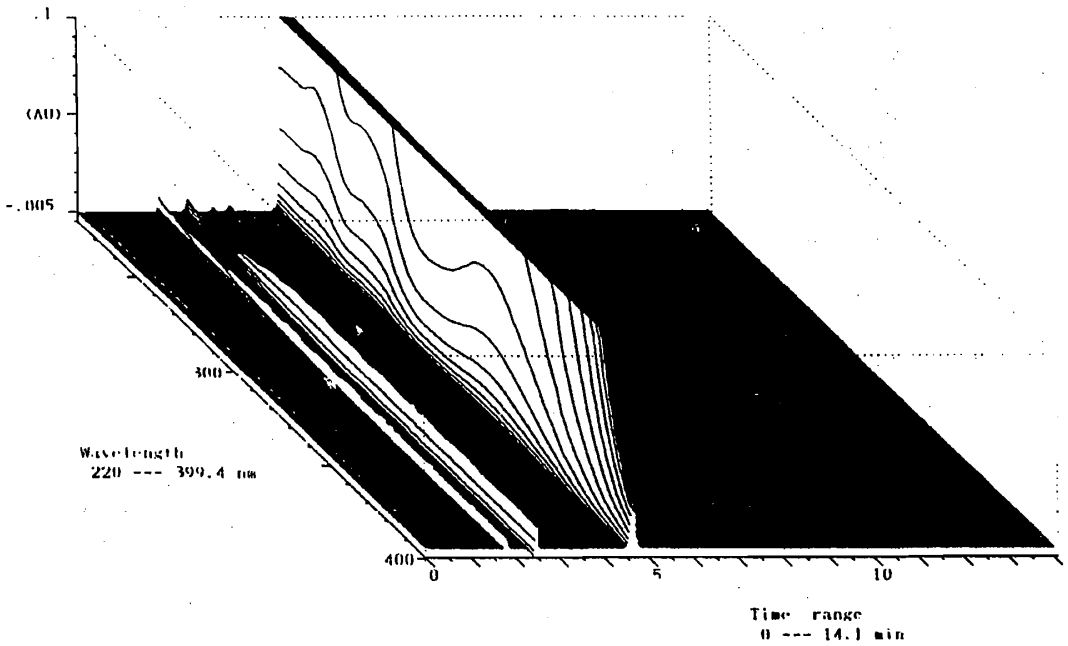
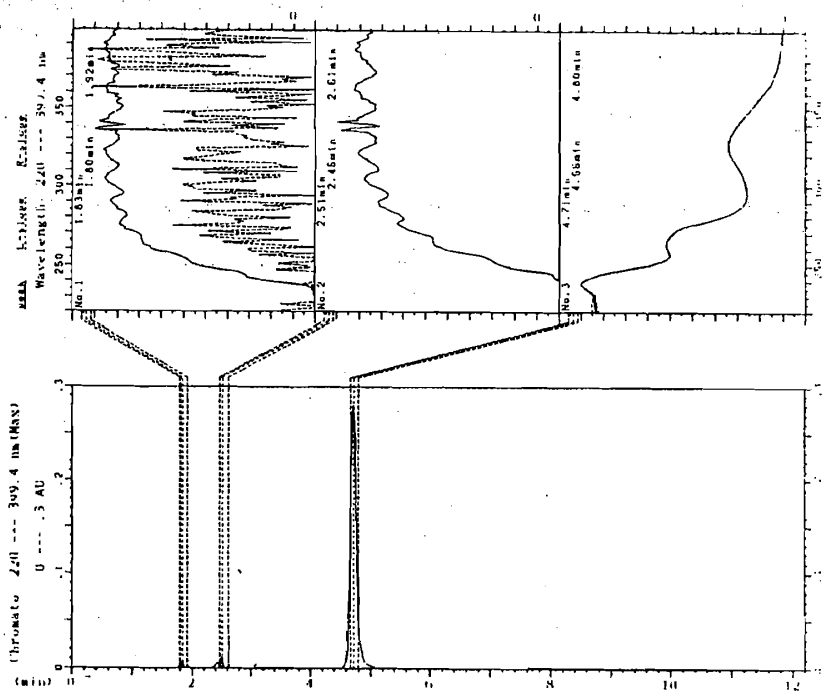


Figure 4. A Representative Conventional HPL Chromatogram with UV Spectra.



When stressed samples are used, an appropriate detector/integrator setting should be selected. For example, to be able to detect low levels, e.g., 0.1% degradation products, the parent peak should be of a size that at least a 0.1% detectability or area count is feasible.

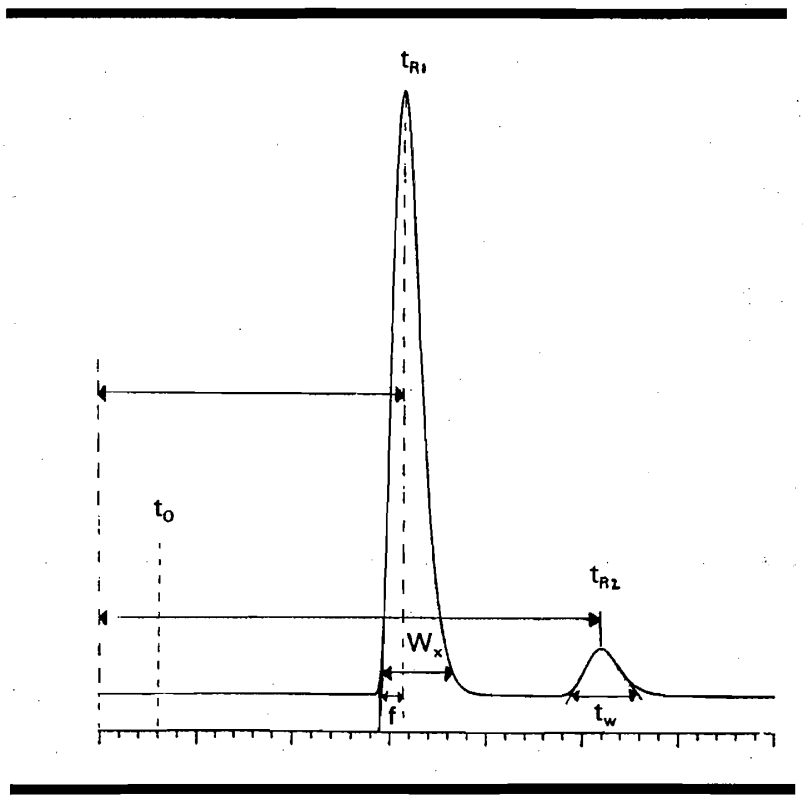
Recommendations:

Representative HPL chromatograms should be submitted for stressed and non-stressed samples that include impurities test method, preservative(s), etc. with the related placebo sample. Representative HPL chromatogram(s) to show selectivity by the addition of known extraneous compounds also should be submitted.

J. System Suitability Specifications and Tests

The accuracy and precision of HPLC data collected begin with a well-behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose. This section explains the terms as indicated in Figure 5, and provides recommendations and illustrations.

Figure 5. Definition of Terms for the System Suitability Parameters.



Where

W_x = width of the peak determined at either 5% (0.05) or 10% (0.10) from the baseline of the peak height

f = distance between peak maximum and peak front at W_x

t_o = elution time of the void volume or non-retained components

t_R = retention time of the analyte

t_W = peak width measured at baseline of the extrapolated straight sides to baseline

t_R = retention time of the analyte
t_W = peak width measured at baseline of the extrapolated straight sides to baseline

1. Capacity factor (k')

   $k' = (t_R - t_O) / t_O$

   The capacity factor is a measure of where the peak of interest is located with respect to the void volume, i.e., elution time of the non-retained components.

   Recommendations:

   The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 2.

2. Precision/Injection repeatability (RSD)

   Injection precision expressed as RSD (relative standard deviation) indicates the performance of the HPL chromatograph which includes the plumbing, column, and environmental conditions, at the time the samples are analyzed. It should be noted that sample preparation and manufacturing variations are not considered.

   Recommendations:

   RSD of ≤ 1% for n ≥ 5 is desirable.

3. Relative retention (α)

   $\alpha = k'_1 / k'_2$

   Relative retention is a measure of the relative location of two peaks. This is not an essential parameter as long as the resolution (R_s) is stated.

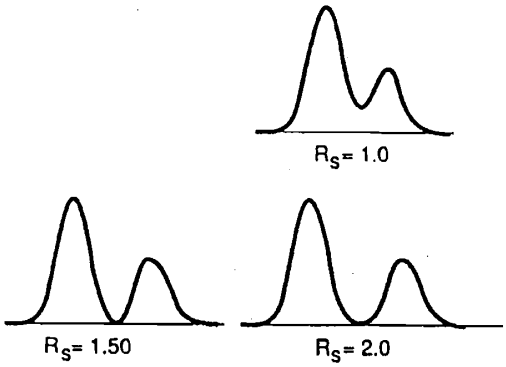
4. Resolution (R_s)

   $R_s = (t_{R2} - t_{R1}) / (1/2) (t_{W1} + t_{W2})$

   R_s is a measure of how well two peaks are separated. For reliable quantitation, well-separated peaks are essential for quantitation. This is a very useful parameter if potential interference peak(s) may be of concern. The closest potential eluting peak to the

R_s is minimally influenced by the ratio of the two compounds being measured. The resolution of peaks as indicated by the R_s values is shown in Figure 6.

Figure 6. Separation of Peaks as Indicated by R_s Values.



Recommendations:

R_s of > 2 between the peak of interest and the closest potential interfering peak (impurity, excipient, degradation product, internal standard, etc.) is desirable.

5. Tailing factor (T)

$T = W_x / 2f$

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak. Integrator variables are preset by the analyst for optimum calculation of the area for the

peak of interest. Figures 7 and 8 illustrate the tailing factors and the effect on quantitation. If the integrator is unable to determine exactly when an upslope or downslope occurs, accuracy drops.

Figure 7. HPLC Peak with Various Tailing Factors.

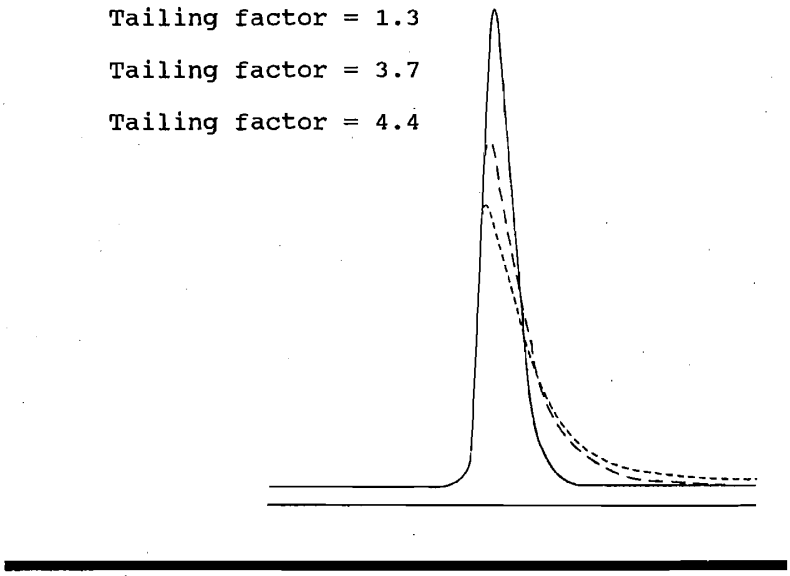
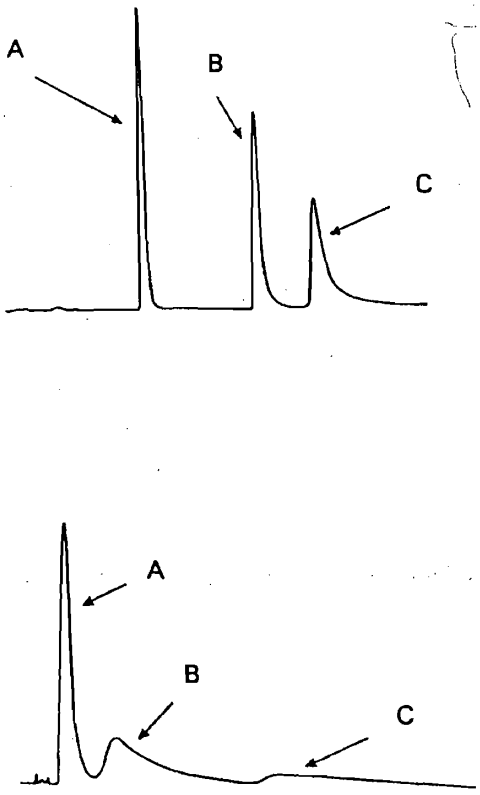


Figure 8. Effect of Peak Tailing on Quantitation.



Recommendations:

T of ≤ 2

6. Theoretical plate number (N)

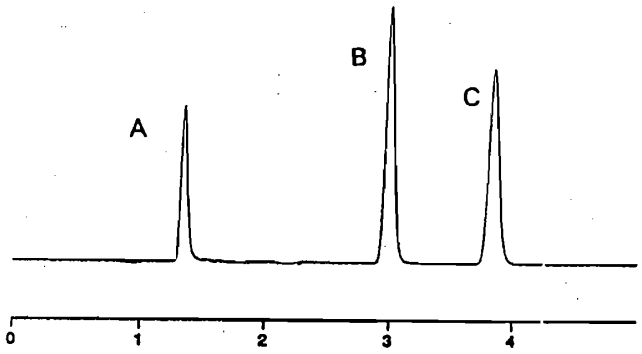
$N = 16 (t_R / t_W)^2 = L / H$

Theoretical plate number is a measure of column efficiency, that is, how many peaks can be located per unit run-time of the chromatogram.

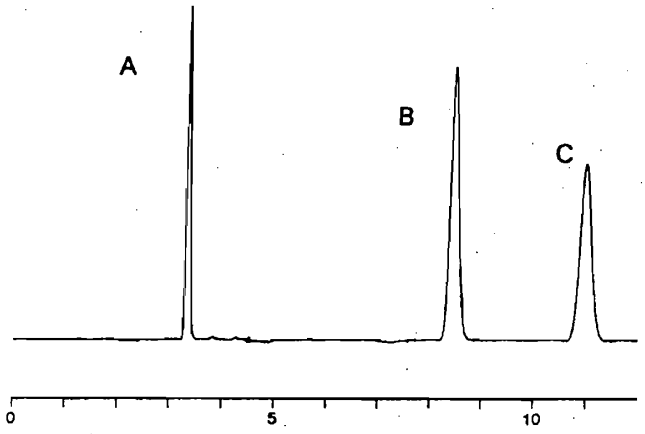
N is fairly constant for each peak on a chromatogram with a fixed set of operating conditions. H, or HETP, the height equivalent of a theoretical plate, measures the column efficiency per unit length (L) of the column. Parameters which can affect N or H include peak position, particle size in column, flow-rate of mobile phase, column temperature, viscosity of mobile phase, and molecular weight of the analyte. Figure 9 shows one set of compounds A, B, and C under two different chromatographic conditions resulting in, e.g., R_ts for B to be 3 and 8.5 minutes, respectively. An examination of peak B indicates that the theoretical plate values are different even though the peaks appear similar visually.

Figure 9. Effect of Retention Times on Theoretical Plates.

	R_t	N	k′
A	1.35	2007	0.51
B	3.00	4702	2.35
C	3.85	5929	3.29



	R_t	N	k′
A	3.36	5076	0.60
B	8.46	7175	3.03
C	10.99	8742	4.23



Recommendations:

The theoretical plate number depends on elution time but in general should be > 2000.

General Recommendation:

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. The amount of testing required will depend on the purpose of the test method. For dissolution or release profile test methods using an external standard method, k', T and RSD are minimum recommended system suitability tests. For acceptance, release, stability, or impurities/degradation methods using external or internal standards, k', T, R_s and RSD are recommended as minimum system suitability testing parameters. In practice, each method submitted for validation should include an appropriate number of system suitability tests defining the necessary characteristics of that system. Additional tests may be selected at the discretion of the applicant or the reviewer.

K. General Points to Consider

Some basic points to note in the test method are:

1. The sample and standard should be dissolved in the mobile phase. If that is not possible, then avoid using too high a level of the organic solvent as compared to the level in the mobile phase.

2. The sample and standard concentrations should be close if not the same.

3. The samples should be bracketed by standards during the analytical procedure.

4. Filtration of the samples before injection is occasionally observed. Filtration will remove particulates (centrifugation performs the same function) that may clog columns. Adhesion of the analyte to the filter can also happen. This will be of importance especially for low level impurities. Data to validate this aspect should be submitted by the applicant.

V. COMMENTS AND CONCLUSIONS

HPL Chromatographic Methods for Drug Substance and Drug Product.

Methods should not be validated as a one-time situation, but methods should be validated and designed by the developer or user to ensure ruggedness or robustness throughout the life of the method.

The variations due to the drug product manufacturing process, the laboratory sample preparation procedure and the instrument performance contribute to the accuracy of the data obtained from the analysis. With proper validation and tight chromatographic performance (system suitability) criteria, an improvement in the reliability of the data can be obtained. Variations, except from the drug product manufacturing process, will be minimized. Only with good reliable validated methods, can data that are generated for release, stability, pharmacokinetics be trust-worthy.

VI. ACKNOWLEDGEMENTS

Comments from Drs. Hoiberg, Poochikian, Blumenstein, Schroeder, Look, Tolgyesi (HFD-150); Dr. Layoff (HFH-300); Drs. Zimmerman and Piechocki (HFD-110); and the Analytical Methods Technical Committee: Dr. Sheinin, Mr. Shostak, Ms. Cunningham, Ms. Jongedyk, Mr. Leutzinger, Dr. Seggel, Ms. Sharkey and Mr. Smela are appreciated.

VII. REFERENCES

1. Guideline for Submitting Samples and Analytical Data for Methods Validation, February 1987.

2. United States Pharmacopeia, XXII, 1990. <1225>.

3. Text on Validation of Analytical Procedures, International Conference on Harmonization, September 1993.

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Certificate of Analysis
September 2, 1999

Characterization of the Drug Substance UT-15		
TP No.: TP-LRR-0008 **Phase: Phase 13**	**Company: United Therapeutics, Corporation Research and Development**	**Product: UT-15 Drug Substance**
A 2-page testing summary is included for lot UT15-99H001.		

All data generated at Magellan Laboratories and described within this report were collected under the direction of the Study Director and were audited by the Quality Assurance Unit of Magellan Laboratories Incorporated for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

Linda Carlisle 9-3-99
Quality Assurance Date

William E. Weiser, Ph.D. 9/3/99
Study Director Date

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-99H001

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	White powder
Identification		
IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.3%, w/w	0.02%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.8%, w/w
Residual Solvents by Gas Chromatography		
Ethyl Acetate	Not more than 1.0%, w/w	ND[1]
Ethanol	Not more than 0.5%, w/w	<0.1%
Acetic Acid	Not more than 1.0%, w/w	<0.1%
Total	Not more than 2.5%, w/w	<0.1%
Total Volatiles	Not more than 4.0%, w/w	0.8%, w/w
Melting Range	Not less than 118 °C and not more than 126 °C	122.2 - 125.1 °C
Specific Rotation	Not less than +31.0° and not more than +35.0° at 589 nm and 25°, volatiles-free basis	$[\alpha]_{589}^{25}$ = +32.4° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

[1]ND = Not Detected

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-99H001

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	0.05%
2AU90	Not more than 1.0%	<0.05%
97W86 (Benzindene Triol)	Not more than 1.0%	ND[1]
3AU90	Not more than 2.0%	ND
UT-15 Methyl Ester	Not more than 1.0%	0.06%
98W86	Not more than 1.0%	<0.05%
UT-15 Ethyl Ester	Not more than 1.0%	0.1%
750W93	Not more than 2.0%	0.5%
751W93	Not more than 2.0%	0.3%
Total Unidentified Impurities	Not more than 2.0%	ND
Total Related Substances	Not more than 5.0%	1.0%
Assay (HPLC)	Not less than 94.0 and not more than 101.0%, w/w, on the volatiles-free basis	98.4%
Factor	Factor the material on the basis of assay and total volatiles content	1.024 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

[1]ND = Not Detected

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February 17, 2004

Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239 **Lot No:** UT15-031202	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Date of Manufacture: 12-23-03 **Retest Date: 12-2005** **Date Received: 01-08-04** **Date Testing Completed: 01-25-04**	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing **Method:** ATM-LRR-M0002.18

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Physical Examination NB 2, PDR 1	A white to cream-colored powder	White powder
Identification by IR NB 2, PDR 15	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC NB 1, PDR 8	The retention time for the principal peak in the sample chromatogram agrees with the retention time of the principal peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition NB 3, PDR 4	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer) NB 2, PDR 8	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography TTP-LRR-M0242, Sample Analysis NB 3, LDR 21	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol <0.1% Acetic Acid ND
Melting Range NB 2, PDR 11	Not less than 120.0 °C and not more than 126.0 °C	122.1 °C - 123.9 °C
Specific Rotation NB 2, PDR 12	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 47.3 °
Heavy Metals NB 3, PDR 8	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00 NB 6, PDR 6	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031202		
Date of Manufacture: 12-23-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 12-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC) NB 1, LDR 68 - 72		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.2%
750W93	Not more than 1.5%	0.07%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances NB 1, LDR 72	Not more than 3.0%	0.5%
Assay (HPLC) NB 1, LDR 67	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.7%
Factor NB 1, PDR 9	Factor the material on the basis of assay and total volatiles content	1.005
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00 NB 5, PDR 5	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
[1]All results conform to specifications.
ND = None detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

17Feb2004
Quality Assurance — Date

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031201		
Date of Manufacture: 12-11-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 12-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Physical Examination NB 2, PDR 1	A white to cream-colored powder	White powder
Identification by IR NB 2, PDR 15	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensitics to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC NB 1, PDR 8	The retention time for the principal peak in the sample chromatogram agrees with the retention time of the principal peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition NB 3, PDR 4	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer) NB 2, PDR 8	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography TTP-LRR-M0242, Sample Analysis NB 3, LDR 20	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol <0.1% Acetic Acid ND
Melting Range NB 2, PDR 11	Not less than 120.0 °C and not more than 126.0 °C	122.5 °C - 123.9 °C
Specific Rotation NB 2, PDR 12	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 47.3 °
Heavy Metals NB 3, PDR 8	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00 NB 6, PDR 6	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031201		
Date of Manufacture: 12-11-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 12-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC) NB 1, LDR 62 - 66		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.09%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances NB 1, LDR 66	Not more than 3.0%	0.4%
Assay (HPLC) NB 1, LDR 61	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.5%
Factor NB 1, PDR 9	Factor the material on the basis of assay and total volatiles content	0.9956
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00 NB 5, PDR 5	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
[1]All results conform to specifications.
ND = None detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

17 Feb 2004
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2/17/04
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Study Director

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031102		
Date of Manufacture: 12-02-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 12-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 02-05-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[3]
Physical Examination NB 2, PDR 1	A white to cream-colored powder	White powder
Identification by IR NB 2, PDR 15	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC NB 1, PDR 8	The retention time for the principal peak in the sample chromatogram agrees with the retention time of the principal peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition NB 3, PDR 4	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer) NB 2, PDR 8	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography TTP-LRR-M0242, Sample Analysis NB 3, LDR 19	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol 0.1% Acetic Acid ND
Melting Range NB 2, PDR 11	Not less than 120.0 °C and not more than 126.0 °C	121.8 °C - 124.0 °C
Specific Rotation NB 2, PDR 12	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 47.2 °
Heavy Metals NB 3, PDR 8	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00 NB 6, PDR 6	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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February 17, 2004

Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031102		
Date of Manufacture: 12-02-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 12-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 02-05-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[3]	
Chromatographic Purity (HPLC)[1] NB 1, LDR 113 - 117			
1AU90	Not more than 0.5%	ND	
2AU90	Not more than 0.5%	ND	
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%	
3AU90	Not more than 1.0%	0.1%	
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%	
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%	
750W93	Not more than 1.5%	0.1%	
751W93	Not more than 1.3%	0.06%	
Unidentified	Not more than 0.1% AUC each		
Unidentified @ RRT 1.12		0.06%	
Total Related Substances NB 1, LDR 117	Not more than 3.0%	0.4%	
Assay (HPLC)[1] NB 1, LDR 55, 112	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	Prep 1	101.6%[2]
		Prep 2	102.8%[2]
		Avg.	102.2%[2]
		Prep 1	99.9%
		Prep 2	100.6%
		Prep 3	100.2%
		Prep 4	100.5%
		Prep 5	100.4%
		Prep 6	99.9%
		Avg.	100.3%
Factor[1] NB 1, PDR 18	Factor the material on the basis of assay and total volatiles content	0.9988	

[1] The assay, impurity, and factor results are the average of six sample preparations.

[2] The results were out of specification. After confirming the results by reanalysis, these results were investigated by preparing an additional three samples per preparation. Upon investigation, the cause was a suspected but unconfirmed preparation error. These results are not included in the calculation of the average result.

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031102		
Date of Manufacture: 12-02-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 12-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 02-05-04		
TEST/REFERENCE	**SPECIFICATIONS**	**RESULTS[3]**
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00 NB 5, PDR 5	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
[3]All results, except assay, conform to specifications.
ND = None detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — Date: 17 Feb 2004

Wei Pan, Ph.D.
Study Director — Date: 2/17/04

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239 **Lot No:** UT15-031101	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Date of Manufacture: 11-18-03 **Retest Date: 11-2005** **Date Received: 01-08-04** **Date Testing Completed: 01-25-04**	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing **Method:** ATM-LRR-M0002.18

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Physical Examination NB 2, PDR 1	A white to cream-colored powder	White powder
Identification by IR NB 2, PDR 15	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC NB 1, PDR 8	The retention time for the principal peak in the sample chromatogram agrees with the retention time of the principal peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition NB 3, PDR 4	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer) NB 2, PDR 8	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography TTP-LRR-M0242, Sample Analysis NB 3, LDR 18	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol <0.1% Acetic Acid ND
Melting Range NB 2, PDR 11	Not less than 120.0 °C and not more than 126.0 °C	122.6 °C - 124.2 °C
Specific Rotation NB 2, PDR 12	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 47.4 °
Heavy Metals NB 3, PDR 8	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00 NB 6, PDR 6	NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	<2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239 **Lot No:** UT15-031101	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Date of Manufacture: 11-18-03 **Retest Date: 11-2005** **Date Received: 01-08-04** **Date Testing Completed: 01-25-04**	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing **Method:** ATM-LRR-M0002.18

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC) NB 1, LDR 50 - 54		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.2%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	
Unidentified @ RRT 1.12		0.06%
Total Related Substances NB 1, LDR 54	Not more than 3.0%	0.5%
Assay (HPLC) NB 1, LDR 49	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.0%
Factor NB 1, PDR 9	Factor the material on the basis of assay and total volatiles content	1.001
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00 NB 5, PDR 5	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than

*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid

[1]All results conform to specifications.

ND = None detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — Date: 17 Feb 2004

Wei Pan, Ph.D.
Study Director — Date: 2/17/04

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031003		
Date of Manufacture: 11-07-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 11-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Physical Examination NB 2, PDR 1	A white to cream-colored powder	White powder
Identification by IR NB 2, PDR 15	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC NB 1, PDR 8	The retention time for the principal peak in the sample chromatogram agrees with the retention time of the principal peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition NB 3, PDR 4	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer) NB 2, PDR 8	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography TTP-LRR-M0242, Sample Analysis NB 3, LDR 17	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol 0.1% Acetic Acid ND
Melting Range NB 2, PDR 11	Not less than 120.0 °C and not more than 126.0 °C	121.3 °C - 123.6 °C
Specific Rotation NB 2, PDR 12	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 47.7 °
Heavy Metals NB 3, PDR 8	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00 NB 6, PDR 6	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031003		
Date of Manufacture: 11-07-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 11-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC) NB 1, LDR 44 - 48		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.2%
750W93	Not more than 1.5%	0.1%
751W93	Not more than 1.3%	0.06%
Unidentified	Not more than 0.1% AUC each	
Unidentified @ RRT 1.12		0.07%
Total Related Substances NB 1, LDR 48	Not more than 3.0%	0.6%
Assay (HPLC) NB 1, LDR 43	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.4%
Factor NB 1, PDR 9	Factor the material on the basis of assay and total volatiles content	0.9981
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00 NB 5, PDR 5	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
[1]All results conform to specifications.
ND = None detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031002		
Date of Manufacture: 10-28-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 10-2005		**Method:** ATM-LRR-M0002.18
Date Received: 01-08-04		
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Physical Examination NB 2, PDR 1	A white to cream-colored powder	White powder
Identification by IR NB 2, PDR 15	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC NB 1, PDR 8	The retention time for the principal peak in the sample chromatogram agrees with the retention time of the principal peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition NB 3, PDR 4	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer) NB 2, PDR 8	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography TTP-LRR-M0242, Sample Analysis NB 3, LDR 16	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol 0.1% Acetic Acid ND
Melting Range NB 2, PDR 11	Not less than 120.0 °C and not more than 126.0 °C	121.5 °C - 123.3 °C
Specific Rotation NB 2, PDR 12	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 47.7 °
Heavy Metals NB 3, PDR 8	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00 NB 6, PDR 6	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031002		
Date of Manufacture: 10-28-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 10-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC) NB 1, LDR 38 - 42		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	
Unidentified @ RRT 1.12		0.08%
Total Related Substances NB 1, LDR 42	Not more than 3.0%	0.4%
Assay (HPLC) NB 1, LDR 37	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.5%
Factor NB 1, PDR 9	Factor the material on the basis of assay and total volatiles content	0.9970
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00 NB 5, PDR 5	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
[1]All results conform to specifications.
ND = None detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — 17 Feb 2004 — Date

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031001		
Date of Manufacture: 10-16-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 10-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Physical Examination NB 2, PDR 1	A white to cream-colored powder	White powder
Identification by IR NB 2, PDR 15	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC NB 1, PDR 8	The retention time for the principal peak in the sample chromatogram agrees with the retention time of the principal peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition NB 3, PDR 4	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer) NB 2, PDR 8	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography TTP-LRR-M0242, Sample Analysis NB 3, LDR 15	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol 0.1% Acetic Acid ND
Melting Range NB 2, PDR 11	Not less than 120.0 °C and not more than 126.0 °C	121.9 °C - 123.3 °C
Specific Rotation NB 2, PDR 12	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 45.6 °
Heavy Metals NB 3, PDR 8	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00 NB 6, PDR 6	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Analysis of UT-15 Drug Substance		
TTP: TTP-LRR-M0239	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-031001		
Date of Manufacture: 10-16-03	**Storage/Packaging:** 5 °C/Ambient Amber HDPE bottle with NCR screw cap	**Phase:** Release Testing
Retest Date: 10-2005		
Date Received: 01-08-04		**Method:** ATM-LRR-M0002.18
Date Testing Completed: 01-25-04		

TEST/REFERENCE	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC) NB 1, LDR 32 - 36		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	< 0.05%
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	< 0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.2%
751W93	Not more than 1.3%	0.08%
Unidentified	Not more than 0.1% AUC each	
Unidentified @ RRT 1.12		0.05%
Total Related Substances NB 1, LDR 36	Not more than 3.0%	0.6%
Assay (HPLC) NB 1, LDR 31	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.4%
Factor NB 1, PDR 9	Factor the material on the basis of assay and total volatiles content	0.9975
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00 NB 5, PDR 5	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
[1]All results conform to specifications.
ND = None detected

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Quality Assurance Date

2/17/04
Wei Pan, Ph.D. Date
Study Director

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Revised Certificate of Analysis

Supercedes Certificate of Analysis Dated August 14, 2003
November 5, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117 Lot No: UT15-030602	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance***
Date of Manufacture: 06-24-03 **Retest Date: 06-24-05** **Date Testing Started: 07-21-03** **Date Testing Completed: 08-06-03**	**Storage/Packaging:** **5 °C/Ambient** **Amber Nalgene bottle with amber Nalgene screw cap**	**Phase:** **RT-Phase 14** **Method:** **ATM-LRR-M0002.17**

TEST	SPECIFICATIONS	RESULTS[1]
Physical Examination	A white to cream-colored powder	White powder
Identification by IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%
Residual Solvents by Gas Chromatography	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol <0.1% Acetic Acid ND
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.8 °C - 123.1 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 45.7 °
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	0 CFU/g 0 CFU/g Absent Absent Absent Absent

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Revised Certificate of Analysis

Supercedes Certificate of Analysis Dated August 14, 2003
November 5, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-030602		
Date of Manufacture: 06-24-03	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 14
Retest Date: 06-24-05		
Date Testing Started: 07-21-03		**Method:** ATM-LRR-M0002.17
Date Testing Completed: 08-06-03		

TEST	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%, w/w	<0.05%
2AU90	Not more than 0.5%, w/w	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%, w/w	<0.05%
3AU90	Not more than 1.0%, w/w	0.2%
Treprostinil Methyl Ester	Not more than 0.2%, w/w	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%, w/w	0.1%
750W93	Not more than 1.5%, w/w	0.06%
751W93	Not more than 1.3%, w/w	<0.05%
Unidentified	Not more than 0.1% AUC each	NR
Total Related Substances	Not more than 3.0%	0.4%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.1%
Factor	Factor the material on the basis of assay and total volatiles content	1.002
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	< 60 EU/mg

[1]All results conform to specifications.
NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
ND = None detected
NR = Not reportable
Total Related Substances value was calculated using unrounded numbers.
Note: Report revised to recalculate Assay and Factor results with the corrected reference standard purity. See memo dated 09-08-03.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

5 Nov 2003
Quality Assurance Date

5 Nov 2003
William E. Weiser, Ph.D. Date
Study Director

Cardinal Health
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Revised Certificate of Analysis

Supercedes Certificate of Analysis Dated September 2, 2003
November 5, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance***
Lot No: **UT15-030601**		
Date of Manufacture: 06-17-03	**Storage/Packaging:** **5 °C/Ambient** **Amber Nalgene bottle with amber Nalgene screw cap**	**Phase:** **RT-Phase 14**
Retest Date: 06-17-05		
Date Testing Started: 07-21-03		**Method:** **ATM-LRR-M0002.17**
Date Testing Completed: 08-06-03		

TEST	SPECIFICATIONS	RESULTS[1]
Physical Examination	A white to cream-colored powder	White powder
Identification by IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%
Residual Solvents by Gas Chromatography	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol <0.1% Acetic Acid ND
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	120.5 °C - 122.8 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 46.5 °
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 0 CFU/g 0 CFU/g Absent Absent Absent Absent

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-030601		
Date of Manufacture: 06-17-03	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 14
Retest Date: 06-17-05		
Date Testing Started: 07-21-03		**Method:** ATM-LRR-M0002.17
Date Testing Completed: 08-06-03		

TEST	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%, w/w	<0.05%
2AU90	Not more than 0.5%, w/w	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%, w/w	<0.05%
3AU90	Not more than 1.0%, w/w	0.2%
Treprostinil Methyl Ester	Not more than 0.2%, w/w	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%, w/w	0.09%
750W93	Not more than 1.5%, w/w	<0.05%
751W93	Not more than 1.3%, w/w	<0.05%
Unidentified	Not more than 0.1% AUC each	NR
Total Related Substances	Not more than 3.0%	0.3%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.1%
Factor	Factor the material on the basis of assay and total volatiles content	1.002
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	< 60 EU/mg

[1]All results conform to specifications.
NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
ND = None detected
NR = Not reportable
Total Related Substances value was calculated using unrounded numbers.

Note: Report revised to recalculate Assay and Factor results with the corrected reference standard purity. See memo dated 09-08-03.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

5 Nov 2003
Quality Assurance — Date

5 Nov 2003
William E. Weiser, Ph.D. — Date
Study Director

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	Company: United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	Product: Treprostinil Drug Substance*
Lot No: UT15-030504		
Date of Manufacture: 06-10-03	Storage/Packaging: 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	Phase: RT-Phase 14
Retest Date: 06-10-05		
Date Testing Started: 07-21-03		Method: ATM-LRR-M0002.17
Date Testing Completed: 08-06-03		

TEST	SPECIFICATIONS	RESULTS[1]
Physical Examination	A white to cream-colored powder	White powder
Identification by IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%
Residual Solvents by Gas Chromatography	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate <0.1% Ethanol <0.1% Acetic Acid ND
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.7 °C - 123.3 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 47.5 °
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 2 CFU/g 0 CFU/g Absent Absent Absent Absent

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	Company: United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	Product: Treprostinil Drug Substance*
Lot No: UT15-030504		
Date of Manufacture: 06-10-03	Storage/Packaging: 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	Phase: RT-Phase 14
Retest Date: 06-10-05		
Date Testing Started: 07-21-03		Method: ATM-LRR-M0002.17
Date Testing Completed: 08-06-03		

TEST	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%, w/w	<0.05%
2AU90	Not more than 0.5%, w/w	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%, w/w	<0.05%
3AU90	Not more than 1.0%, w/w	0.2%
Treprostinil Methyl Ester	Not more than 0.2%, w/w	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%, w/w	0.1%
750W93	Not more than 1.5%, w/w	0.06%
751W93	Not more than 1.3%, w/w	<0.05%
Unidentified	Not more than 0.1% AUC each	NR
Total Related Substances	Not more than 3.0%	0.4%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.0%
Factor	Factor the material on the basis of assay and total volatiles content	1.003
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	< 60 EU/mg

[1]All results conform to specifications.
NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
ND = None detected
NR = Not reportable
Total Related Substances value was calculated using unrounded numbers.
Note: Report revised to recalculate Assay and Factor results with the corrected reference standard purity. See memo dated 09-08-03.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — 5 Nov 2003 — Date

William E. Weiser, Ph.D. — Study Director — 5 Nov 2003 — Date

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February 17, 2004

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117 **Lot No:** UT15-030503	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Date of Manufacture: 05-30-03 **Retest Date: 05-30-05** **Date Testing Started: 06-04-03** **Date Testing Completed: 06-23-03**	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13 **Method:** ATM-LRR-M0002.17

TEST	SPECIFICATIONS	RESULTS[1]
Physical Examination	A white to cream-colored powder	White powder
Identification by IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%
Residual Solvents by Gas Chromatography	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate 0.0% Ethanol 0.1% Acetic Acid 0.0%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	120.3 °C – 121.3°C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+ 44.4°
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	2 CFU/g 2 CFU/g Absent Absent Absent Absent

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February 17, 2004

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-030503		
Date of Manufacture: 05-30-03	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13
Retest Date: 05-30-05		
Date Testing Started: 06-04-03		**Method:** ATM-LRR-M0002.17
Date Testing Completed: 06-23-03		

TEST	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.3%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.2%
750W93	Not more than 1.5%	0.2%
751W93	Not more than 1.3%	0.1%
Unidentified	Not more than 0.1% AUC each	
Unidentified at RRT 1.12		0.09%
Total Related Substances	Not more than 3.0%	0.9%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.9%
Factor	Factor the material on the basis of assay and total volatiles content	1.004
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	< 60 EU/mg

[1] All results conform to specifications.
NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
ND = None detected
Note: Report revised to correct Total Related Substances value to match current methodology.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

17 Feb 2004
Quality Assurance — Date

2/17/04
William E. Weiser, Ph.D. — Date
Study Director

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February 17, 2004

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-030502		
Date of Manufacture: 05-23-03	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13
Retest Date: 05-23-05		
Date Testing Started: 06-04-03		**Method:** ATM-LRR-M0002.17
Date Testing Completed: 06-23-03		

TEST	SPECIFICATIONS	RESULTS[1]
Physical Examination	A white to cream-colored powder	White powder
Identification by IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%
Residual Solvents by Gas Chromatography	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate 0.0% Ethanol 0.1% Acetic Acid 0.0%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	120.7 °C – 121.5°C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.2°
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 0 CFU/g 2 CFU/g Absent Absent Absent Absent

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February 17, 2004

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-030502		
Date of Manufacture: 05-23-03	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13
Retest Date: 05-23-05		
Date Testing Started: 06-04-03		**Method:** ATM-LRR-M0002.17
Date Testing Completed: 06-23-03		

TEST	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.3%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.1%
751W93	Not more than 1.3%	0.06%
Unidentified	Not more than 0.1% AUC each	
Unidentified at RRT 1.12		0.08%
Total Related Substances	Not more than 3.0%	0.6%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.5%
Factor	Factor the material on the basis of assay and total volatiles content	1.008
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	< 60 EU/mg

[1]All results conform to specifications.
NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
ND = None detected
Total Related Substances value was calculated using unrounded numbers.
Note: Report revised to correct Total Related Substances value to match current methodology.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

17 Feb 2004
Quality Assurance — Date

WEW 2/17/04
William E. Weiser, Ph.D. — Date
Study Director

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117 **Lot No:** UT15-030501	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Date of Manufacture: 05-15-03 **Retest Date: 05-15-05** **Date Testing Started: 06-04-03** **Date Testing Completed: 06-23-03**	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13 **Method:** ATM-LRR-M0002.17

TEST	SPECIFICATIONS	RESULTS[3]
Physical Examination	A white to cream-colored powder	White powder
Identification by IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%
Residual Solvents by Gas Chromatography	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate 0.0% Ethanol 0.1% Acetic Acid 0.0%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	120.1 °C - 121.0 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.0°
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 0 CFU/g[1] 2 CFU/g Absent Absent Absent Absent

[1]This is the retest value. The original result was 12 CFU/g. Per sponsor request, sample was retested according to USP guidelines using 2.5 times the sample weight. Both values are being reported.

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117 **Lot No:** UT15-030501	**Company:** United Therapeutics Corporation, 2225 W. Harrison Street, Chicago, IL 60612, (312) 421-1819	**Product:** Treprostinil Drug Substance*
Date of Manufacture: 05-15-03 **Retest Date: 05-15-05** **Date Testing Started: 06-04-03** **Date Testing Completed: 06-23-03**	**Storage/Packaging:** 5 °C/Ambient, Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13 **Method:** ATM-LRR-M0002.17
TEST	**SPECIFICATIONS**	**RESULTS[3]**
Chromatographic Purity (HPLC) 1AU90 2AU90 97W86 (Benzindene Triol) 3AU90 Treprostinil Methyl Ester Treprostinil Ethyl Ester 750W93 751W93 Unidentified Unidentified at RRT 1.12	 Not more than 0.5% Not more than 0.5% Not more than 0.2% Not more than 1.0% Not more than 0.2% Not more than 0.6% Not more than 1.5% Not more than 1.3% Not more than 0.1% AUC each	 ND ND ND 0.3% <0.05% 0.1% 0.1% 0.07% 0.07%
Total Related Substances	Not more than 3.0%	0.6%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.9%
Factor	Factor the material on the basis of assay and total volatiles content	1.004
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	< 60 EU/mg

NMT = Not more than

*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid

[3]All results conform to specifications.

ND = None detected

Note: Report revised to correct Total Related Substances value to match current methodology.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

17 Feb 2004
Quality Assurance — Date

WEW 2/17/04
William E. Weiser, Ph.D., Study Director — Date

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February 17, 2004

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117 **Lot No:** UT15-030401	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Date of Manufacture: 05-09-03 **Retest Date: 05-09-05** **Date Testing Started: 06-04-03** **Date Testing Completed: 06-23-03**	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13 **Method:** ATM-LRR-M0002.17

TEST	SPECIFICATIONS	RESULTS[1]
Physical Examination	A white to cream-colored powder	White powder
Identification by IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
Identification by HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%
Residual Solvents by Gas Chromatography	Ethyl Acetate Not more than 0.5%, w/w Ethanol Not more than 0.5%, w/w Acetic Acid Not more than 0.5%, w/w	Ethyl Acetate 0.0% Ethanol <0.1% Acetic Acid 0.0%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.3°C - 122.0 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.1°
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	0 CFU/g 6 CFU/g Absent Absent Absent Absent

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CardinalHealth

Revised Certificate of Analysis

Supercedes Certificate of Analysis Dated September 30, 2003
February 17, 2004

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP: TTP-LRR-M0117	**Company:** United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	**Product:** Treprostinil Drug Substance*
Lot No: UT15-030401		
Date of Manufacture: 05-09-03	**Storage/Packaging:** 5 °C/Ambient Amber Nalgene bottle with amber Nalgene screw cap	**Phase:** RT-Phase 13
Retest Date: 05-09-05		
Date Testing Started: 06-04-03		**Method:** ATM-LRR-M0002.17
Date Testing Completed: 06-23-03		

TEST	SPECIFICATIONS	RESULTS[1]
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.3%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.2%
750W93	Not more than 1.5%	0.06%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	NR
Total Related Substances	Not more than 3.0%	0.6%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.1%
Factor	Factor the material on the basis of assay and total volatiles content	1.002
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	< 60 EU/mg

[1]All results conform to specifications.
NMT = Not more than
*[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
ND = None detected
NR = Not reportable
Total Related Substances value was calculated using unrounded numbers.
Note: Report revised to correct Total Related Substances value to match current methodology.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — Date: 17Feb2004

William E. Weiser, Ph.D., Study Director — Date: 2/17/04

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Certificate of Analysis
October 31, 2000

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 1**	**Company: United Therapeutics, Corporation Research and Development**	**Product: UT-15 Drug Substance** **Method: ATM-LRR-M0002.13**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000901

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.16%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 Not Detected <0.1% Not Detected
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.3 - 124.5 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +45.0° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000901

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.5%	Not Detected
3AU90	Not more than 1.0%	0.3%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
UT-15 Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 2.0%	0.05%
751W93	Not more than 2.0%	<0.05%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 5.0%	0.5%
Assay (HPLC)	Not less than 95.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.8%
Factor	Factor the material on the basis of assay and total volatiles content	1.004 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were audited by the Quality Assurance Unit of Magellan Laboratories Incorporated for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

Marlene E. Rasetta 10/31/00
Quality Assurance Date

William Edward Weiser, Ph.D. 10/31/00
Study Director Date

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Certificate of Analysis
November 22, 2000

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117	**Company: United Therapeutics, Corporation Research and Development**	**Product: UT-15 Drug Substance**
Phase: RT-Phase 2		**Method: ATM-LRR-M0002.13**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-001001

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.01%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	123.4 - 124.9 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +45.4° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-001001

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	<0.05%
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.5%	Not Detected
3AU90	Not more than 1.0%	0.2%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
UT-15 Ethyl Ester	Not more than 0.6%	0.09%
750W93	Not more than 2.0%	0.09%
751W93	Not more than 2.0%	0.06%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 5.0%	0.4%
Assay (HPLC)	Not less than 95.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.8%
Factor	Factor the material on the basis of assay and total volatiles content	1.003 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were audited by the Quality Assurance Unit of Magellan Laboratories Incorporated for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

Kimberly A. Yongue 11/22/00
Quality Assurance Date

William E Weiser 11/22/2000
William Edward Weiser, Ph.D. Date
Study Director

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Revised Certificate of Analysis [1,2]
May 7, 2001
Supersedes Certificate of Analysis dated March 26, 2001
Retest needed by March 26, 2002

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase:** RT-Phase 4	**Company:** United Therapeutics Corporation Research and Development	**Product:** UT-15 Drug Substance **Method:** ATM-LRR-M0002.15

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010201

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR HPLC	 The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5% Not more than 0.5% Not more than 0.5%	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	123.2 - 124.0 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +47.4° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Conforms

[1]Revised to add endotoxin results, which were tested under TTP-LRR-M0117 RT-Phase 4 AT.
[2]Revised to add header on second page.

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Revised Certificate of Analysis [1,2]
May 7, 2001
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Retest needed by March 26, 2002

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117	**Company:** United Therapeutics Corporation	**Product:** UT-15 Drug Substance
Phase: RT-Phase 4	Research and Development	**Method:** ATM-LRR-M0002.15

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010201

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.2%
UT-15 Methyl Ester	Not more than 0.2%	Not Detected
98W86 (Methoxy Diol)	Not more than 0.1%	Not Detected
UT-15 Ethyl Ester	Not more than 0.6%	0.09%
750W93	Not more than 1.5%	0.06%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 3.0%	0.4%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.3%
Factor	Factor the material on the basis of assay and total volatiles content	1.007 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15
Endotoxins (1:2000 dilution)	< 104 EU/mg	< 60 EU/mg

[1]Revised to add endotoxin results, which were tested under TTP-LRR-M0117 RT-Phase 4 AT.
[2]Revised to add header on second page.

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

K. Ashmore 5/7/01
Quality Assurance Date

Jonathan S. Green, Ph.D. 5/7/01
Study Director Date

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Revised Certificate of Analysis [1,2]
May 7, 2001
Supersedes Certificate of Analysis dated March 26, 2001
Retest needed by March 26, 2002

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117	**Company: United Therapeutics Corporation Research and Development**	**Product: UT-15 Drug Substance**
Phase: RT-Phase 4		**Method: ATM-LRR-M0002.15**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010202

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5% Not more than 0.5% Not more than 0.5%	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	123.0 - 124.0 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +47.2° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Conforms

[1]Revised to add endotoxin results, which were tested under TTP-LRR-M0117 RT-Phase 4 AT.
[2]Revised to add header on second page.

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Revised Certificate of Analysis [1,2]
May 7, 2001
Supersedes Certificate of Analysis dated March 26, 2001
Retest needed by March 26, 2002

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117	**Company:** United Therapeutics Corporation Research and Development	**Product:** UT-15 Drug Substance
Phase: RT-Phase 4		**Method:** ATM-LRR-M0002.15

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010202

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.2%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	Not Detected
UT-15 Ethyl Ester	Not more than 0.6%	0.09%
750W93	Not more than 1.5%	0.06%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 3.0%	0.4%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.8%
Factor	Factor the material on the basis of assay and total volatiles content	1.002 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15
Endotoxins (1:2000 dilution)	< 104 EU/mg	< 60 EU/mg

[1]Revised to add endotoxin results, which were tested under TTP-LRR-M0117 RT-Phase 4 AT.
[2]Revised to add header on second page.

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

K. Ashmore 5/7/01
Quality Assurance Date

5/7/01
Jonathan S. Green, Ph.D. Date
Study Director

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Revised Certificate of Analysis [1,2]
May 7, 2001
Supersedes Certificate of Analysis dated March 26, 2001
Retest needed by March 26, 2002

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117	**Company: United Therapeutics Corporation Research and Development**	**Product: UT-15 Drug Substance**
Phase: RT-Phase 4		**Method: ATM-LRR-M0002.15**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010203

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification		
IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography		
Ethyl Acetate	Not more than 0.5%	<0.1%
Ethanol	Not more than 0.5%	<0.1%
Acetic Acid	Not more than 0.5%	Not Detected
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	123.3 - 124.4 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +47.1° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Conforms

[1]Revised to add endotoxin results, which were tested under TTP-LRR-M0117 RT-Phase 4 AT.
[2]Revised to add header on second page.

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Revised Certificate of Analysis [1,2]
May 7, 2001
Supersedes Certificate of Analysis dated March 26, 2001
Retest needed by March 26, 2002

Release Testing for Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase:** RT-Phase 4	**Company:** United Therapeutics Corporation Research and Development	**Product:** UT-15 Drug Substance **Method:** ATM-LRR-M0002.15

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010203

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	0.2%
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	0.08%
3AU90	Not more than 1.0%	0.3%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
UT-15 Ethyl Ester	Not more than 0.6%	0.4%
750W93	Not more than 1.5%	0.3%
751W93	Not more than 1.3%	0.2%
Unidentified Unid @ RRT 0.59	Not more than 0.1% each	0.06%
Total Related Substances	Not more than 3.0%	1.5%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	98.1%
Factor	Factor the material on the basis of assay and total volatiles content	1.021 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15
Endotoxins (1:2000 dilution)	< 104 EU/mg	< 60 EU/mg

[1]Revised to add endotoxin results, which were tested under TTP-LRR-M0117 RT-Phase 4 AT.
[2]Revised to add header on second page.

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

K. Ashmore 5/7/01
Quality Assurance Date

Jonathan S. Green, Ph.D. 5/7/01
Study Director Date

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Magellan LABORATORIES

Revised Certificate of Analysis[1]

August 30, 2001
Supercedes Certificate of Analysis issued August 27, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 5**	**Company: United Therapeutics Corporation Research and Development**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.15**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010301
Manufactured 3/15/2001 and Retest 3/15/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR HPLC	 The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5% Not more than 0.5% Not more than 0.5%	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	123.2 – 124.0 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +45.1° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

[1]Revised to include manufacturing dates and retest dates per Sponsor request and to change the drug substance name to Treprostinil.

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August 27, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 5**	**Company: United Therapeutics Corporation Research and Development**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.15**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010301
Manufactured 3/15/2001 and Retest 3/15/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	Not Detected
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.3%
Treprostinil Methyl Ester	Not more than 0.2%	Not Detected
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.09%
750W93	Not more than 1.5%	0.07%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 3.0%	0.5%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.1%
Factor	Factor the material on the basis of assay and total volatiles content	1.011 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution)	< 104 EU/mg	< 60 EU/mg

[1]Revised to include manufacturing dates and retest dates per Sponsor request and to change the drug substance name to Treprostinil.

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Jean D Mase 8/30/01
Quality Assurance Date

William T Green /JSG 30 Aug 2001
Jonathan S. Green, Ph.D. Date
Study Director

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 5**	**Company: United Therapeutics Corporation Research and Development**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.15**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010302
Manufactured 3/27/2001 and Retest 3/27/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR HPLC	 The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5% Not more than 0.5% Not more than 0.5%	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	123.1 - 123.9 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +47.5° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

[1]Revised to include manufacturing dates and retest dates per Sponsor request and to change the drug substance name to Treprostinil.

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase:** RT-Phase 5	**Company:** United Therapeutics Corporation Research and Development	**Product:** Treprostinil Drug Substance **Method:** ATM-LRR-M0002.15

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010302
Manufactured 3/27/2001 and Retest 3/27/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	<0.05%
2AU90	Not more than 0.5%	Not Detected
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	Not Detected
98W86 (Methoxy Diol)	Not more than 0.1%	Not Detected
Treprostinil Ethyl Ester	Not more than 0.6%	0.08%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 3.0%	0.3%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.6%
Factor	Factor the material on the basis of assay and total volatiles content	1.005 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution)	< 104 EU/mg	< 60 EU/mg

[1]Revised to include manufacturing dates and retest dates per Sponsor request and to change the drug substance name to Treprostinil.

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Jean D Mase 8/30/01
Quality Assurance Date

JSG 30 Aug 2001
Jonathan S. Green, Ph.D. Date
Study Director

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 5**	**Company: United Therapeutics Corporation Research and Development**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.15**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010303
Manufactured 4/10/2001 and Retest 4/10/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR HPLC	 The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5% Not more than 0.5% Not more than 0.5%	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	123.0 - 123.9 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +47.5° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

[1]Revised to include manufacturing dates and retest dates per Sponsor request and to change the drug substance name to Treprostinil.

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 5**	**Company: United Therapeutics Corporation Research and Development**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.15**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010303
Manufactured 4/10/2001 and Retest 4/10/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	Not Detected
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	Not Detected
98W86 (Methoxy Diol)	Not more than 0.1%	Not Detected
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 3.0%	0.3%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.0%
Factor	Factor the material on the basis of assay and total volatiles content	1.002 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution)	< 104 EU/mg	< 60 EU/mg

[1]Revised to include manufacturing dates and retest dates per Sponsor request and to change the drug substance name to Treprostinil.

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Jean D Mase 8/30/01
Quality Assurance Date

William C Mc JSG 30 Aug 2001
Jonathan S. Green, Ph.D. Date
Study Director

RPT-A-LRR-M0117-007-01.00

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Release Testing for Commercial Lots of Treprostinil Drug Substance

Technical TP Number: TTP-LRR-M0117
Study Phase: RT-Phase 6
October 23, 2001

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October 15, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 6	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.16

[[(1*R*,2*R*,3a*S*,9a*S*)-2;3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010802
Manufactured 8/28/2001 and Retest 8/28/2002
Testing started 9/24/01 and completed 10/10/01

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification		
IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography		
Ethyl Acetate	Not more than 0.5%	<0.1%
Ethanol	Not more than 0.5%	<0.1%
Acetic Acid	Not more than 0.5%	<0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.4 - 124.8 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[a]_{589}^{25}$ = +45.7° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits		
Total Aerobic Count Total Yeast and Mold Count	NMT 10 CFU/g	2 CFU/g
Escherichia coli	NMT 10 CFU/g	2 CFU/g
Salmonella species	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Staphylococcus aureus	Absent	Absent
ATM-LRR-M0007.00	Absent	Absent

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October 15, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 6	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.16

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010802
Manufactured 8/28/2001 and Retest 8/28/2002
Testing started 9/24/01 and completed 10/10/01

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	< 0.05%
2AU90	Not more than 0.5%	< 0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	Not Detected
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	< 0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	< 0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	< 0.05%
750W93	Not more than 1.5%	< 0.05%
751W93	Not more than 1.3%	< 0.05%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 3.0%	0.2%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.7%
Factor	Factor the material on the basis of assay and total volatiles content	1.005 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

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Jean D Mase 10/15/2001
Quality Assurance Date

William E. Weiser 15 Oct 2001
William E. Weiser, Ph.D. Date
Study Director

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October 15, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 6	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.16

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010803
Manufactured 9/13/2001 and Retest 9/13/2002
Testing started 9/24/01 and completed 10/10/01

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification		
IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography		
Ethyl Acetate	Not more than 0.5%	<0.1%
Ethanol	Not more than 0.5%	0.1%
Acetic Acid	Not more than 0.5%	<0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.6 - 124.6 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[a]_{589}^{25}$ = +45.2° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits		
Total Aerobic Count	NMT 10 CFU/g	< 2 CFU/g
Total Yeast and Mold Count	NMT 10 CFU/g	< 2 CFU/g
Escherichia coli	Absent	Absent
Salmonella species	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Staphylococcus aureus	Absent	Absent
ATM-LRR-M0007.00		

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October 15, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 6	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.16

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010803
Manufactured 9/13/2001 and Retest 9/13/2002
Testing started 9/24/01 and completed 10/10/01

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	<0.05%
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	Not Detected
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.07%
750W93	Not more than 1.5%	0.1%
751W93	Not more than 1.3%	0.06%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 3.0%	0.4%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.7%
Factor	Factor the material on the basis of assay and total volatiles content	1.006 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

Quality Assurance — Date 10/15/2001

William E. Weiser, Ph.D. — Study Director — Date 15 Oct 2001

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Certificate of Analysis

November 20, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 7	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010901
Manufactured 9/20/2001 and Retest 9/20/2002
Testing started 10/30/01 and completed 11/14/01

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	 The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% 0.1% Not Detected
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.3 – 124.0 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+45.6° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus*	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g 2 CFU/g Absent Absent Absent Absent

ATM-LRR-M0007.00

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Certificate of Analysis

November 20, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 7	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010901
Manufactured 9/20/2001 and Retest 9/20/2002
Testing started 10/30/01 and completed 11/14/01

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	0.07%
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	Not Detected
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.09%
750W93	Not more than 1.5%	0.1%
751W93	Not more than 1.3%	0.08%
Unidentified RRT 1.12	Not more than 0.1% AUC each	0.08%
Total Related Substances	Not more than 3.0%	0.6%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.1%
Factor	Factor the material on the basis of assay and total volatiles content	1.011 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

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Quality Assurance — 20-Nov-2001 — Date

William E. Weiser, Ph.D. — 20 Nov 2001 — Date
Study Director

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November 20, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 7	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010902
Manufactured 10/2/2001 and Retest 10/2/2002
Testing started 10/30/01 and completed 11/14/01

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% Not Detected
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.6 – 124.4 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +45.7° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 2 CFU/g <2 CFU/g Absent Absent Absent Absent

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November 20, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 7	Company: United Therapeutics Corporation Research and Development	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010902
Manufactured 10/2/2001 and Retest 10/2/2002
Testing started 10/30/01 and completed 11/14/01

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	Not Detected
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	Not Detected
98W86 (Methoxy Diol)	Not more than 0.1%	Not Detected
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified RRT 1.12	Not more than 0.1% AUC each	0.07%
Total Related Substances	Not more than 3.0%	0.4%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.5%
Factor	Factor the material on the basis of assay and total volatiles content	1.006 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

20-Nov-2001
Quality Assurance Date

30 Nov 2001
William E. Weiser, Ph.D. Date
Study Director

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November 20, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase:** RT-Phase 7	**Company:** United Therapeutics Corporation Research and Development	**Product:** Treprostinil Drug Substance **Method:** ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-011001
Manufactured 10/11/2001 and Retest 10/11/2002
Testing started 10/30/01 and completed 11/14/01

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% 0.1% Not Detected
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.7 – 124.2 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +45.8° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 < 2 CFU/g < 2 CFU/g Absent Absent Absent Absent

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November 20, 2001

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 7**	**Company: United Therapeutics Corporation Research and Development**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-011001
Manufactured 10/11/2001 and Retest 10/11/2002
Testing started 10/30/01 and completed 11/14/01

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.3%
Treprostinil Methyl Ester	Not more than 0.2%	Not Detected
98W86 (Methoxy Diol)	Not more than 0.1%	Not Detected
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.08%
751W93	Not more than 1.3%	<0.05%
Unidentified RRT 1.12	Not more than 0.1% AUC each	0.07%
Total Related Substances	Not more than 3.0%	0.6%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.4%
Factor	Factor the material on the basis of assay and total volatiles content	1.008 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

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Quality Assurance — 20-Nov-2001 — Date

William E. Weiser, Ph.D. — 20 Nov 2001 — Date
Study Director

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Certificate of Analysis (Part 1)[1]

March 14, 2002

Release Testing for Commercial Lots of Treprostinil Drug Substance			
TTP No.: TTP-LRR-M0117	**Company:** United Therapeutics Corporation	**Product:**	Treprostinil Drug Substance
Phase: RT-Phase 8	Research and Development	**Method:**	ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010801-RP
Manufactured 02/05/2002 and Retest 02/05/2003
Testing started 02/20/02 and completed 03/04/02

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	Average ND <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.4 – 124.6 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.7° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* *(ATM-LRR-M0007.00)*	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 2 CFU/g 2 CFU/g Absent Absent Absent Absent

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Certificate of Analysis (Part 1)[1]

March 14, 2002

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase:** RT-Phase 7	**Company:** United Therapeutics Corporation Research and Development	**Product:** Treprostinil Drug Substance **Method:** ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-010801-RP
Manufactured 02/05/2002 and Retest 02/05/2003
Testing started 02/20/02 and completed 03/04/02

Test	Specifications	Results
Chromatographic Purity (HPLC)		Average
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.1%
Treprostinil Methyl Ester	Not more than 0.2%	ND
98W86 (Methoxy Diol)	Not more than 0.1%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.2%
750W93	Not more than 1.5%	0.2%
751W93	Not more than 1.3%	0.1%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.6%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	98.8%
Factor	Factor the material on the basis of assay and total volatiles content	1.014 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) (ATM-LRR-M0012.00)	< 104 EU/mg	< 60 EU/mg

[1]Results for lot UT15-020101 will be reported in Part 2.
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Lisa M Bergen 3-14-2002
Quality Assurance Date

William E. Weiser, Ph.D. 14 Mar 2002
Study Director Date

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Revised Certificate of Analysis

April 25, 2002
Supercedes Certificate of Analysis dated April 11, 2002

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 9	Company: United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020202
Manufactured 02/26/2002 and Retest 02/26/2003
Testing started 03/22/02 and completed 04/04/02

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.0 – 121.6 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+45.7° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus*	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

ATM-LRR-M0007.00

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Revised Certificate of Analysis

April 25, 2002
Supercedes Certificate of Analysis dated April 11, 2002

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 9**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020202
Manufactured 02/26/2002 and Retest 02/26/2003
Testing started 03/22/02 and completed 04/04/02

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.05%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.2%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	98.8%
Factor	Factor the material on the basis of assay and total volatiles content	1.014 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

Note: Revised Microbial and Endotoxin results.

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25-Apr-02
Quality Assurance — Date

William E. Weiser
25 Apr 2002
William E. Weiser, Ph.D. — Date
Study Director

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CardinalHealth

Revised Certificate of Analysis

February 4, 2003
Supercedes Certificate of Analysis dated April 25, 2002

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 9**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020203
Manufactured 03/07/2002 and Retest 03/07/2004
Testing started 03/22/02 and completed 04/04/02

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.0 – 121.8 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+45.7° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus*	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent
ATM-LRR-M0007.00		

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Revised Certificate of Analysis

February 4, 2003
Supercedes Certificate of Analysis dated April 25, 2002

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 9**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020203
Manufactured 03/07/2002 and Retest 03/07/2004
Testing started 03/22/02 and completed 04/04/02

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.05%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.08%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.2%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	98.9%
Factor	Factor the material on the basis of assay and total volatiles content	1.014 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

Note: Revised to extend retest date for Lot UT15-020203 per Sponsor request.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — 4 Feb 2003 — Date

William E. Weiser, Ph.D. — Study Director — 4 Feb 2003 — Date

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February 4, 2003
Supersedes Certificate of Analysis dated April 26, 2002

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 10**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020301
Manufactured 03/19/2002 and Retest 3/19/2004
Testing started 04/15/2002 and completed 04/22/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% ND[1]
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.5 – 123.5 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+45.6° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* (ATM-LRR-M0007.00)	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 10**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020301
Manufactured 03/19/2002 and Retest 03/19/2004
Testing started 04/15/2002 and completed 04/22/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		Average
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	ND
98W86 (Methoxy Diol)	Not more than 0.1%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.3%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.7%
Factor	Factor the material on the basis of assay and total volatiles content	1.006 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) (ATM-LRR-M0012.00)	< 104 EU/mg	< 60 EU/mg

[1]ND = Not Detected.

Note: Revised to extend retest date per Sponsor request.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

4 Feb 2003
Quality Assurance — Date

4 Feb 2003
William E. Weiser, Ph.D. — Date
Study Director

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 10**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020302
Manufactured 3/28/2002 and Retest 03/28/2004
Testing started 4/15/2002 and completed 04/22/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% ND[1]
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.6 – 124.2 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+45.6° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 10**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020302
Manufactured 03/28/2002 and Retest 03/28/2004
Testing started 04/15/2002 and completed 04/22/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	ND
98W86 (Methoxy Diol)	Not more than 0.1%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.06%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.4%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.6%
Factor	Factor the material on the basis of assay and total volatiles content	1.007 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

[1]ND = Not Detected

Note: Revised to extend retest date per Sponsor request.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

4 Feb 2003
Quality Assurance — Date

4 Feb 2003
William E. Weiser, Ph.D. — Date
Study Director

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 Phase: RT-Phase 10	Company: United Therapeutics Corporation 2225 W. Harrison Street Chicago, IL 60612 (312) 421-1819	Product: Treprostinil Drug Substance Method: ATM-LRR-M0002.17

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020303
Manufactured 04/09/2002 and Retest 04/09/2004
Testing started 04/15/2002 and completed 04/22/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	 The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% ND[1]
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.6 – 123.1 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+45.6° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 <2 CFU/g <2 CFU/g Absent Absent Absent Absent

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 10**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-020303
Manufactured 04/09/2002 and Retest 04/09/2004
Testing started 04/15/2002 and completed 04/22/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	ND
98W86 (Methoxy Diol)	Not more than 0.1%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.3%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.3%
Factor	Factor the material on the basis of assay and total volatiles content	1.011 parts Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	< 104 EU/mg	< 60 EU/mg

[1]ND = Not Detected

Note: Revised to extend retest date per Sponsor request.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

4 Feb 2003
Quality Assurance — Date

4 Feb 2003
William E. Weiser, Ph.D. — Date
Study Director

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January 8, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 11**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021001
Manufactured 10/17/2002 and Retest 10/17/2004
Testing started 11/21/2002 and completed 12/30/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% 0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.1-123.7 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.3°
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* (ATM-LRR-M0007.00)	 NMT[1] 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 2 CFU/g 0 CFU/g Absent Absent Absent Absent

[1]NMT = Not More Than

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 11**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021001
Manufactured 10/17/2002 and Retest 10/17/2004
Testing started 11/21/2002 and completed 12/30/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%
3AU90	Not more than 1.0%	0.4%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.1%
751W93	Not more than 1.3%	0.08%
Unidentified	Not more than 0.1% AUC each Unidentified @ RRT 1.12	0.07%
Total Related Substances	Not more than 3.0%	0.8%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.3%
Factor	Factor the material on the basis of assay and total volatiles content	1.011 Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) (ATM-LRR-M0012.00)	Less than 104 EU/mg	<60 EU/mg

[1]ND = Not Detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Ryan Poling 08 Jan 2003
Quality Assurance Date

William E. Weiser 8 Jan 2003
William E. Weiser, Ph.D. Date
Study Director

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Revised Certificate of Analysis[1]

January 23, 2003
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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 11**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021002
Manufactured 11/5/2002 and Retest 11/5/2004
Testing started 11/21/2002 and completed 1/21/2003

Test	Specifications	Results[2]
Physical Examination	A white to cream-colored powder	White powder
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.1%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.5 °C – 124.6 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.4 °
Heavy Metals	Not more than 0.002%	Not more than 0.002%

[1]Report revised to include investigation of high assay result of Assay prep 1.
[2]All results conform to specifications.

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Revised Certificate of Analysis[1]

January 23, 2003
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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 11**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021002
Manufactured 11/5/2002 and Retest 11/5/2004
Testing started 11/21/2002 and completed 1/21/2003

Test	Specifications	Results[2]	
Chromatographic Purity (HPLC)[3]			
1AU90	Not more than 0.5%	ND[4]	
2AU90	Not more than 0.5%	<0.05%	
97W86 (Benzindene Triol)	Not more than 0.2%	<0.05%	
3AU90	Not more than 1.0%	0.3%	
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%	
Treprostinil Ethyl Ester	Not more than 0.6%	0.2%	
750W93	Not more than 1.5%	0.06%	
751W93	Not more than 1.3%	<0.05%	
Unidentified	Not more than 0.1% AUC each Unidentified @ RRT 1.12	0.05%	
Total Related Substances	Not more than 3.0%	0.6%	
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	Prep[5]	Result
		1	101.2%[6]
		2	99.8%
		3	99.9%
		4	100.1%
		5	99.9%
		6	100.0%
		7	100.1%
		Average(n=6)	100.0%

[1]Report revised to include investigation of high assay result of Assay prep 1.
[2]All results, except Assay (HPLC) Prep 1, conform to specifications.
[3]The impurities reported are the average of n=6.
[4]ND = Not detected.
[5]Preparations one and two were tested on 12-17-2002. Preparations three through seven were tested on 1-17-2003.
[6]This result is out of specifications. An investigation was conducted and an assignable cause was not determined. A laboratory error is suspected but unconfirmed. Five additional sample preps confirm the result of prep two. This result is not included in the reported average.

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 11**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021002
Manufactured 11/5/2002 and Retest 11/5/2004
Testing started 11/21/2002 and completed 1/21/2003

Test	Specifications	Results[2]
Factor	Factor the material on the basis of assay and total volatiles content	1.002 Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Microbial Limits		
Total Aerobic Count	NMT[3] 10 CFU/g	2 CFU/g
Total Yeast and Mold Count	NMT 10 CFU/g	4 CFU/g
Escherichia coli	Absent	Absent
Salmonella species	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Staphylococcus aureus	Absent	Absent
ATM-LRR-M0007.00		
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	<60 EU/mg

[1]Report revised to include investigation of high assay result of Assay prep 1.
[2]All results conform to specifications.
[3]NMT = Not More Than

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — 23 Jan 2003 — Date

William E. Weiser, Ph.D. — Study Director — 1/WEW — 1/23/03 — Date

RPT-A-LRR-M0117-012-01.01

REVISED Certificate of Analysis

Release Testing for Commercial Lots of Treprostinil Drug Substance

Technical TP Number: TTP-LRR-M0117
Study Phase: RT-Phase 11

January 23, 2003

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Certificate of Analysis

January 8, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 11**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021003
Manufactured 11/12/2002 and Retest 11/12/2004
Testing started 11/21/2002 and completed 12/30/2002

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	A white powder
Identification IR HPLC	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.7-123.9 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.1°
Heavy Metals	Not more than 0.002%	Not more than 0.002%
Microbial Limits Total Aerobic Count Total Yeast and Mold Count *Escherichia coli* *Salmonella* species *Pseudomonas aeruginosa* *Staphylococcus aureus* ATM-LRR-M0007.00	 NMT[1] 10 CFU/g NMT 10 CFU/g Absent Absent Absent Absent	 2 CFU/g 0 CFU/g Absent Absent Absent Absent

[1]NMT = Not More Than

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January 8, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117 **Phase: RT-Phase 11**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product: Treprostinil Drug Substance** **Method: ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021003
Manufactured 11/12/2002 and Retest 11/12/2004
Testing started 11/21/2002 and completed 12/30/2002

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND[1]
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.4%
Treprostinil Methyl Ester	Not more than 0.2%	<0.05%
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	<0.05%
751W93	Not more than 1.3%	<0.05%
Unidentified	Not more than 0.1% AUC each Unidentified @ RRT 1.12	0.07%
Total Related Substances	Not more than 3.0%	0.6%
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.8%
Factor	Factor the material on the basis of assay and total volatiles content	0.9929 Treprostinil equivalent to 1.00 parts volatiles-free Treprostinil
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	<60 EU/mg

[1] ND = Not Detected

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — 08 Jan 2003 — Date

William E. Weiser, Ph.D. — Study Director — 8 Jan 2003 — Date

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January 16, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 12**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021101
Manufactured 11/21/2002 and Retest 11/21/2004
Testing started 12/17/2002 and completed 1/3/2003

Test	Specifications	Results[1]
Physical Examination	A white to cream-colored powder	White powder
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% ND[2]
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.1 °C – 124.6 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+45.0 °
Heavy Metals	Not more than 0.002%	Not more than 0.002%

[1]All results conform to specifications.
[2]ND = Not Detected

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January 16, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 12**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021101
Manufactured 11/21/2002 and Retest 11/21/2004
Testing started 12/17/2002 and completed 1/3/2003

Test	Specifications	Results[1]
Microbial Limits		
Total Aerobic Count	NMT[2] 10 CFU/g	2 CFU/g
Total Yeast and Mold Count	NMT 10 CFU/g	0 CFU/g
Escherichia coli	Absent	Absent
Salmonella species	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Staphylococcus aureus	Absent	Absent
ATM-LRR-M0007.00		
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND[3]
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	ND
3AU90	Not more than 1.0%	0.2%
Treprostinil Methyl Ester	Not more than 0.2%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.09%
751W93	Not more than 1.3%	0.06%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.5%

[1] All results conform to specifications.
[2] NMT = Not More Than
[3] ND = Not Detected

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 12**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021101
Manufactured 11/21/2002 and Retest 11/21/2004
Testing started 12/17/2002 and completed 1/3/2003

Test	Specifications	Results[1]
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.6%
Factor	Factor the material on the basis of assay and total volatiles content	1.005
Endotoxins (1:2000 dilution) ATM-LRR-M0012.00	Less than 104 EU/mg	<60 EU/mg

[1]All results conform to specifications.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

Quality Assurance — 16 Jan 2003 — Date

William E. Weiser, Ph.D. — Study Director — 1/16/03 — Date

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Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 12**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021102
Manufactured 12/5/2002 and Retest 12/5/2004
Testing started 12/17/2002 and completed 1/3/2003

Test	Specifications	Results[1]
Physical Examination	A white to cream-colored powder	White powder
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of Treprostinil, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.0%
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% 0.1% ND[2]
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	121.2 °C – 123.5 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	+46.4 °
Heavy Metals	Not more than 0.002%	Not more than 0.002%

[1]All results conform to specifications.
[2]ND = Not Detected

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January 16, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: **TTP-LRR-M0117**	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: **RT-Phase 12**	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021102
Manufactured 12/5/2002 and Retest 12/5/2004
Testing started 12/17/2002 and completed 1/3/2003

Test	Specifications	Results[1]
Microbial Limits		
Total Aerobic count	NMT[2] 10 CFU/g	0 CFU/g
Total Yeast and Mold Count	NMT 10 CFU/g	0 CFU/g
Escherichia coli	Absent	Absent
Salmonella species	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Staphylococcus aureus	Absent	Absent
(ATM-LRR-M0007.00)		
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND[3]
2AU90	Not more than 0.5%	ND
97W86 (Benzindene Triol)	Not more than 0.2%	0.07%
3AU90	Not more than 1.0%	0.1%
Treprostinil Methyl Ester	Not more than 0.2%	ND
Treprostinil Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 1.5%	0.2%
751W93	Not more than 1.3%	0.1%
Unidentified	Not more than 0.1% AUC each	ND
Total Related Substances	Not more than 3.0%	0.6%

[1]All results conform to specifications.
[2]NMT = Not More Than
[3]ND = Not Detected

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January 16, 2003

Release Testing for Commercial Lots of Treprostinil Drug Substance		
TTP No.: TTP-LRR-M0117	**Company:** **United Therapeutics Corporation** **2225 W. Harrison Street** **Chicago, IL 60612** **(312) 421-1819**	**Product:** **Treprostinil Drug Substance**
Phase: RT-Phase 12	**Packaging/Storage:** **Amber HDPE bottle** **with NCR screw cap** **5 °C/Ambient RH**	**Method:** **ATM-LRR-M0002.17**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-021102
Manufactured 12/5/2002 and Retest 12/5/2004
Testing started 12/17/2002 and completed 1/3/2003

Test	Specifications	Results[1]
Assay (HPLC)	Not less than 97.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.2%
Factor	Factor the material on the basis of assay and total volatiles content	1.011
Endotoxins (1:2000 dilution) (ATM-LRR-M0012.00)	Less than 104 EU/mg	<60 EU/mg

[1]All results conform to specifications.

All data generated at Cardinal Health and described within this report were collected under the direction of the Study Director and were reviewed for compliance with Good Manufacturing Practices (21 CFR Parts 210 and 211). This report accurately reflects the raw data which have been stored in the Archives of Cardinal Health.

16 Jan 2003
Quality Assurance — Date

1/16/03
William E. Weiser, Ph.D. — Date
Study Director

Certificate of Analysis

Release and Stability Testing for Three Lots of UT-15 Drug Substance

Technical TP Number: TTP-LRR-M0107
Study Phase: RT-Phase 1
September 29, 2000

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September 29, 2000

Release and Stability Testing for Three Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0107 **Phase: RT-Phase 1**	**Company: United Therapeutics, Corporation Research and Development**	**Product: UT-15 Drug Substance** **Method: ATM-LRR-M0002.13**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000701

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.04%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120 °C and not more than 126 °C	123.1-124.7 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +44.1° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000701

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND[1]
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.5%	ND
3AU90	Not more than 1.0%	0.1%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	ND
UT-15 Ethyl Ester	Not more than 0.6%	0.08%
750W93	Not more than 2.0%	0.06%
751W93	Not more than 2.0%	<0.05%
Unidentified	Not more than 0.1% each	ND
Total Related Substances	Not more than 5.0%	0.2%
Assay (HPLC)	Not less than 95.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.0%
Factor	Factor the material on the basis of assay and total volatiles content	1.003 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

[1]ND = Not Detected

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were audited by the Quality Assurance Unit of Magellan Laboratories Incorporated for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

Quality Assurance — Date: 9/29/00

William Edward Weiser, Ph.D. — Study Director — Date: 9/29/2000

RPT-A-LRR-M0107-001-03.00

Certificate of Analysis

Release and Stability Testing for Three Lots of UT-15 Drug Substance

Technical TP Number: TTP-LRR-M0107
Study Phase: RT-Phase 1
September 29, 2000

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Certificate of Analysis
September 29, 2000

Release and Stability Testing for Three Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0107 Phase: RT-Phase 1	Company: United Therapeutics, Corporation Research and Development	Product: UT-15 Drug Substance Method: ATM-LRR-M0002.13

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000801

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.01%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120 °C and not more than 126 °C	123.1-124.7 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +44.7° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000801

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND[1]
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.5%	ND
3AU90	Not more than 1.0%	0.2%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	ND
UT-15 Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 2.0%	0.07%
751W93	Not more than 2.0%	<0.05%
Unidentified Unid@RRT16.41	Not more than 0.1% each	0.05%
Total Related Substances	Not more than 5.0%	0.4%
Assay (HPLC)	Not less than 95.0 and not more than 101.0%, w/w, on the volatiles-free basis	100.0%
Factor	Factor the material on the basis of assay and total volatiles content	1.002 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

[1]ND = Not Detected

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were audited by the Quality Assurance Unit of Magellan Laboratories Incorporated for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

Quality Assurance — 9/29/00 Date

William Edward Weiser, Ph.D. — 9/29/2000 Date
Study Director

Certificate of Analysis

Release and Stability Testing for Three Lots of UT-15 Drug Substance

Technical TP Number: TTP-LRR-M0107
Study Phase: RT-Phase 1
September 29, 2000

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Certificate of Analysis
September 29, 2000

Release and Stability Testing for Three Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0107 Phase: RT-Phase 1	Company: United Therapeutics, Corporation Research and Development	Product: UT-15 Drug Substance Method: ATM-LRR-M0002.13

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000802

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR HPLC	 The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained. The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	 Conforms Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.00%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.3%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% <0.1% <0.1%
Melting Range	Not less than 120 °C and not more than 126 °C	123.1-124.7 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +44.0° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000802

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	ND[1]
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.5%	ND
3AU90	Not more than 1.0%	0.1%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	ND
UT-15 Ethyl Ester	Not more than 0.6%	0.07%
750W93	Not more than 2.0%	0.1%
751W93	Not more than 2.0%	0.07%
Unidentified	Not more than 0.1% each	ND
Total Related Substances	Not more than 5.0%	0.3%
Assay (HPLC)	Not less than 95.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.9%
Factor	Factor the material on the basis of assay and total volatiles content	1.004 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

[1]ND = Not Detected

All data generated at Magellan Laboratories Incorporated and described within this report were collected under the direction of the Study Director and were audited by the Quality Assurance Unit of Magellan Laboratories Incorporated for compliance with Good Laboratory Practices (21 CFR Part 58) and Good Manufacturing Practices (21 CFR Part 211). This report accurately reflects the raw data which have been stored in the Archives of Magellan Laboratories Incorporated.

Quality Assurance — 9/29/00 — Date

William Edward Weiser, Ph.D. — 9/29/2000 — Date
Study Director

RPT-A-LRR-M0113-001-02.00

Certificate of Analysis

Release and Stability Testing for Two Commercial Lots of UT-15 Drug Substance

Technical TP Number: TTP-LRR-M0113
Study Phase: Release Testing
October 31, 2000

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Certificate of Analysis
October 31, 2000

Release and Stability Testing for Two Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0113	**Company: United Therapeutics, Corporation Research and Development**	**Product: UT-15 Drug Substance**
Phase: Release Testing		**Method: ATM-LRR-M0002.13**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000902

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.07%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.1%, w/w
Residual Solvents by Gas Chromatography Ethyl Acetate Ethanol Acetic Acid	 Not more than 0.5%, w/w Not more than 0.5%, w/w Not more than 0.5%, w/w	 <0.1% 0.1% <0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.8 - 125.2 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +45.9° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000902

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.5%	Not Detected
3AU90	Not more than 1.0%	0.2%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
UT-15 Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 2.0%	0.1%
751W93	Not more than 2.0%	0.06%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 5.0%	0.5%
Assay (HPLC)	Not less than 95.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.8%
Factor	Factor the material on the basis of assay and total volatiles content	1.004 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

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Marlene E. Baseta 10/31/00
Quality Assurance Date

William Edward Weiser, Ph.D. 10/31/00
Study Director Date

RPT-A-LRR-M0113-001-02.00

Certificate of Analysis

Release and Stability Testing for Two Commercial Lots of UT-15 Drug Substance

Technical TP Number: TTP-LRR-M0113
Study Phase: Release Testing
October 31, 2000

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Certificate of Analysis
October 31, 2000

Release and Stability Testing for Two Commercial Lots of UT-15 Drug Substance		
TTP No.: TTP-LRR-M0113	**Company: United Therapeutics, Corporation Research and Development**	**Product: UT-15 Drug Substance**
Phase: Release Testing		**Method: ATM-LRR-M0002.13**

[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl] -1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000803

Test	Specifications	Results
Physical Examination	A white to cream-colored powder	Conforms
Identification		
IR	The infrared spectrum exhibits maxima which are only at the same wavenumbers as, and have similar relative intensities to, those in the reference standard spectrum of UT-15, similarly obtained.	Conforms
HPLC	The retention time for the principle peak in the sample chromatogram agrees with the retention time of the principle peak in the standard chromatogram, using the chromatographic purity method.	Conforms
Residue on Ignition	Not more than 0.2%, w/w	0.11%, w/w
Water (Karl Fischer)	Not more than 2.0%, w/w	0.2%, w/w
Residual Solvents by Gas Chromatography		
Ethyl Acetate	Not more than 0.5%, w/w	Not Detected
Ethanol	Not more than 0.5%, w/w	0.1%
Acetic Acid	Not more than 0.5%, w/w	<0.1%
Melting Range	Not less than 120.0 °C and not more than 126.0 °C	122.4 - 124.9 °C
Specific Rotation	Not less than +42.0° and not more than +49.0° at 589 nm and 25 °C, volatiles-free basis	$[\alpha]_{589}^{25}$ = +46.2° (volatiles-free basis)
Heavy Metals	Not more than 0.002%	Not more than 0.002%

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[[(1*R*,2*R*,3a*S*,9a*S*)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3*S*)-3-hydroxyoctyl]-1*H*-benz[*f*]inden-5-yl]oxy]acetic acid
Lot UT15-000803

Test	Specifications	Results
Chromatographic Purity (HPLC)		
1AU90	Not more than 0.5%	Not Detected
2AU90	Not more than 0.5%	<0.05%
97W86 (Benzindene Triol)	Not more than 0.5%	Not Detected
3AU90	Not more than 1.0%	0.2%
UT-15 Methyl Ester	Not more than 0.2%	<0.05%
98W86 (Methoxy Diol)	Not more than 0.1%	<0.05%
UT-15 Ethyl Ester	Not more than 0.6%	0.1%
750W93	Not more than 2.0%	0.2%
751W93	Not more than 2.0%	0.09%
Unidentified	Not more than 0.1% each	Not Detected
Total Related Substances	Not more than 5.0%	0.6%
Assay (HPLC)	Not less than 95.0 and not more than 101.0%, w/w, on the volatiles-free basis	99.7%
Factor	Factor the material on the basis of assay and total volatiles content	1.006 parts UT-15 equivalent to 1.00 parts volatiles-free UT-15

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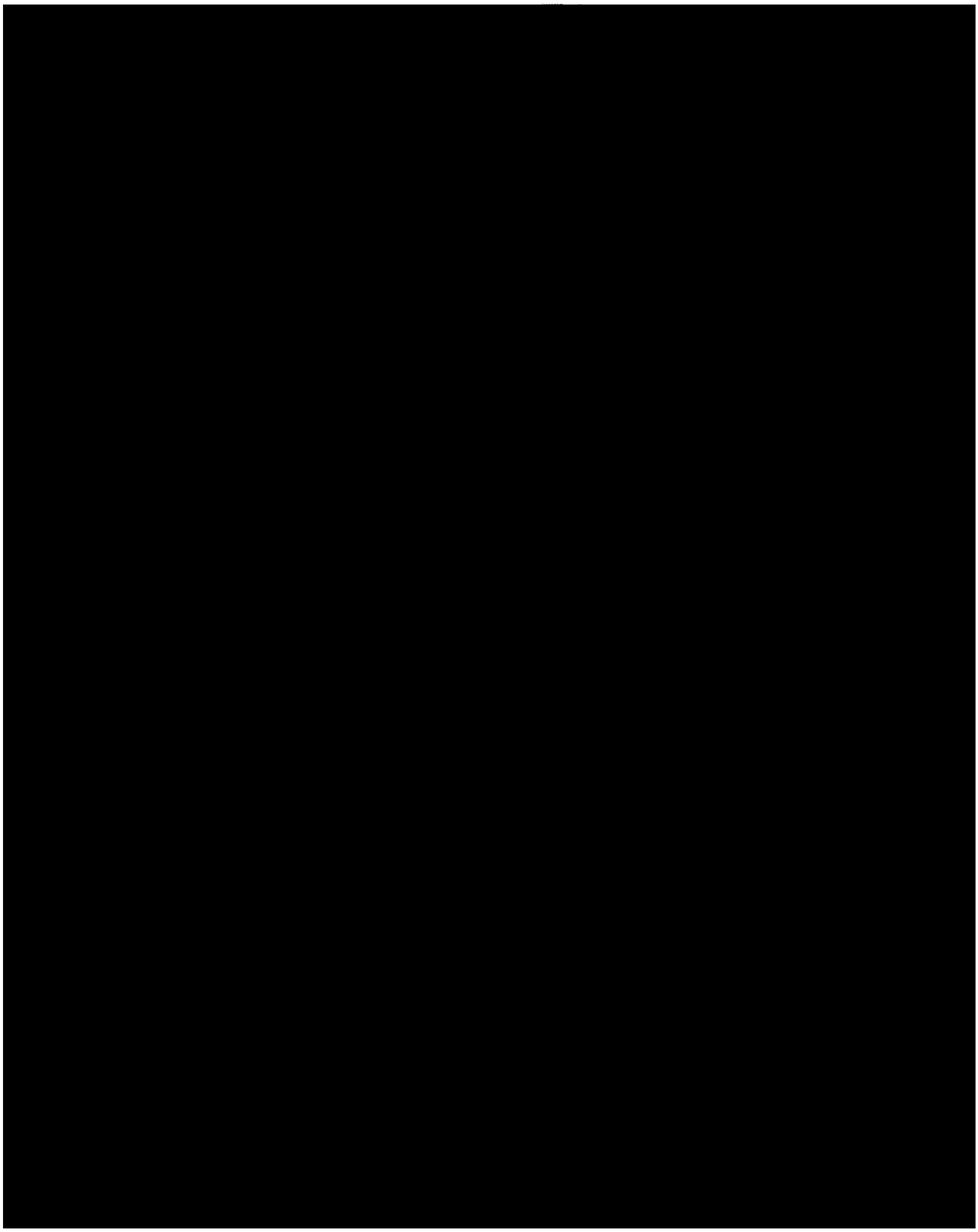
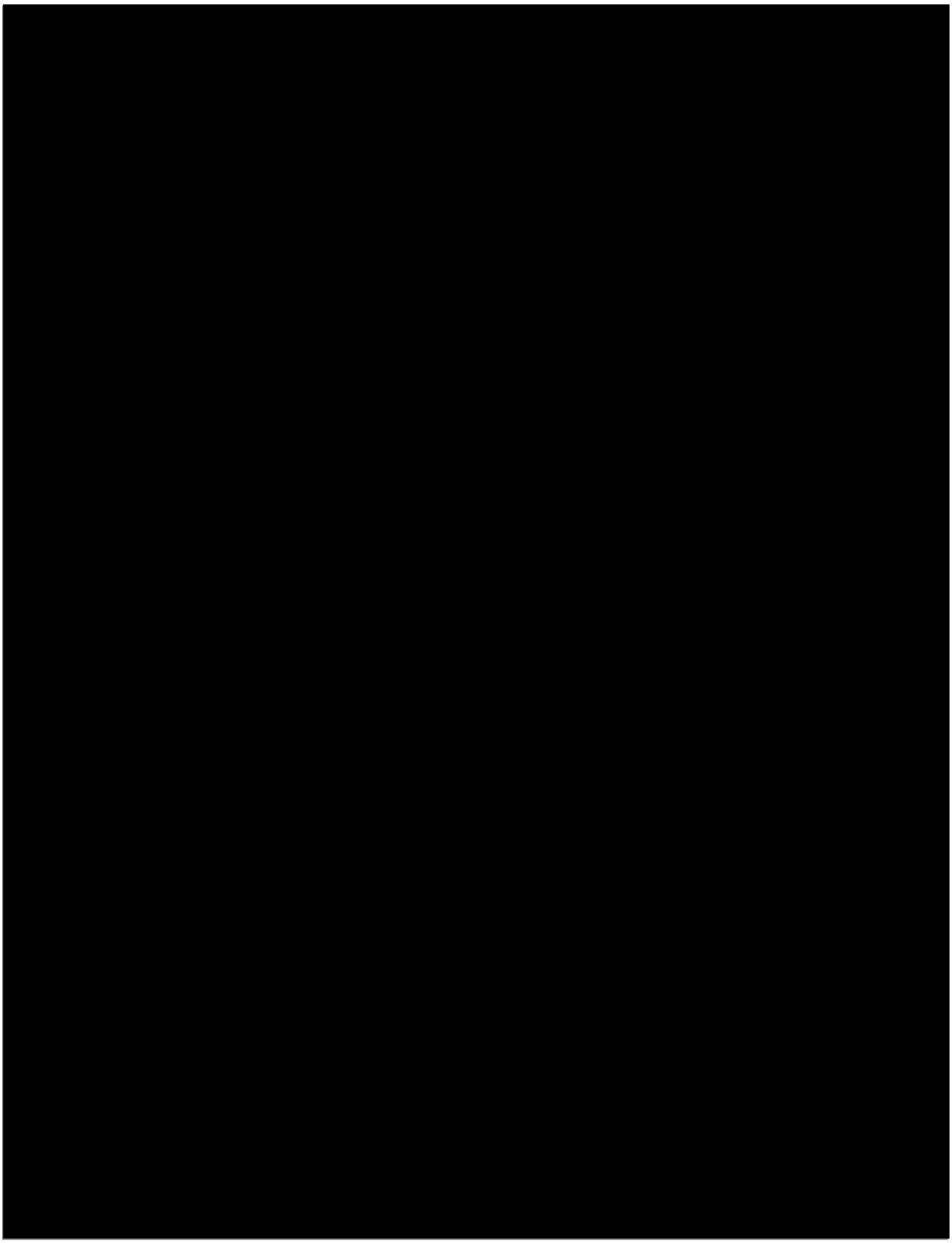
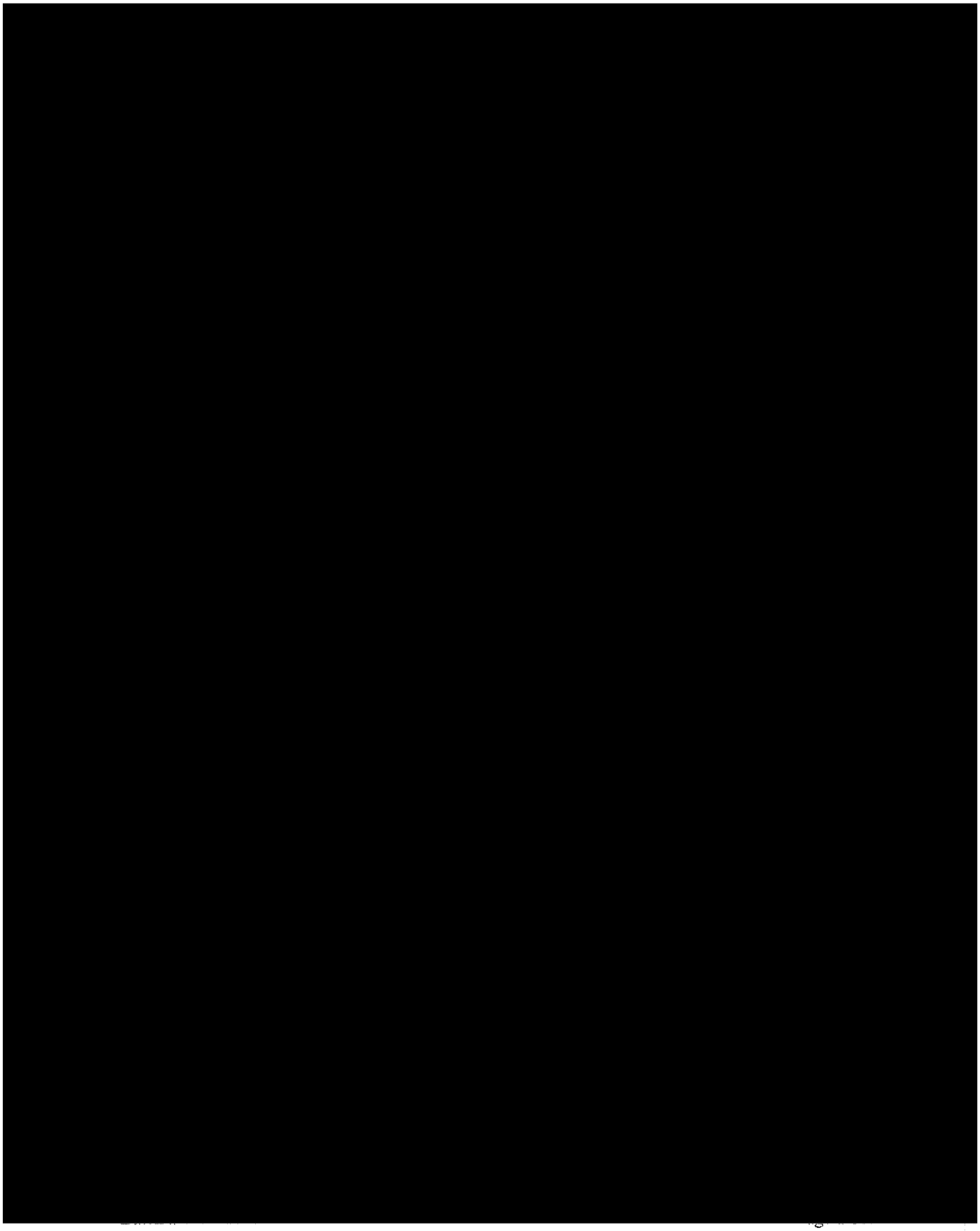
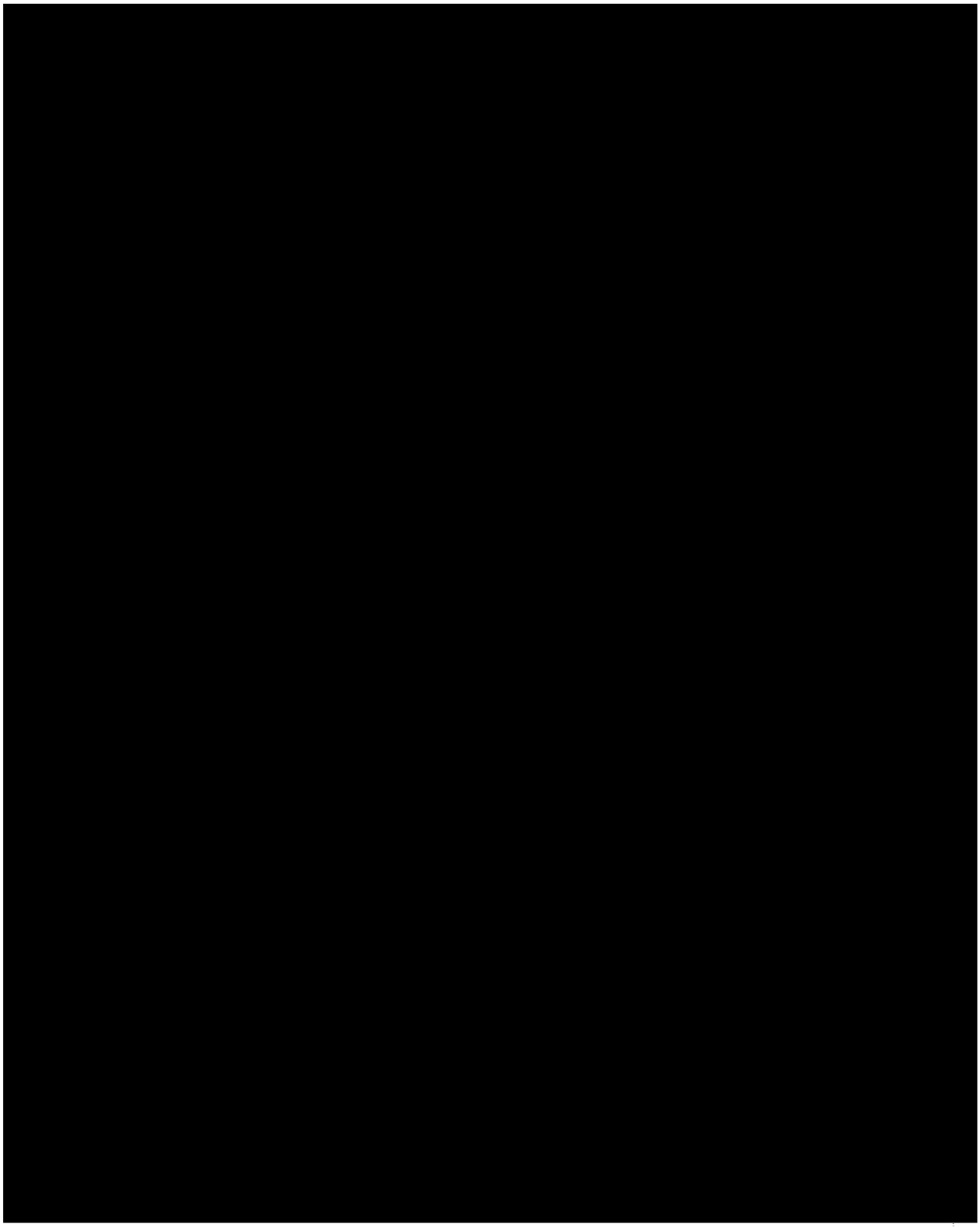
Marlene E. Raseta 10/31/00
Quality Assurance Date

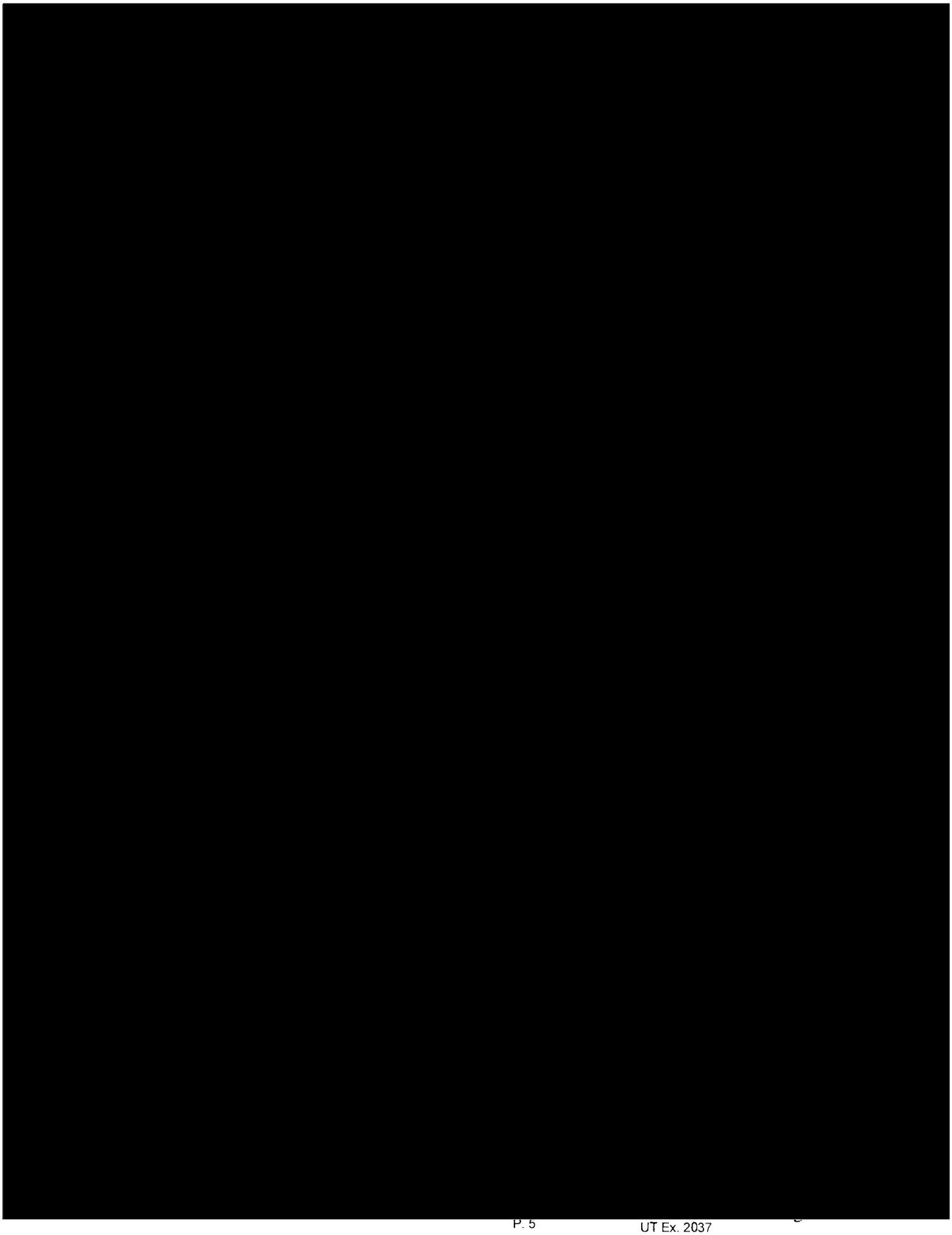
10/31/00
William Edward Weiser, Ph.D. Date
Study Director

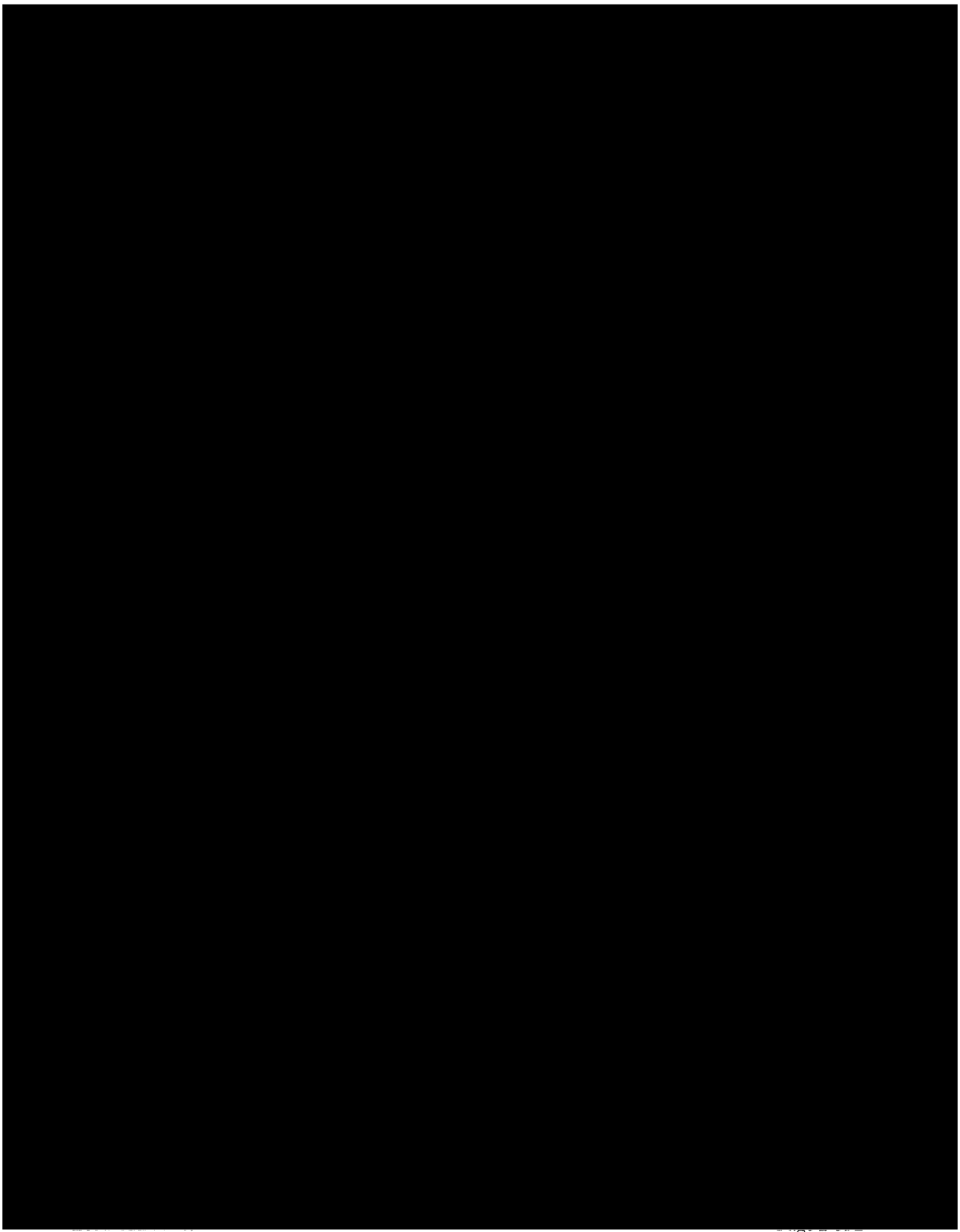


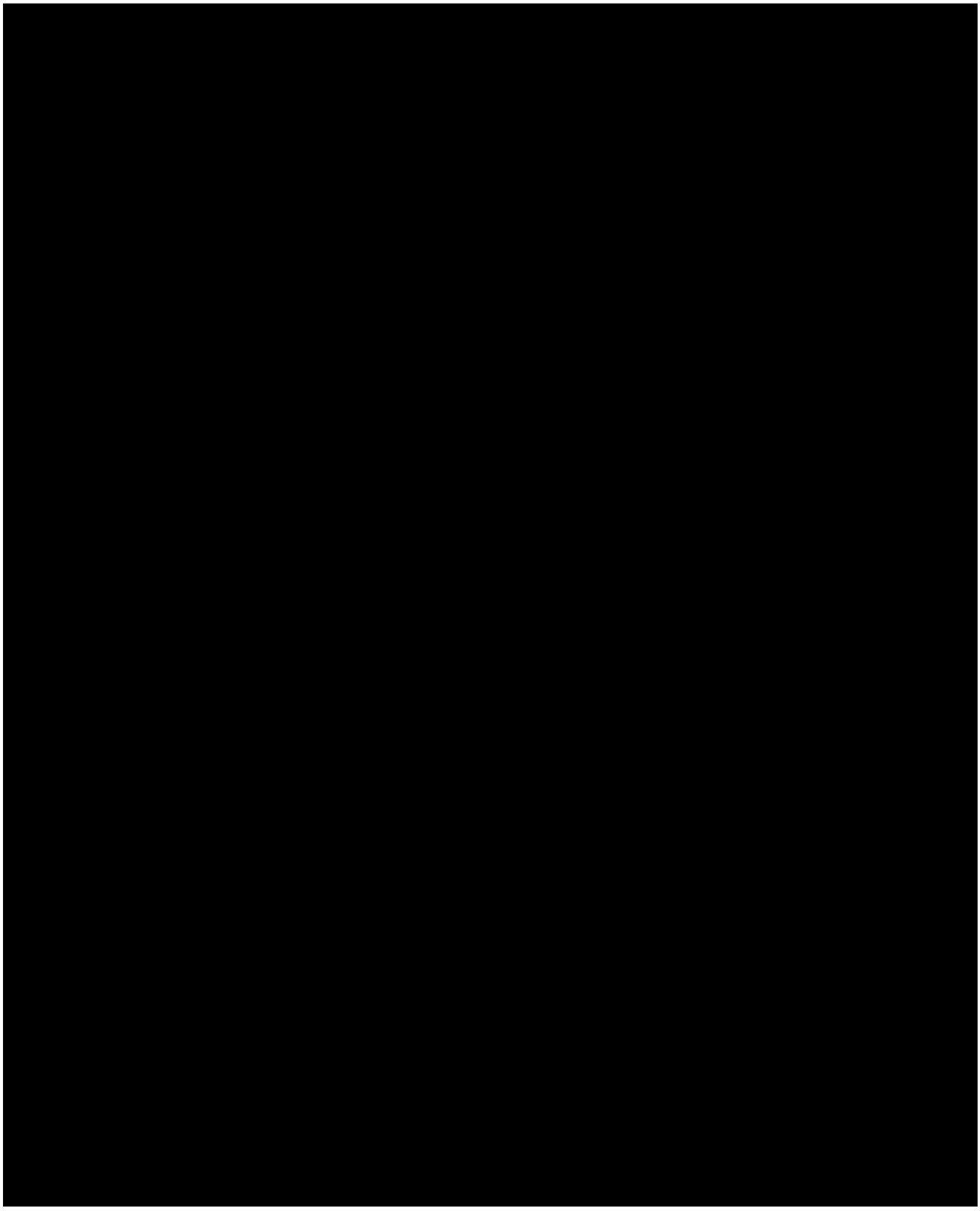


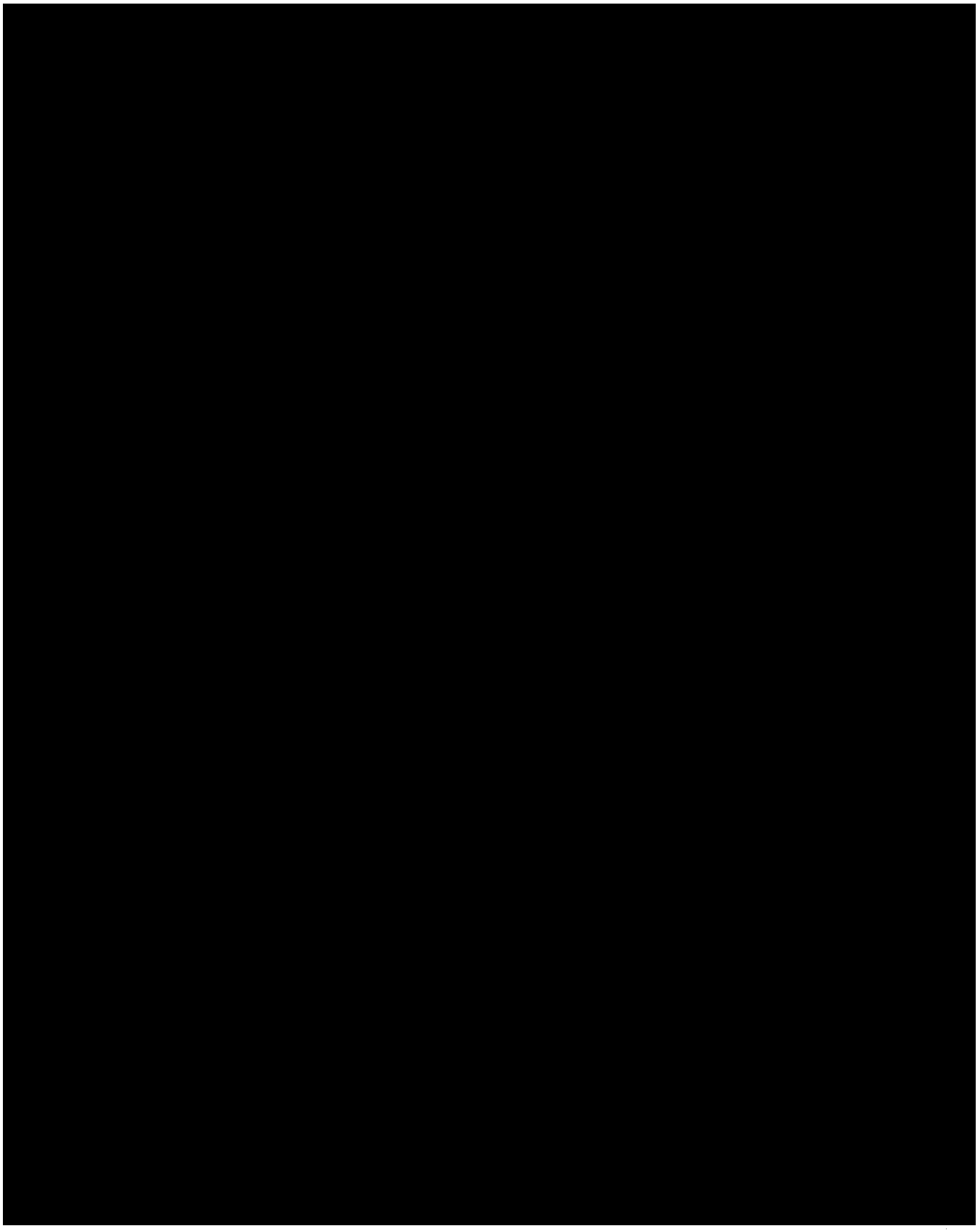


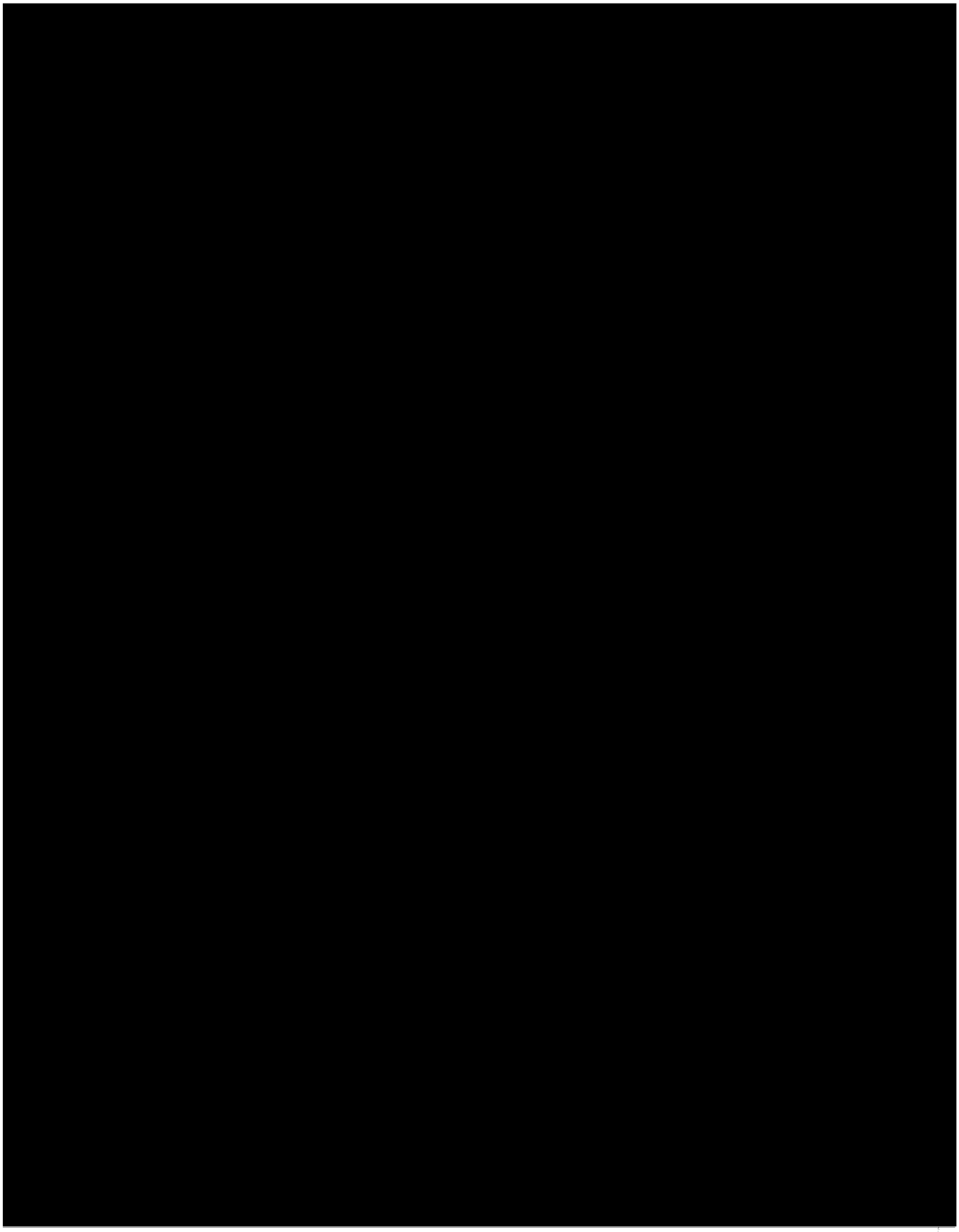


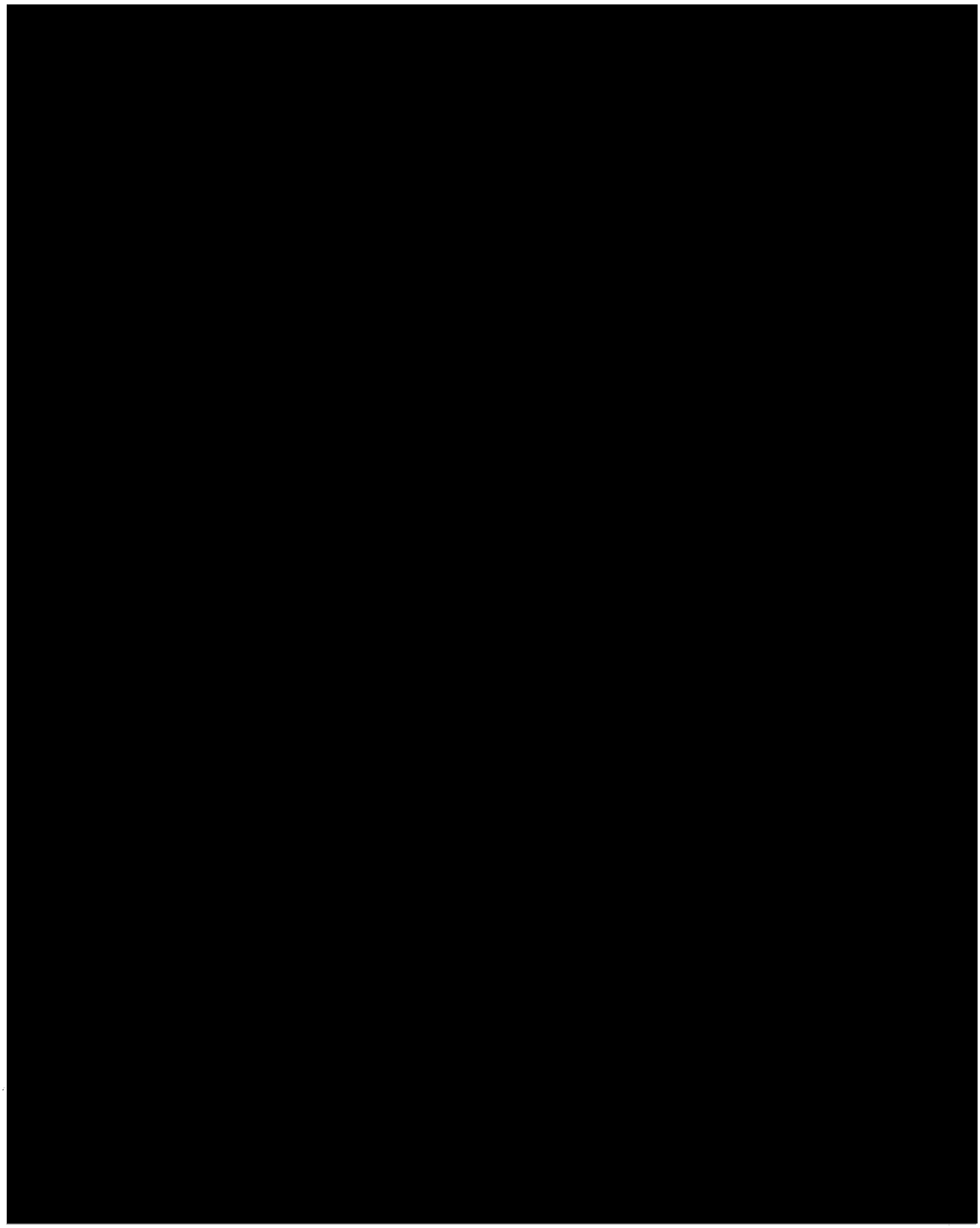


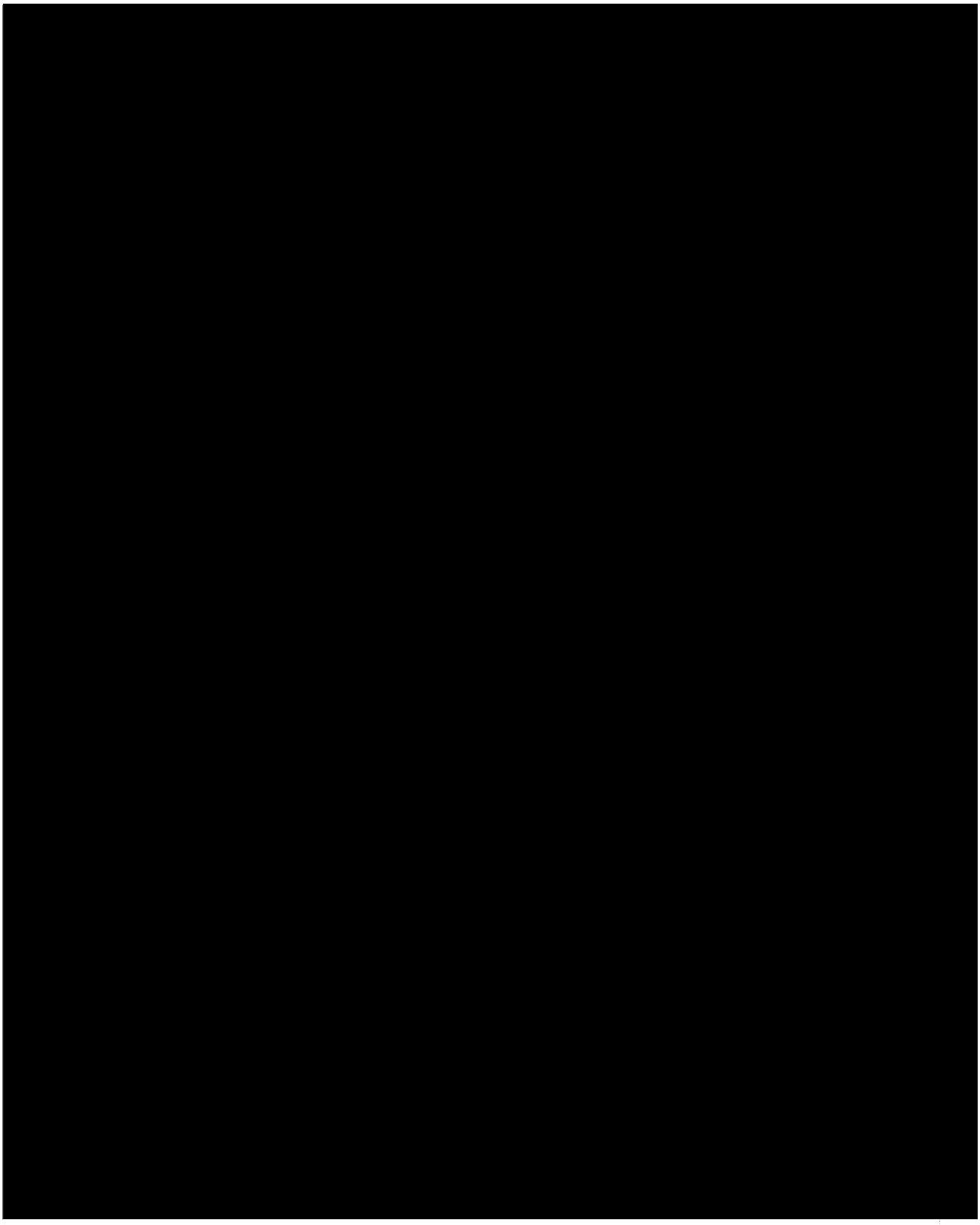


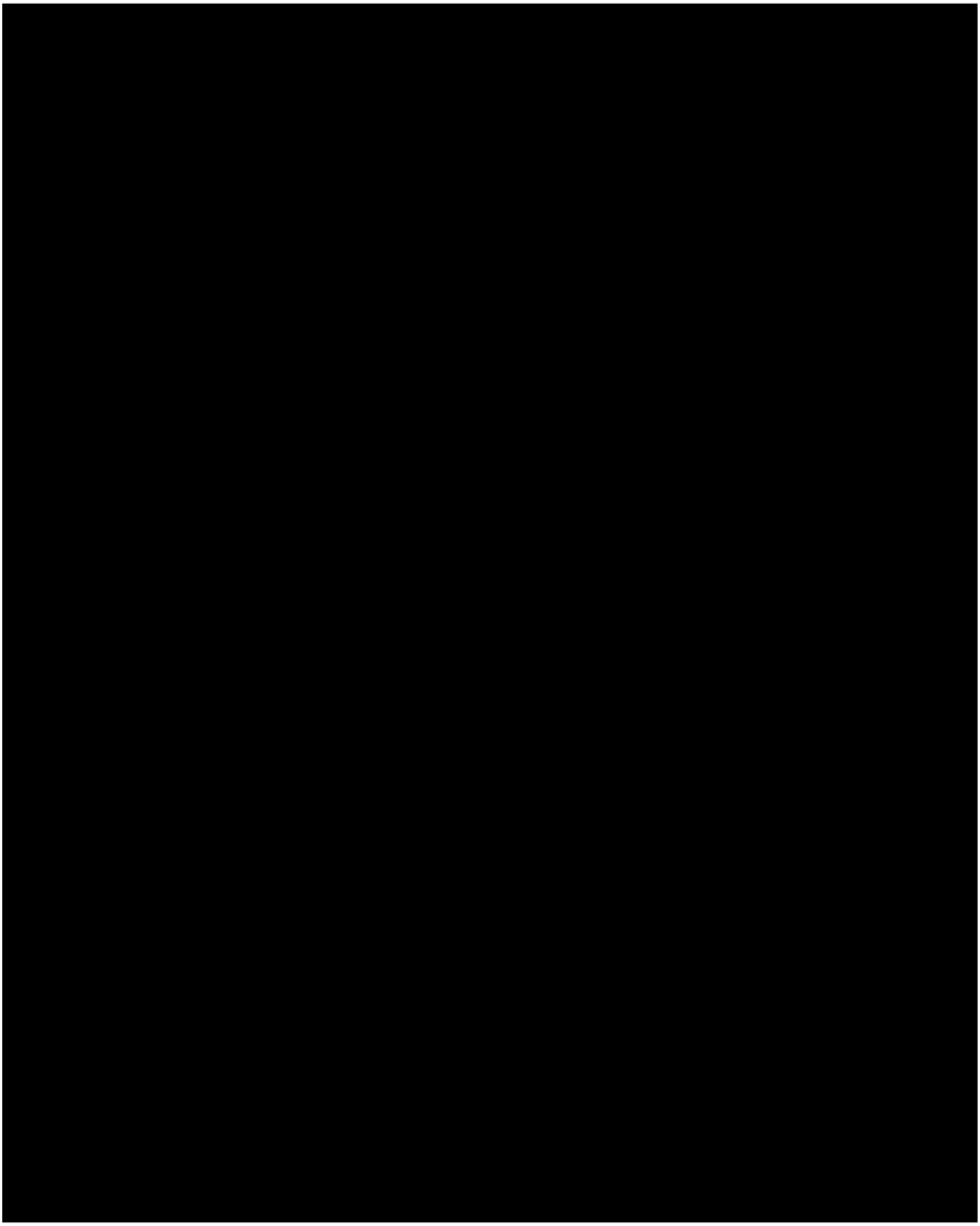


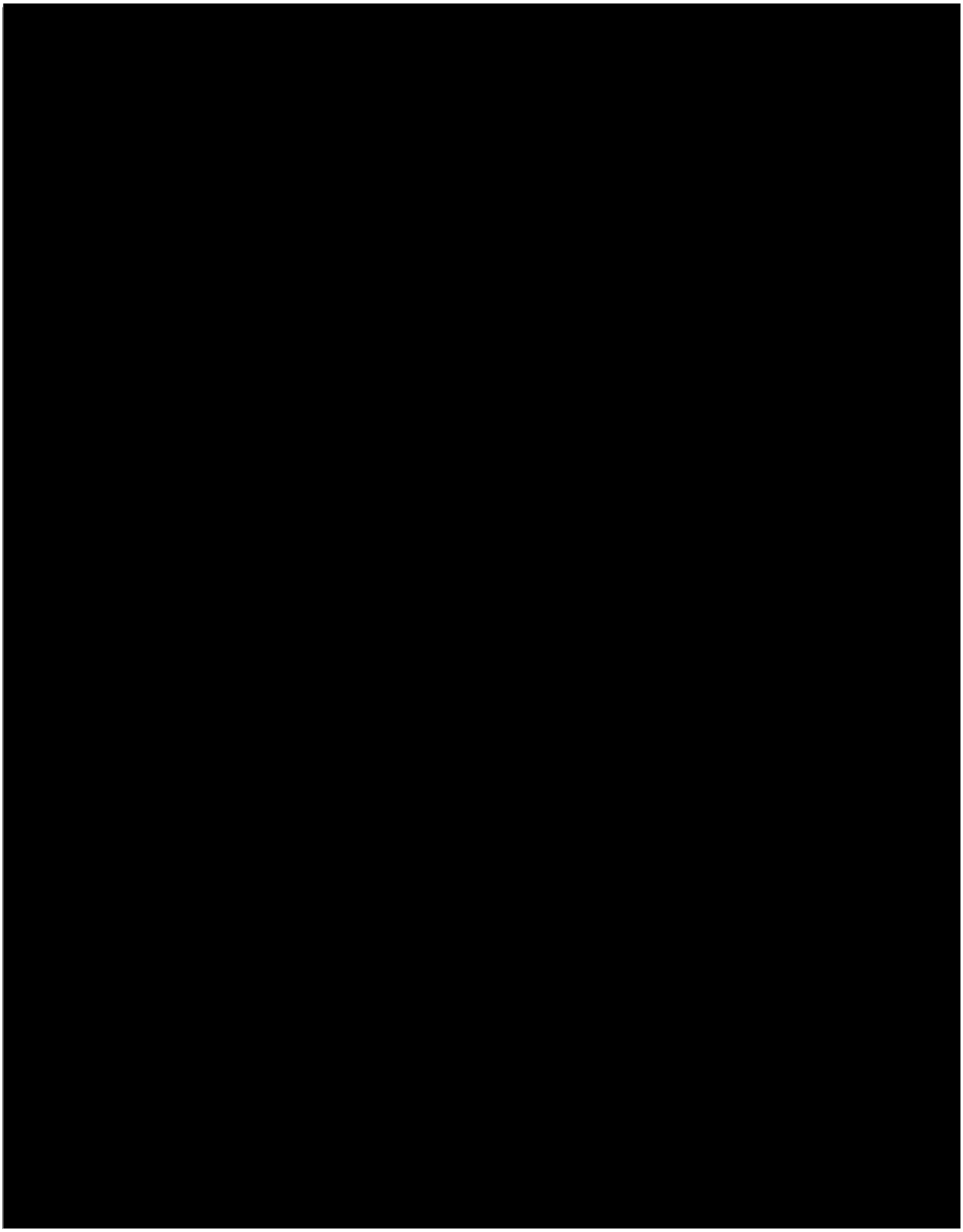


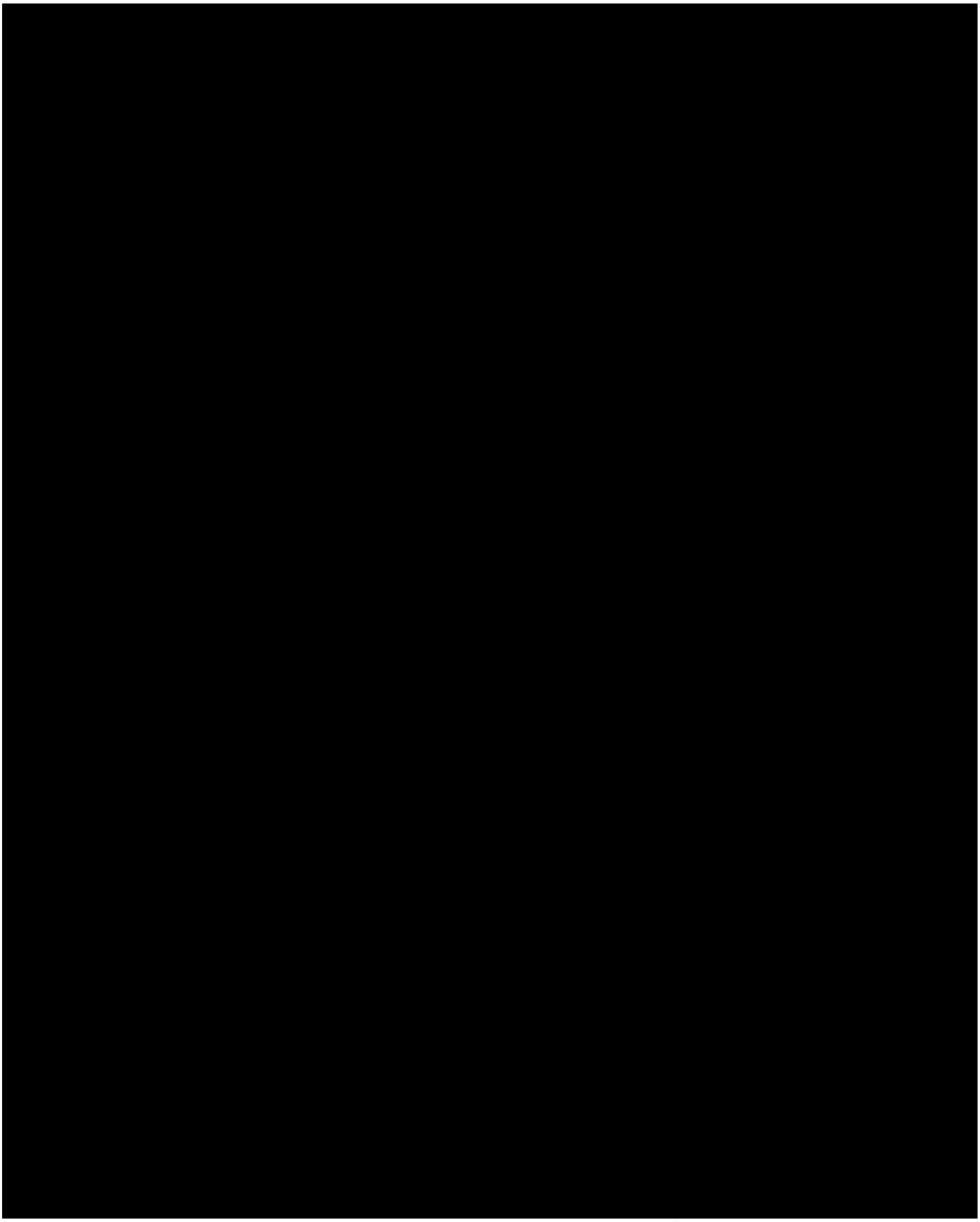


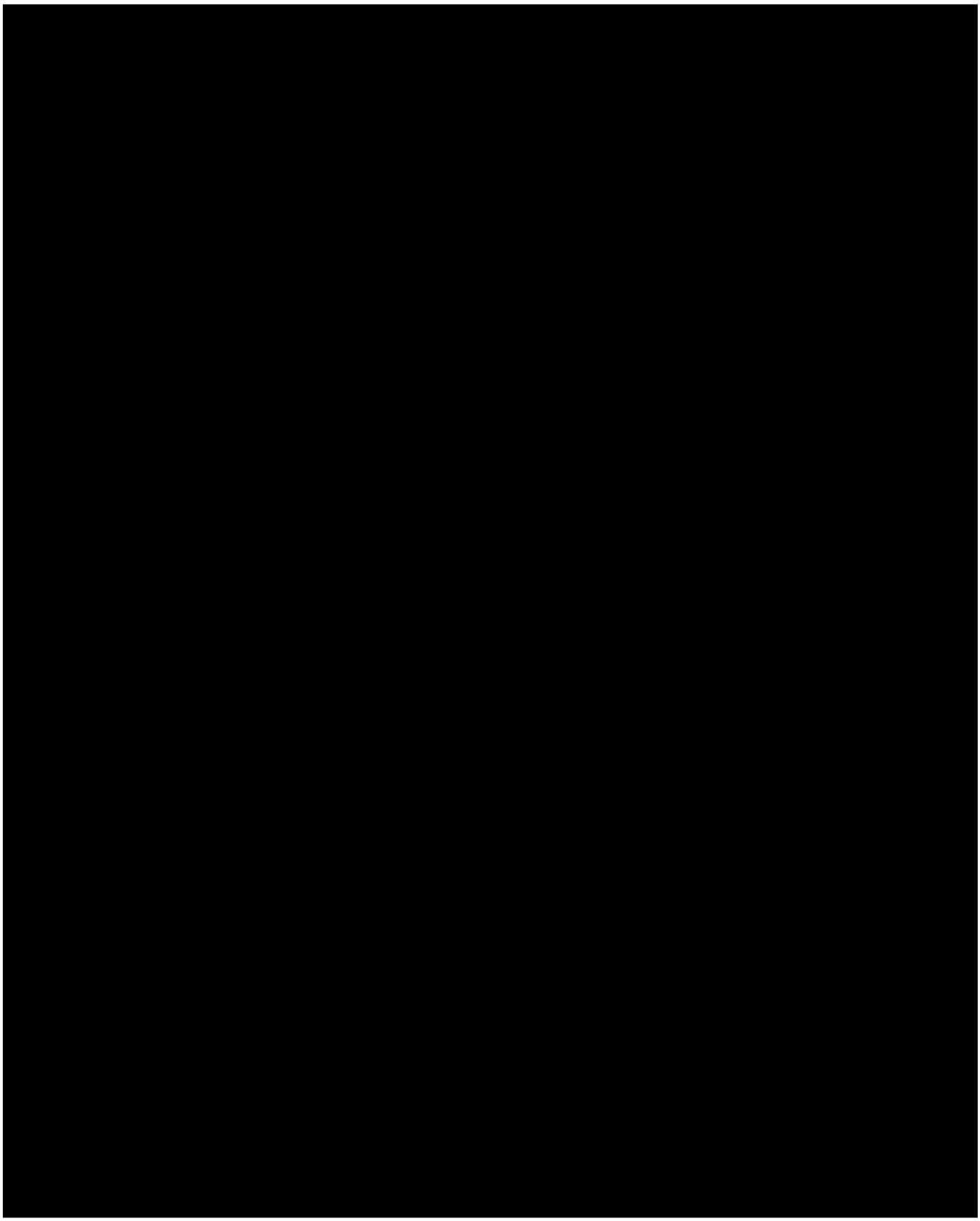


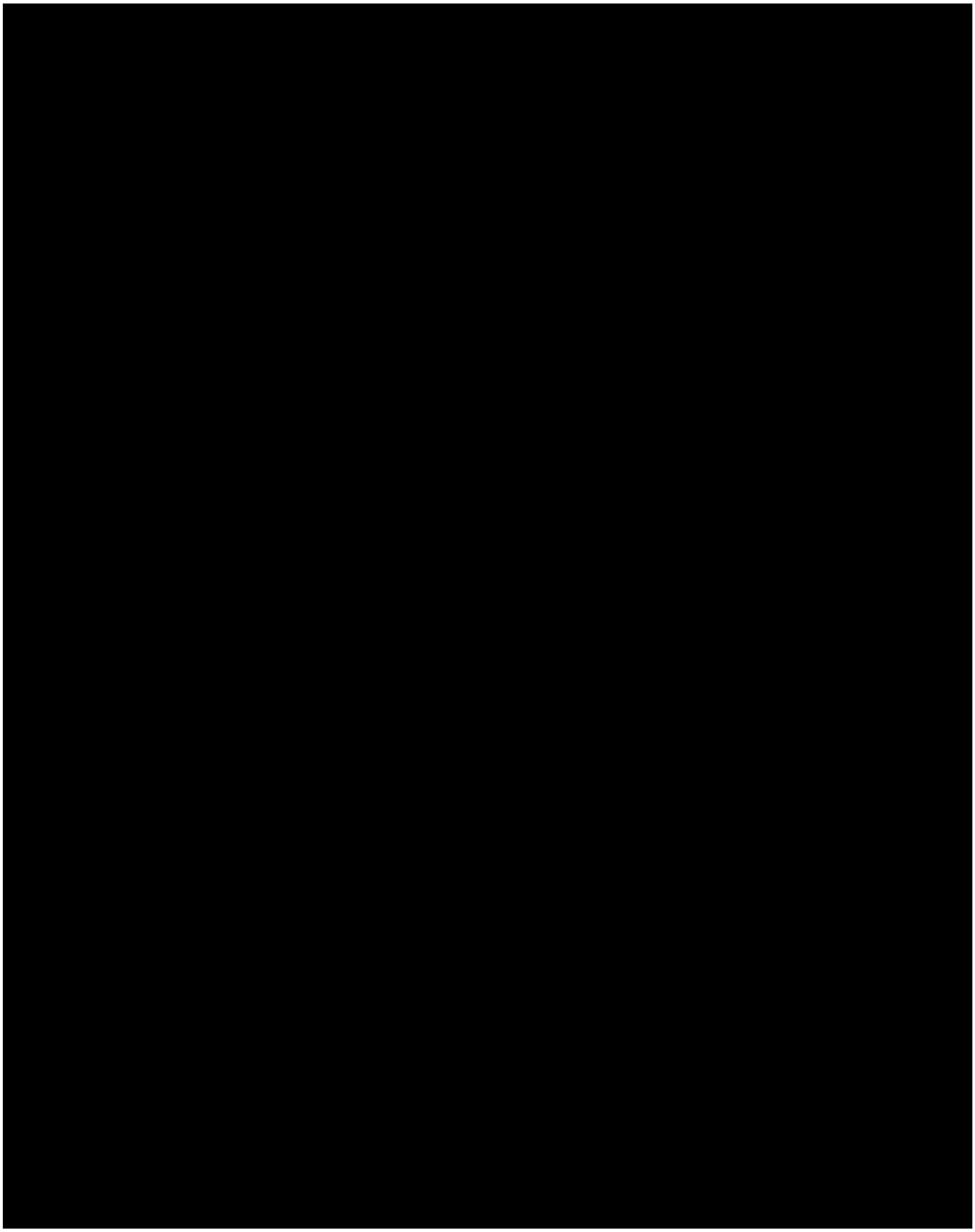


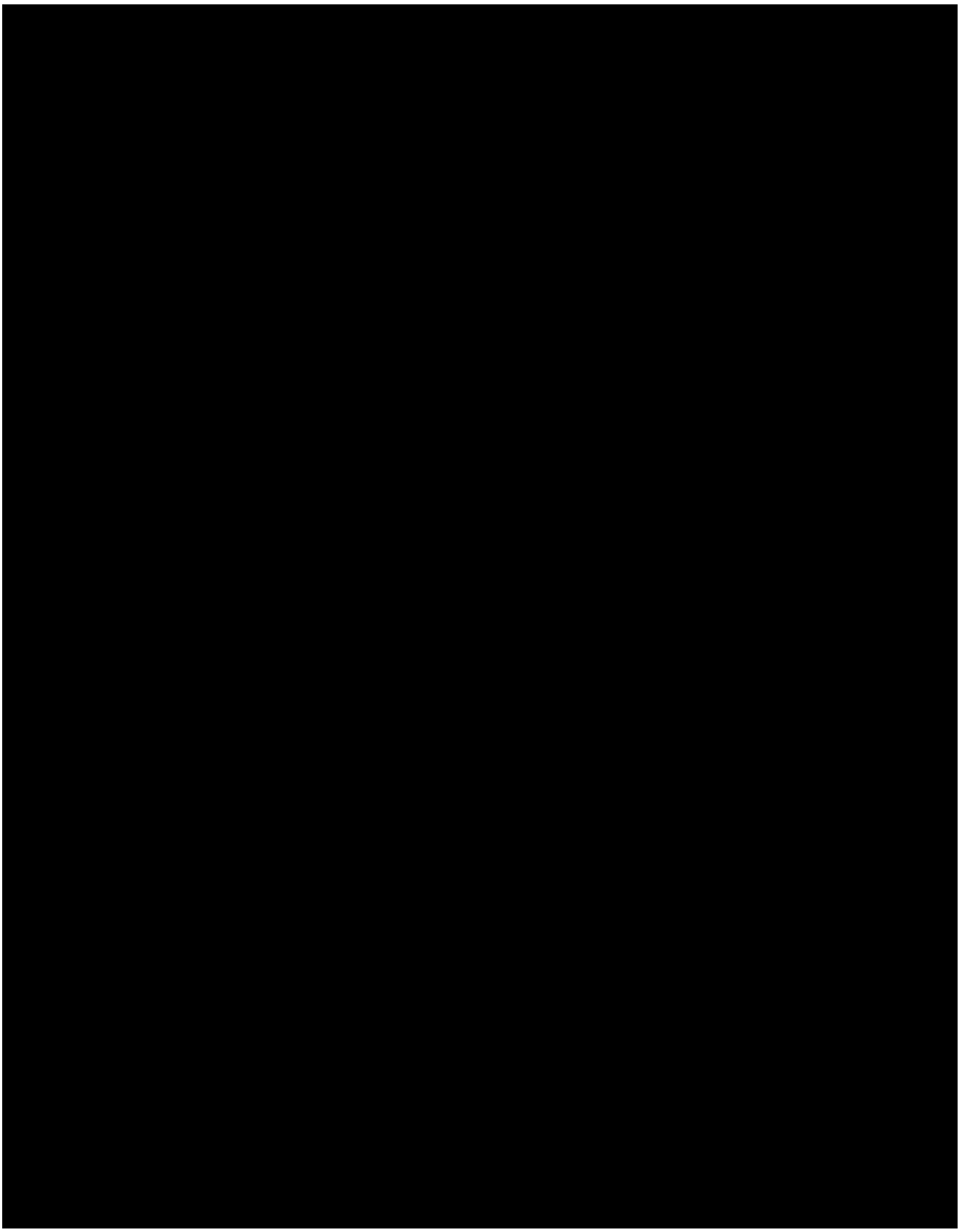


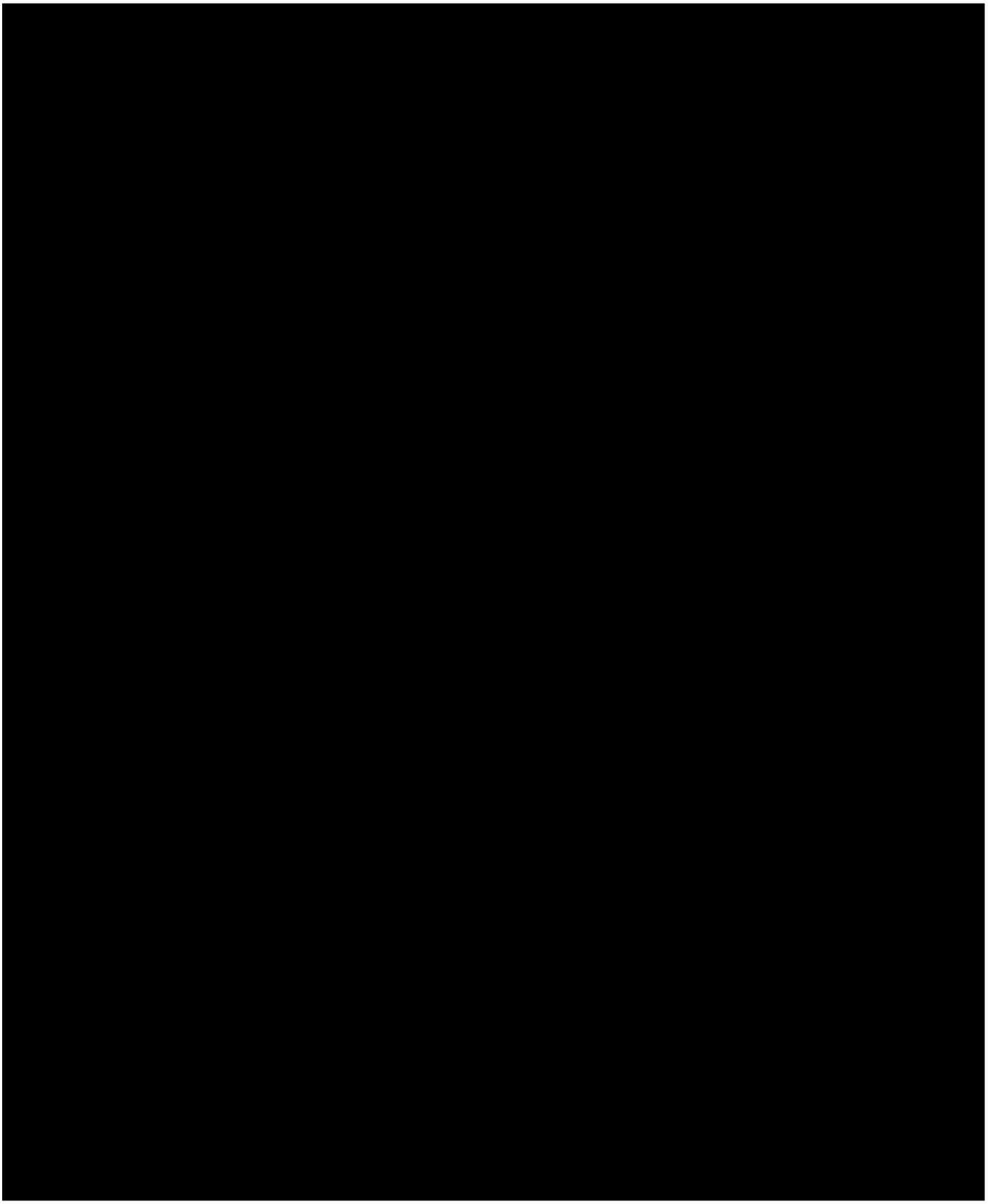


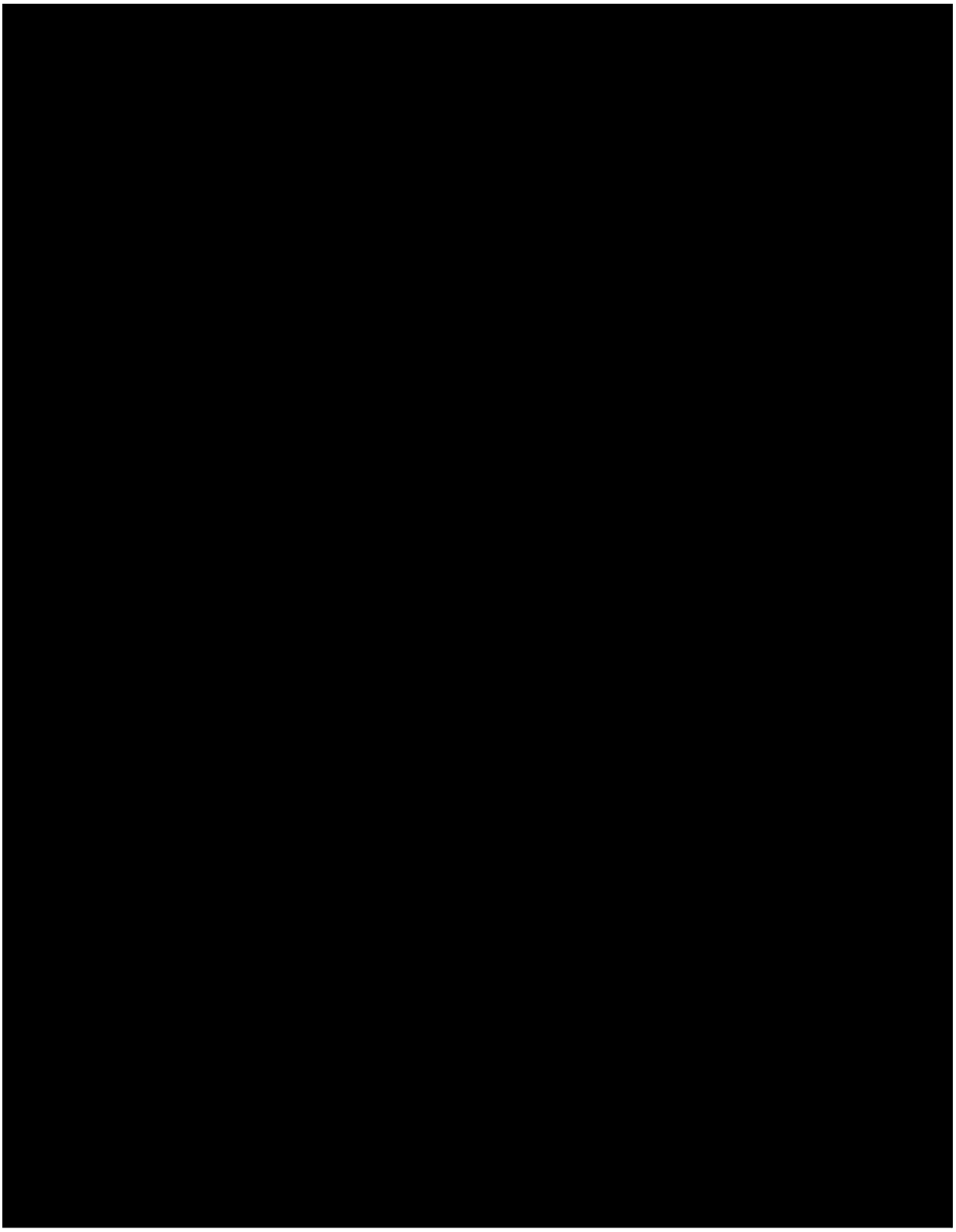


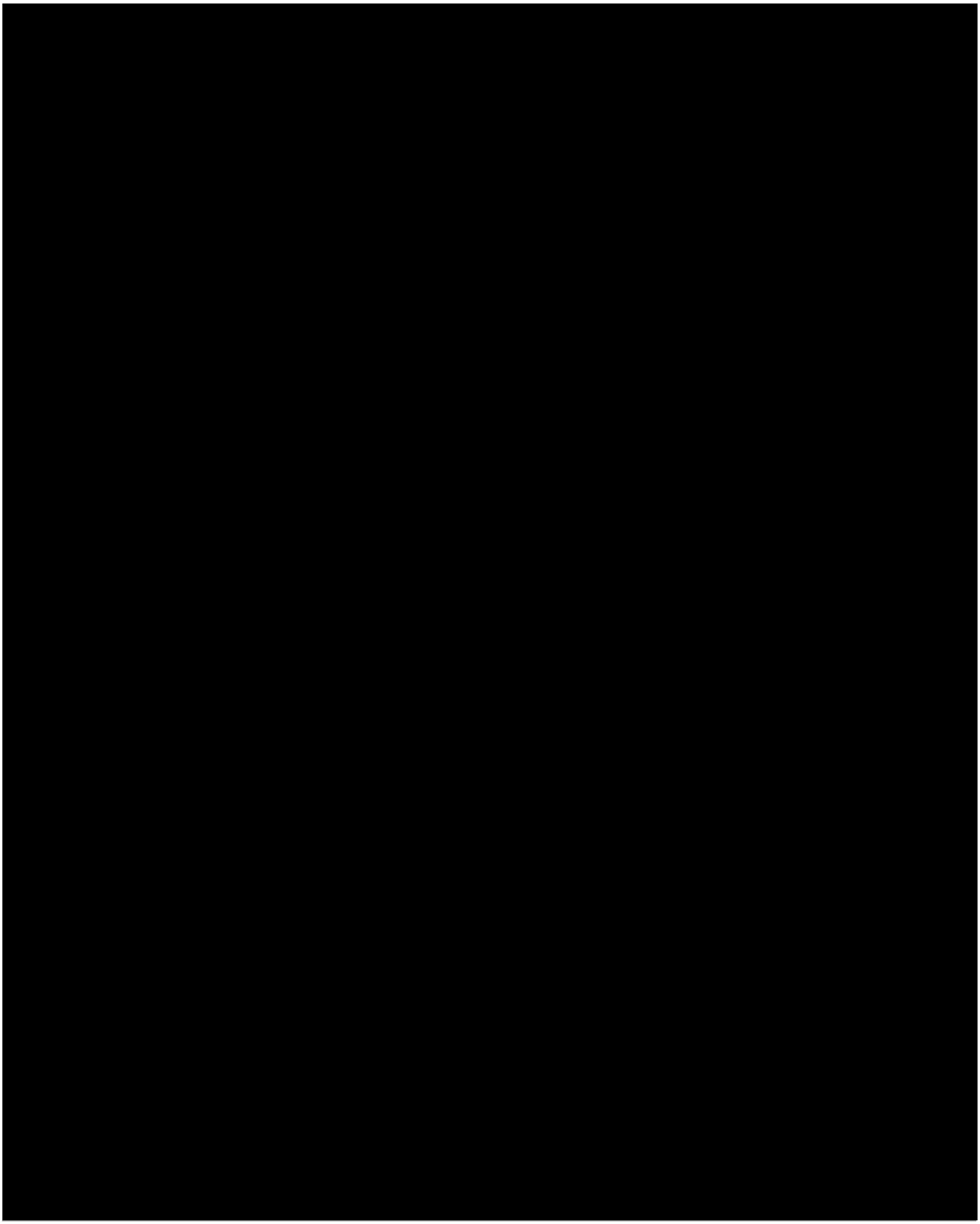


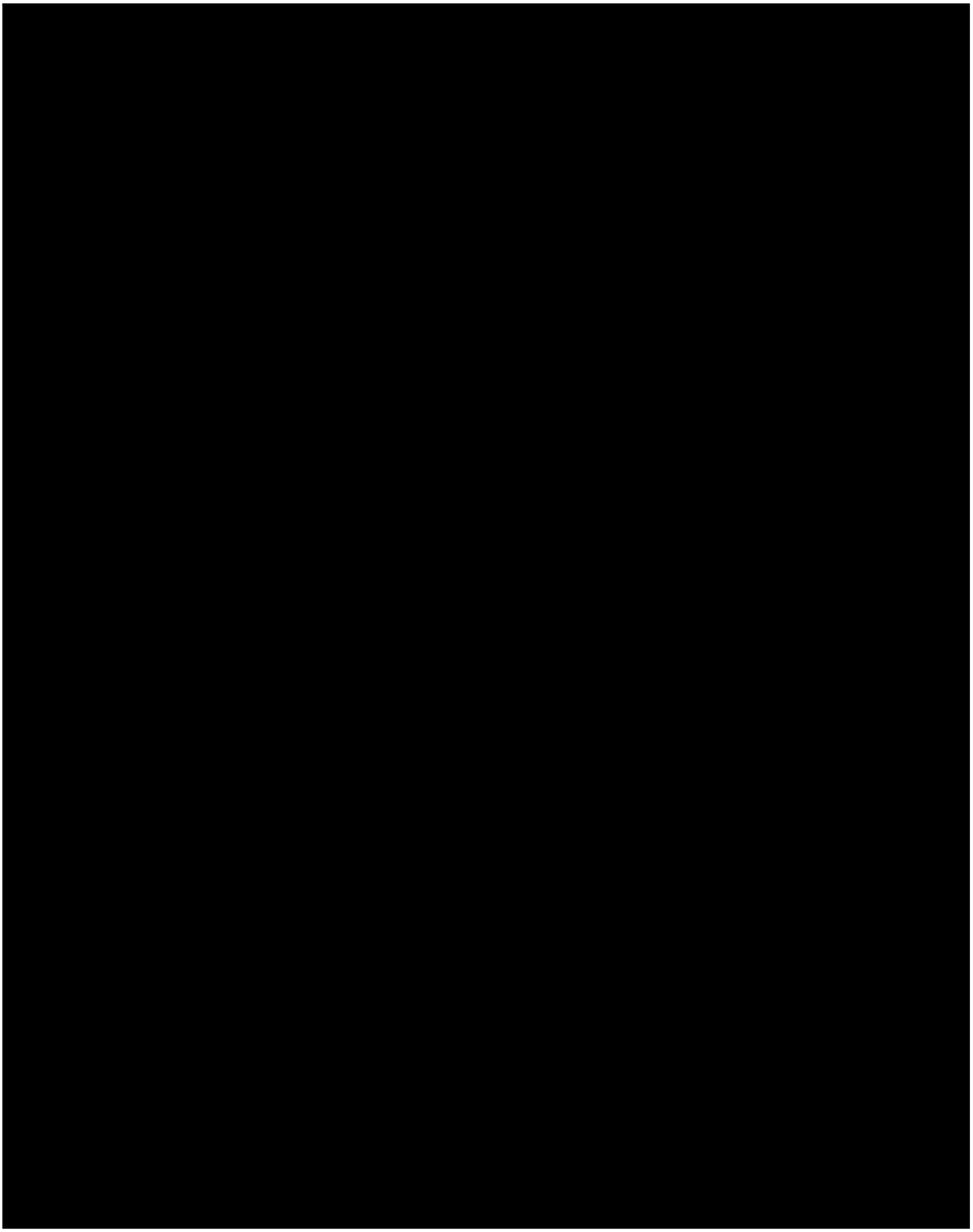


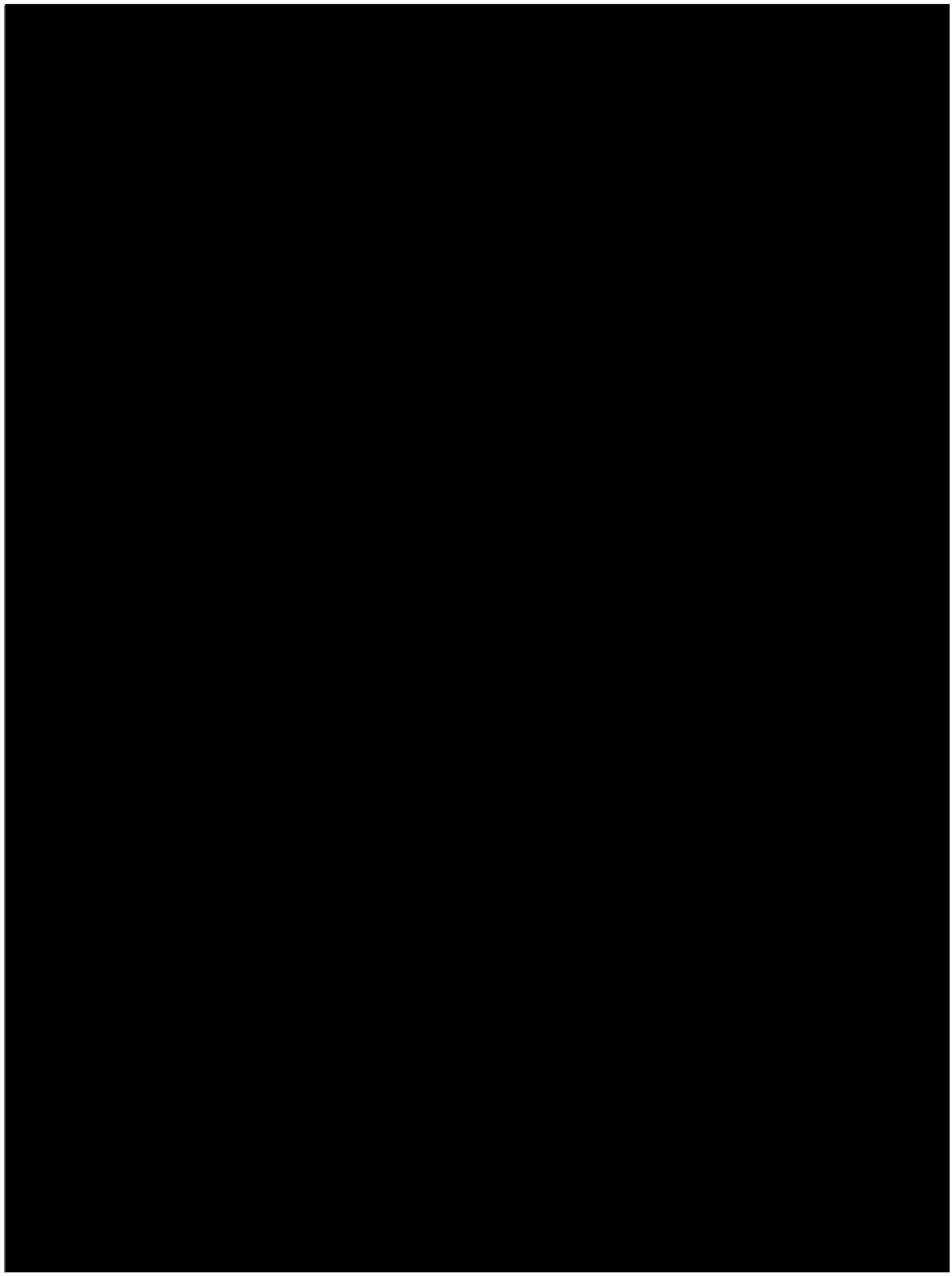


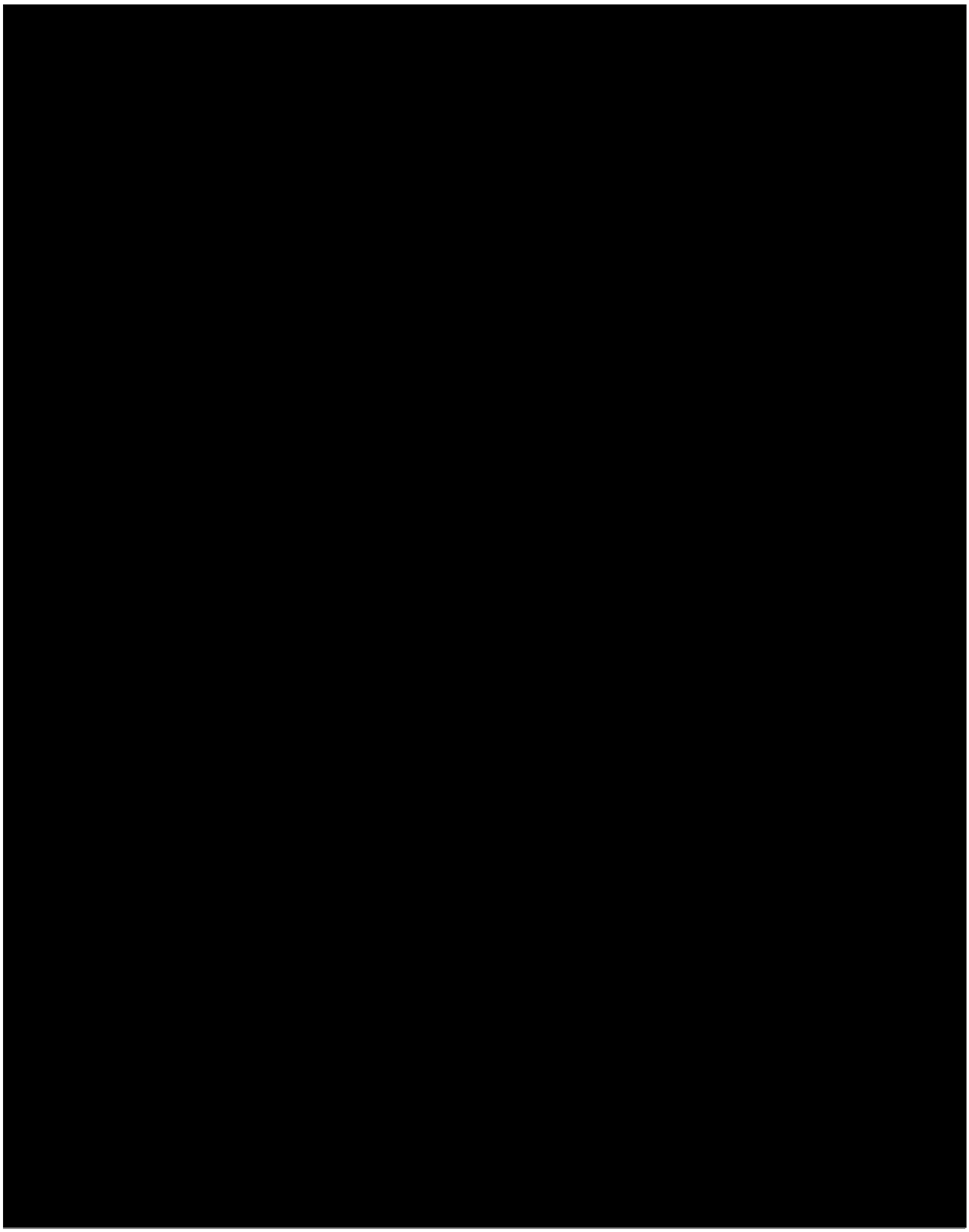


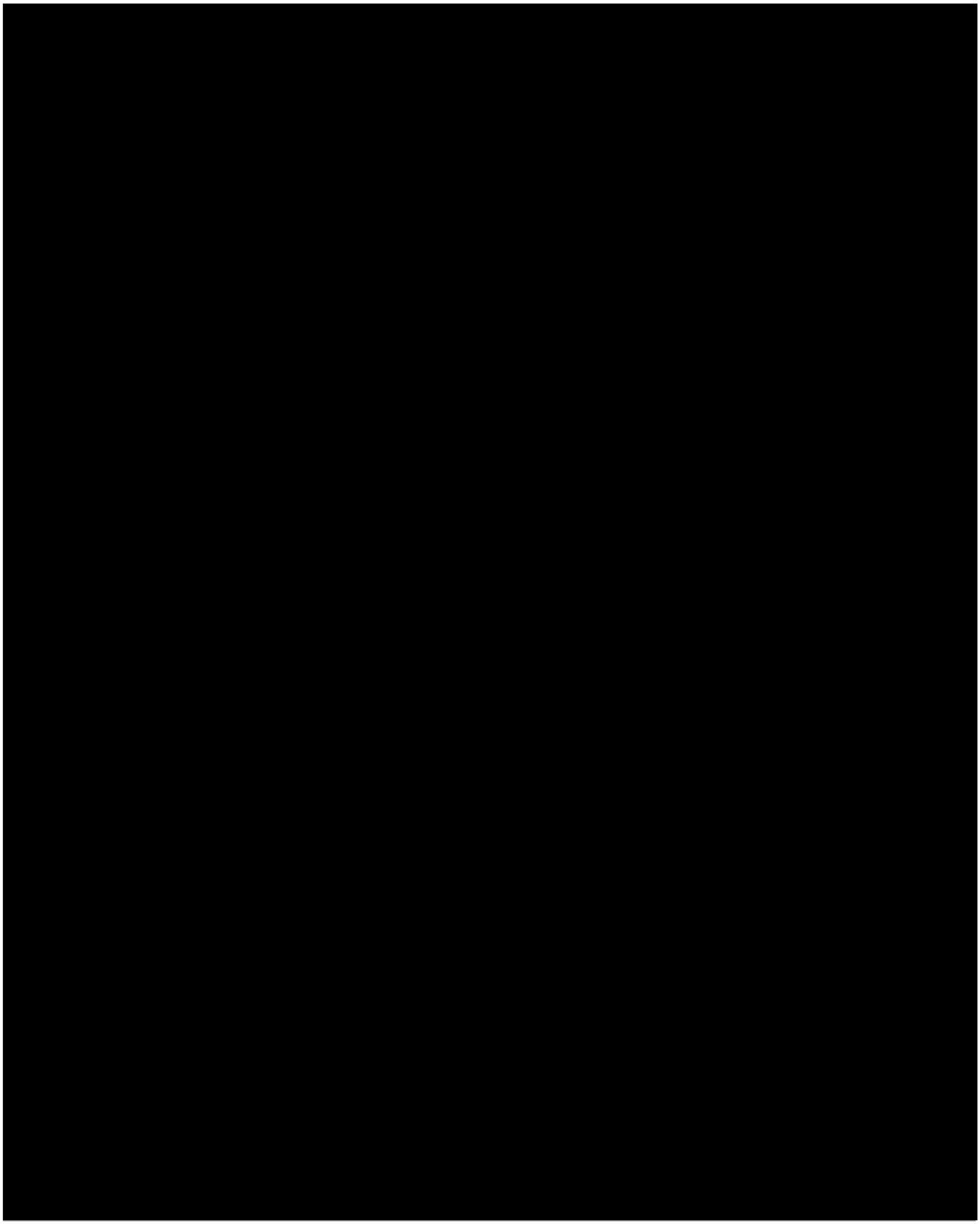


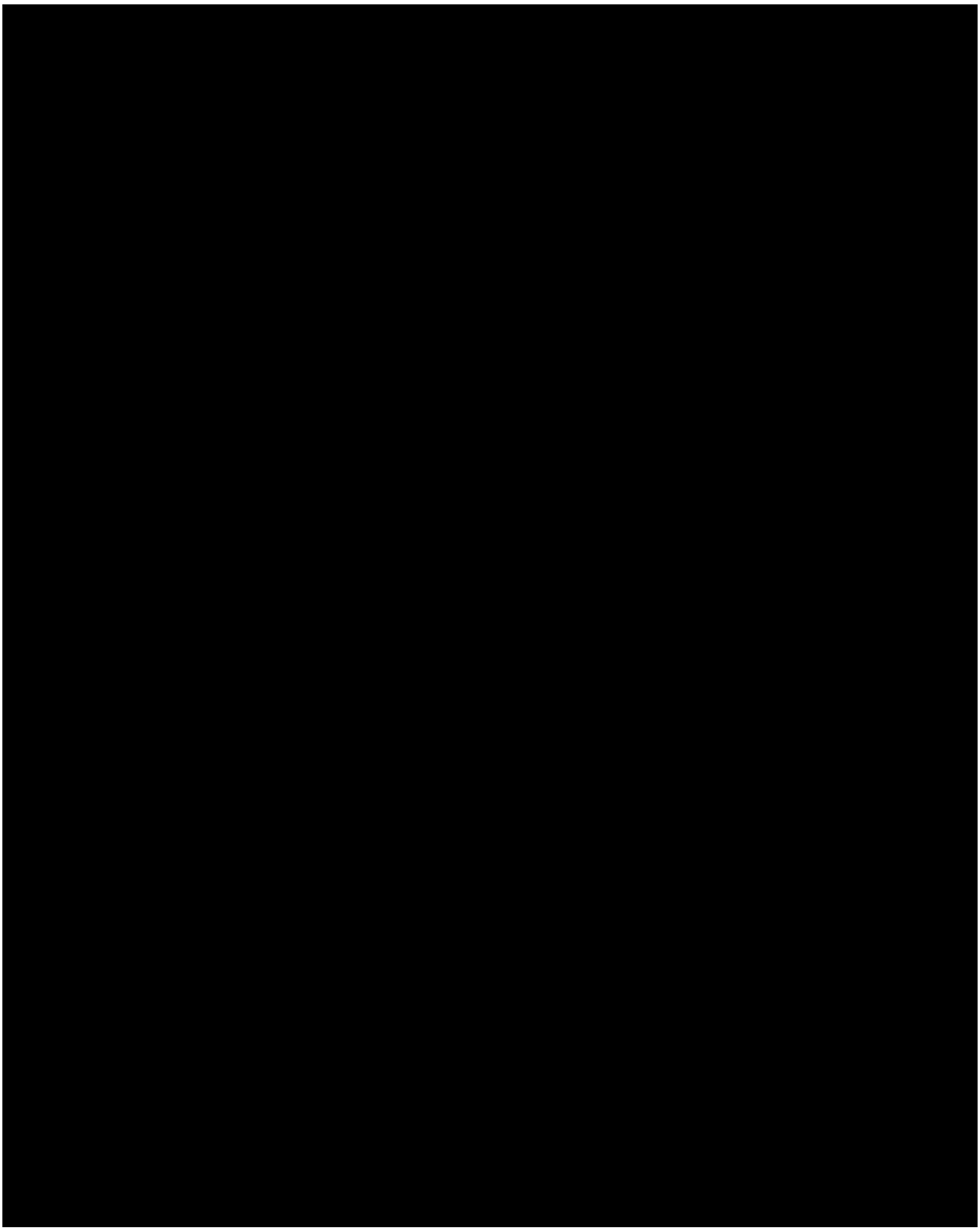


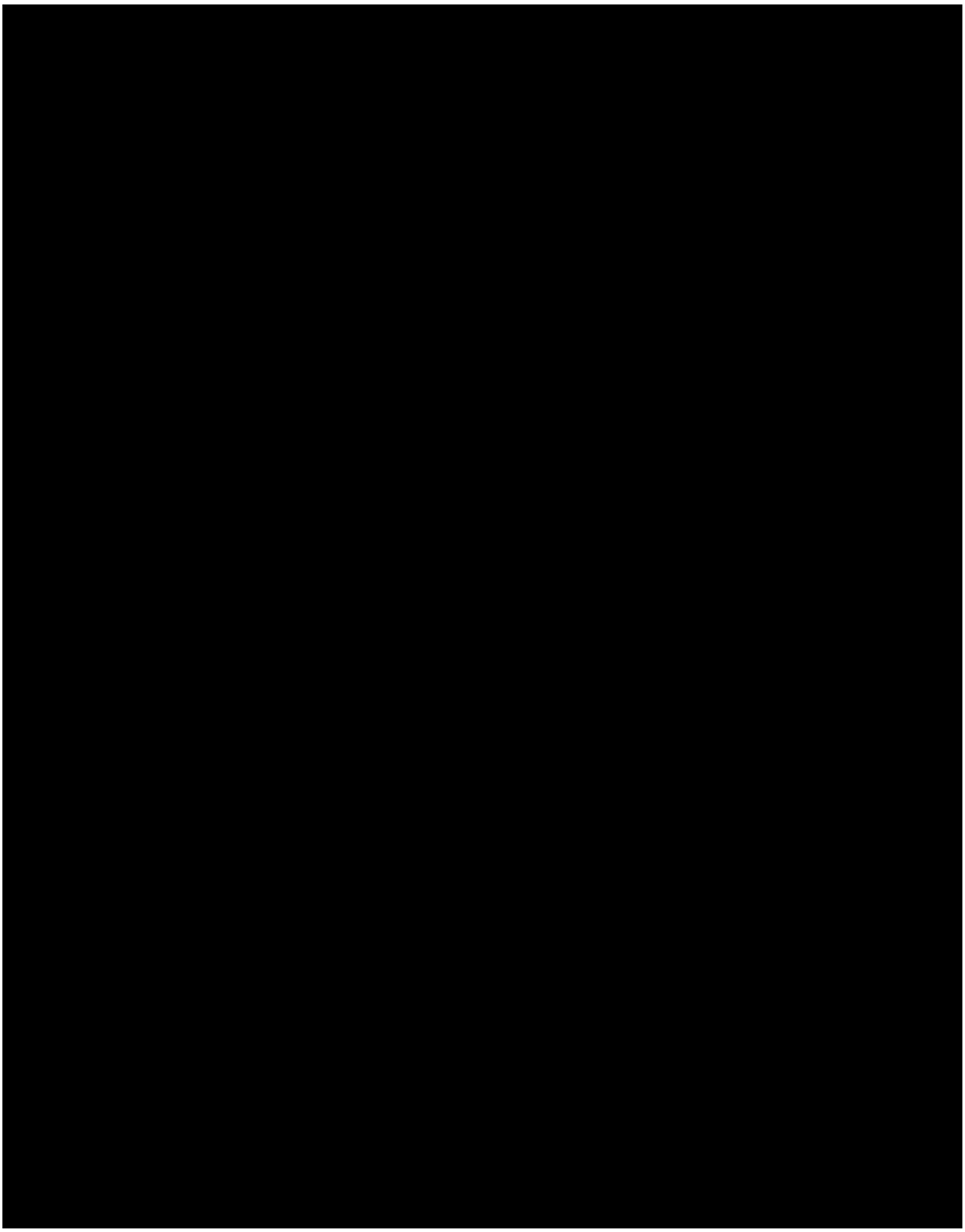


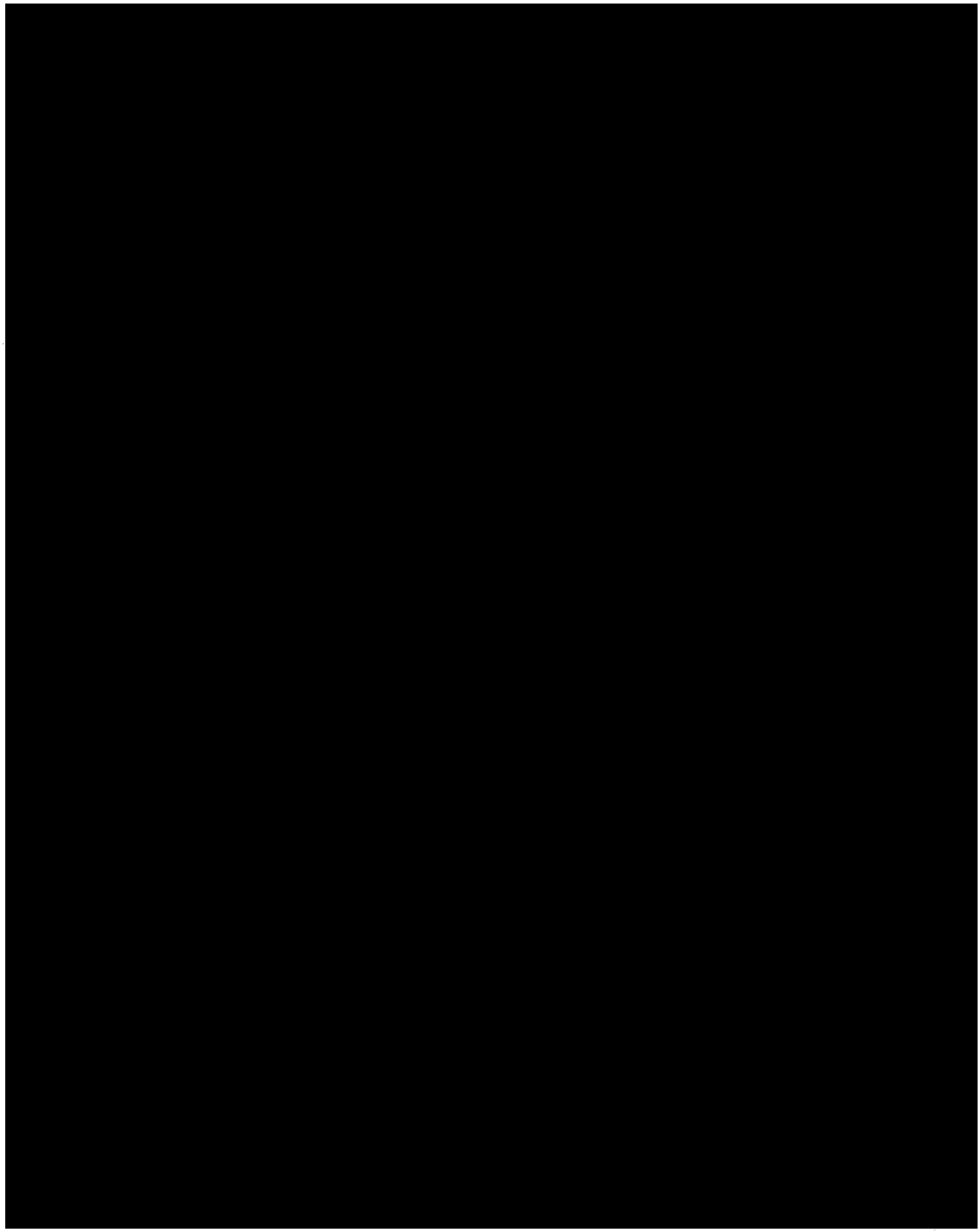


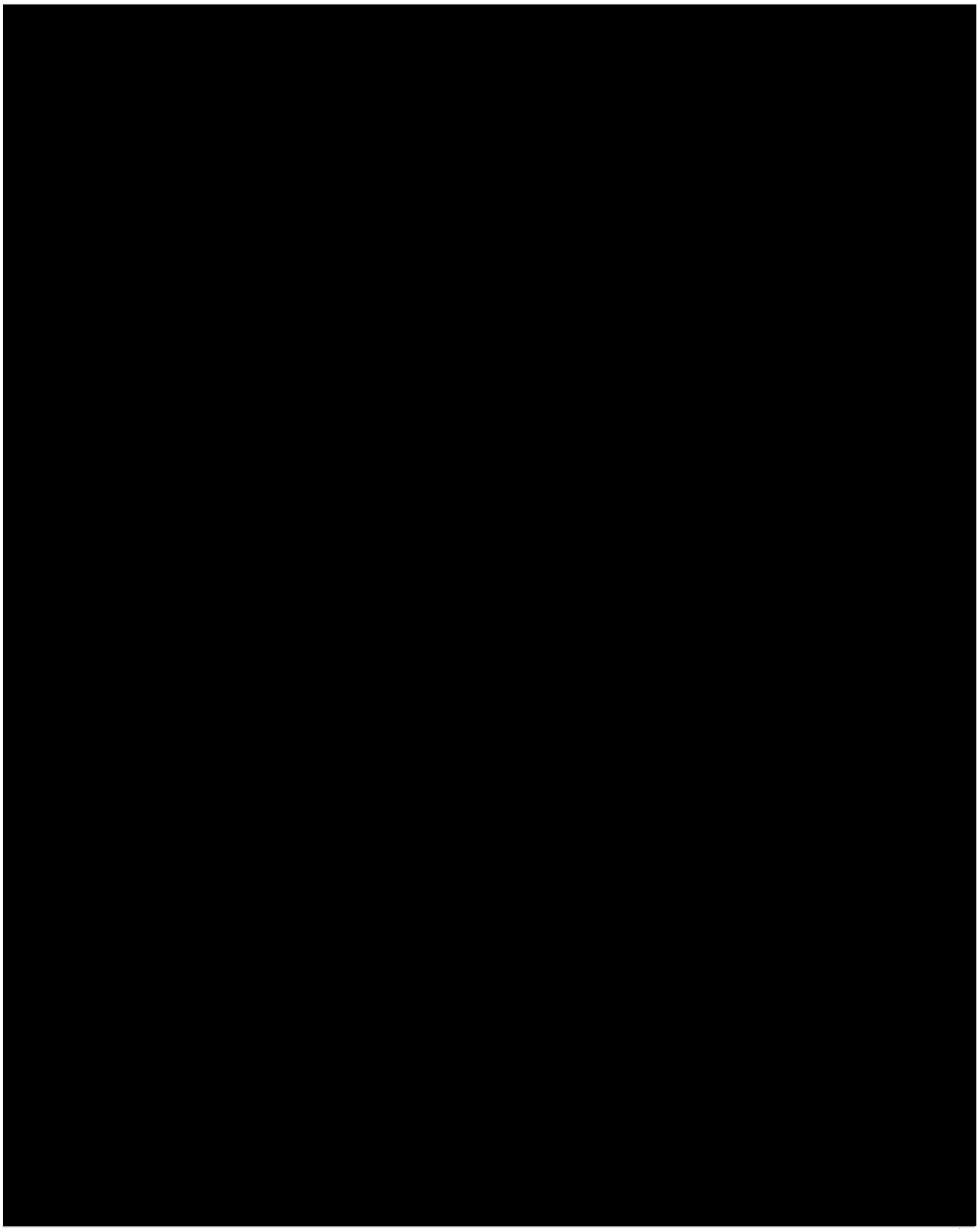


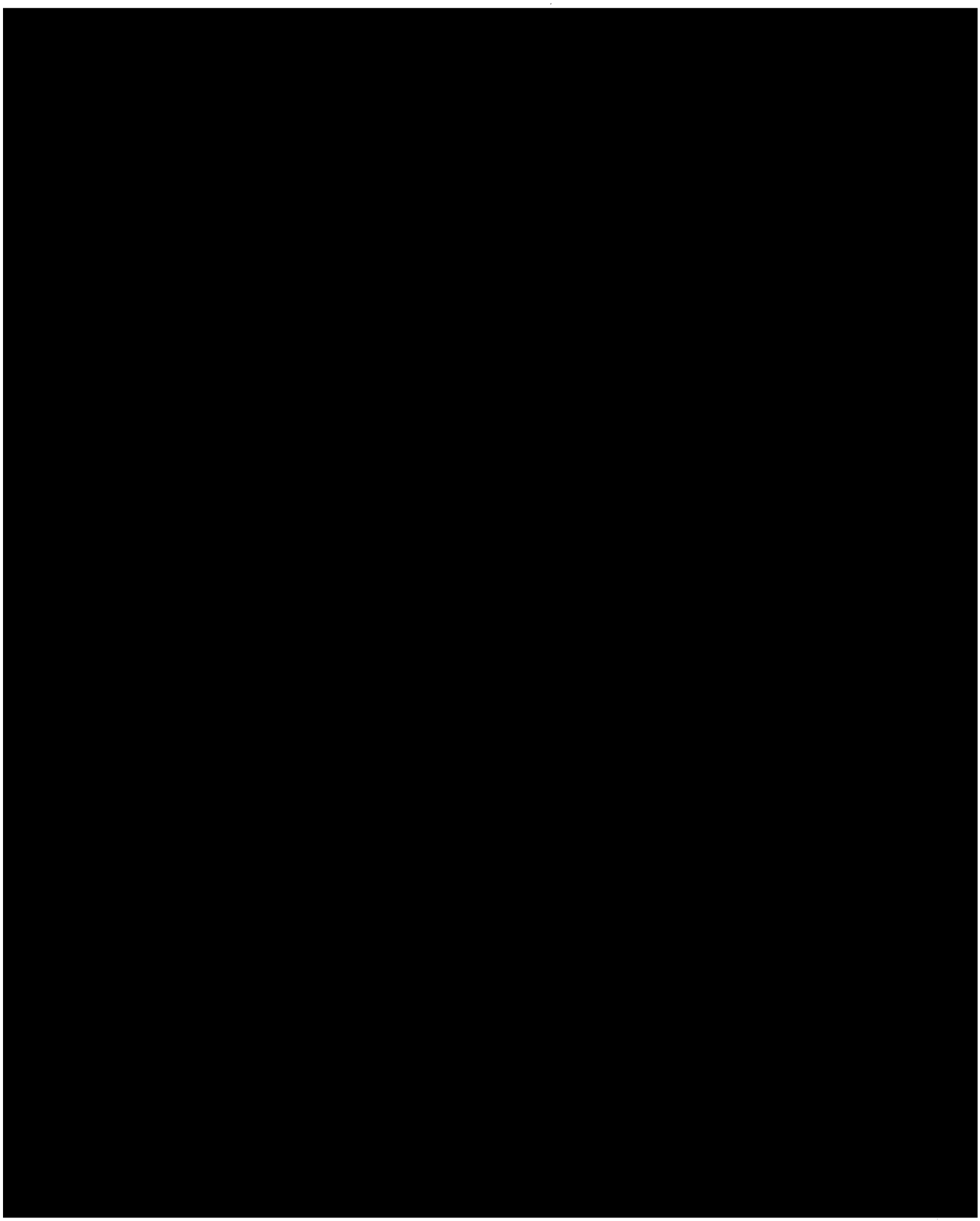


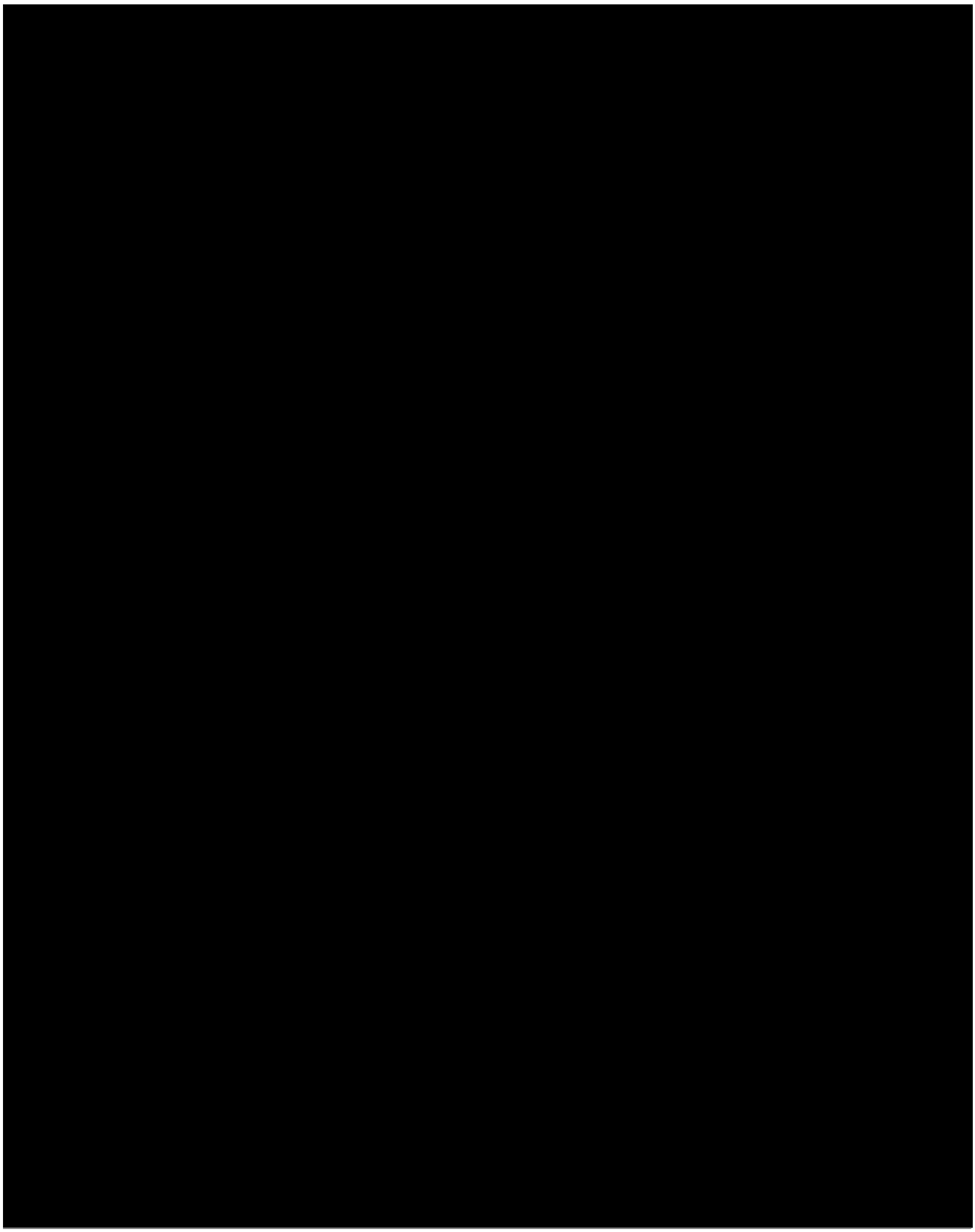


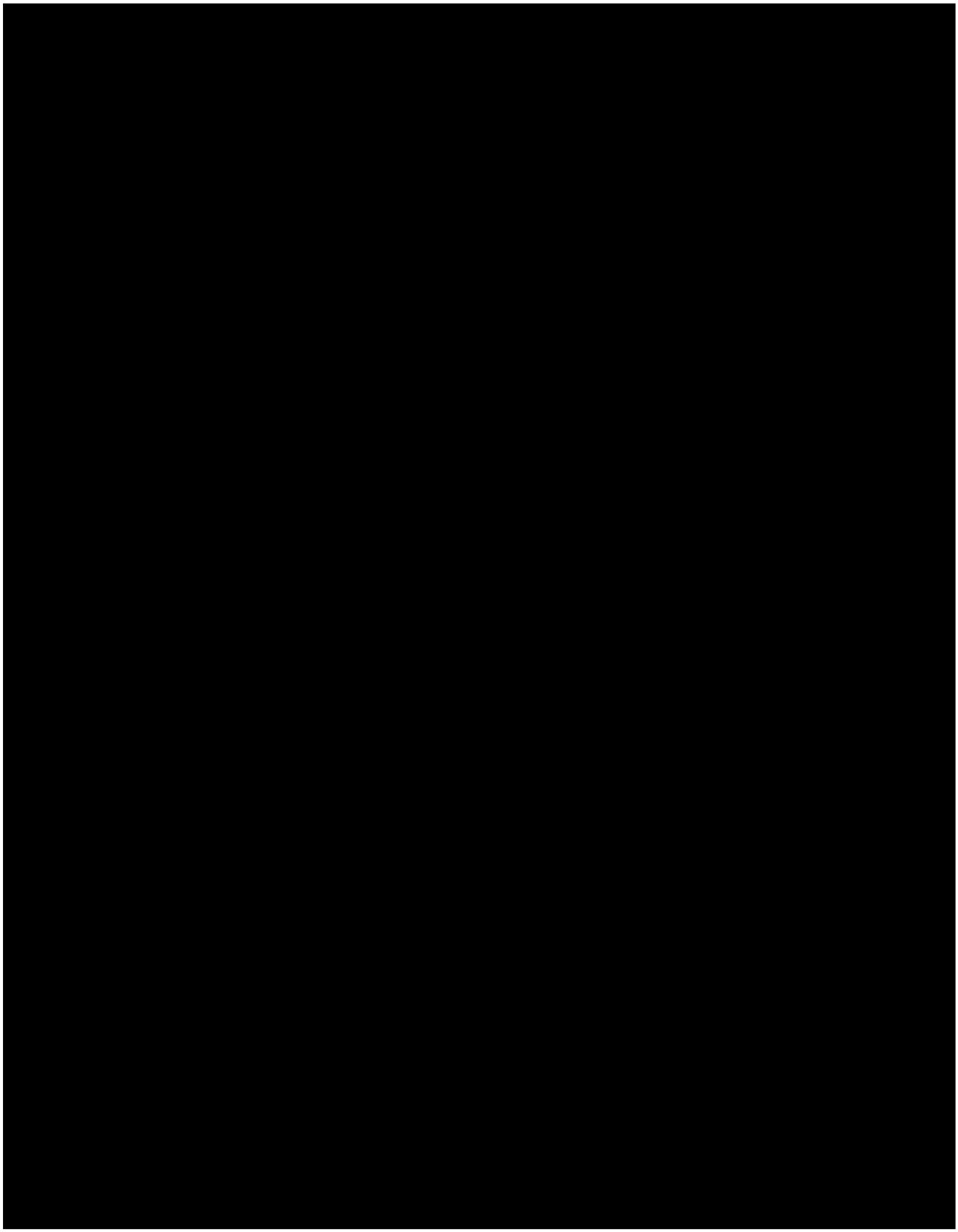


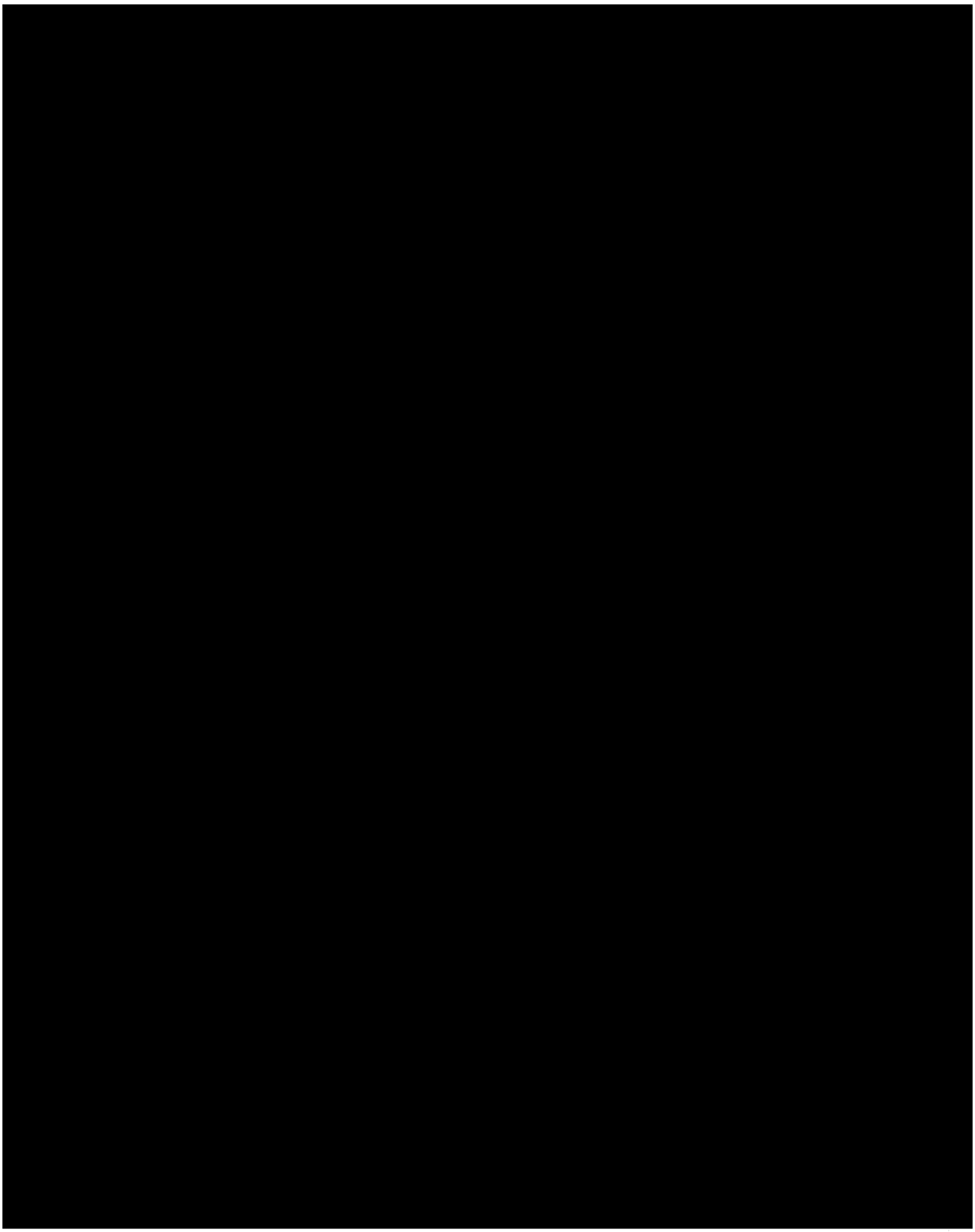


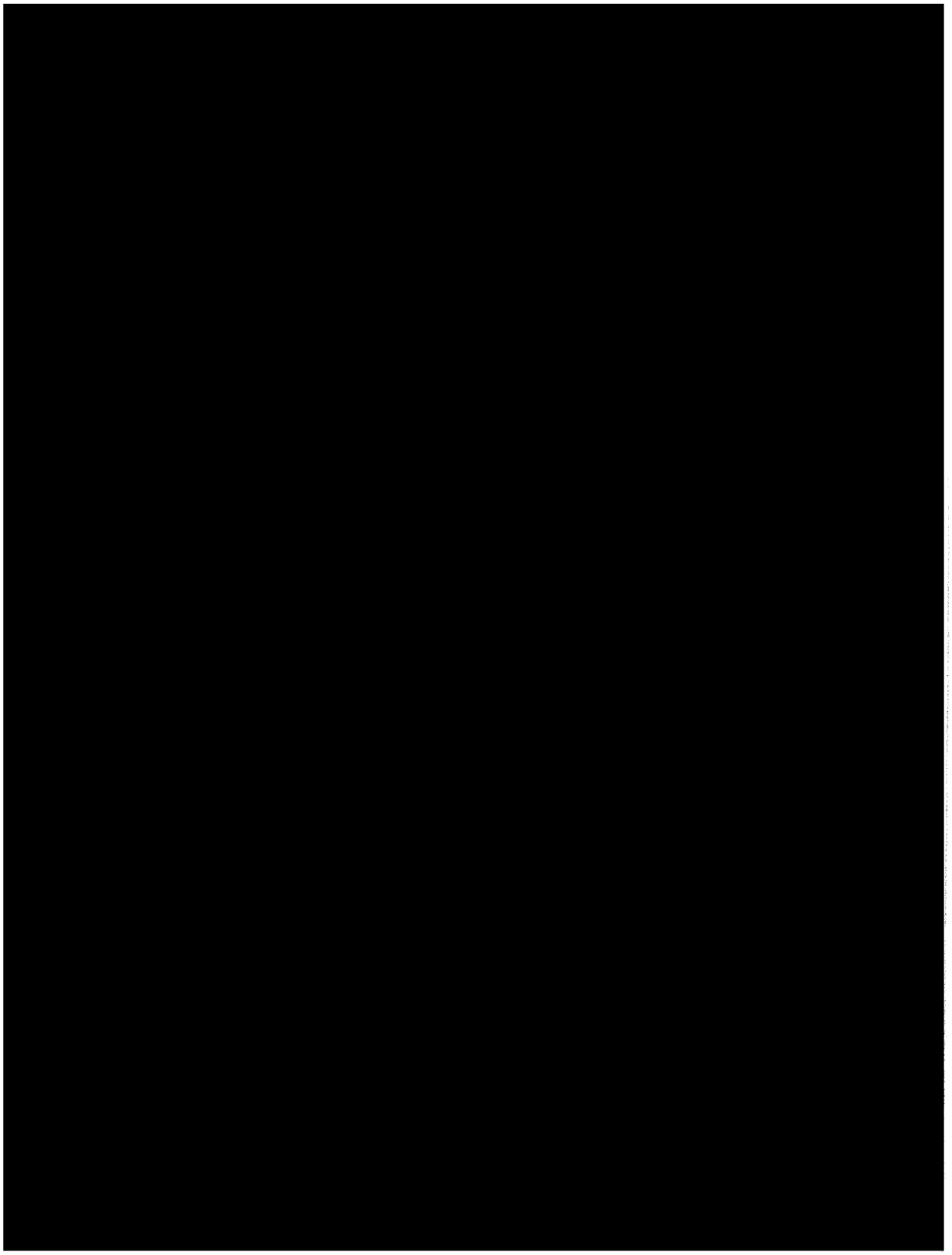












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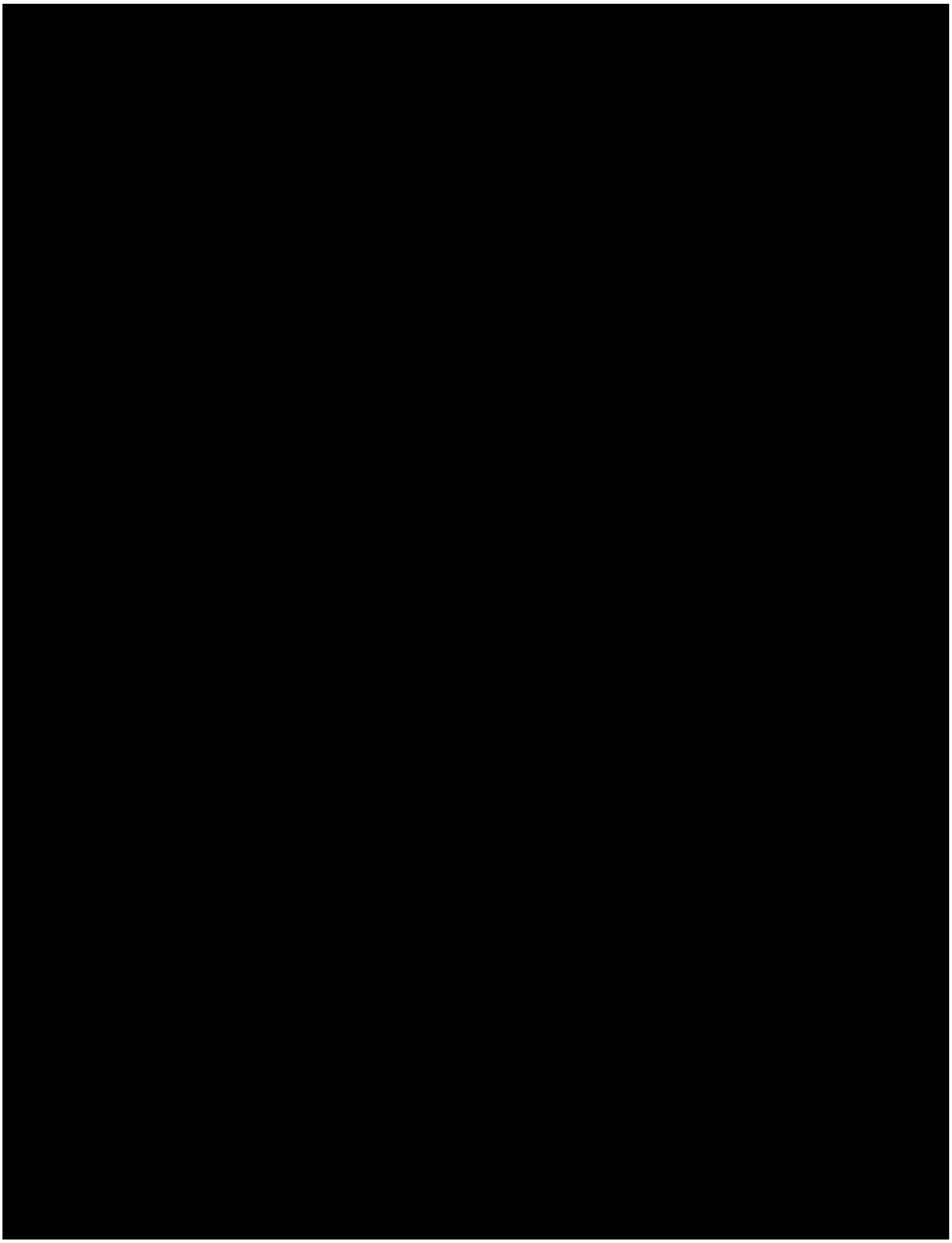

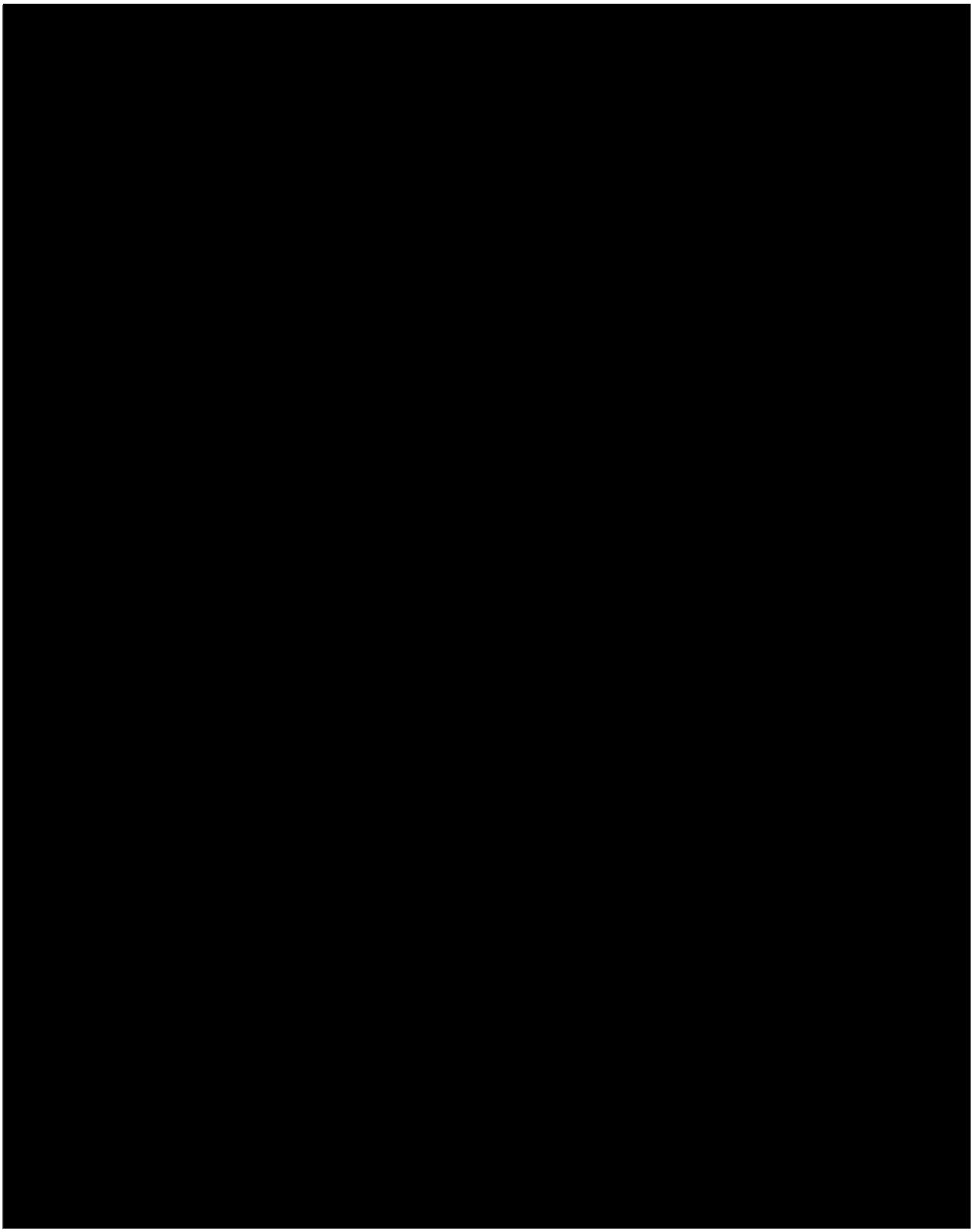

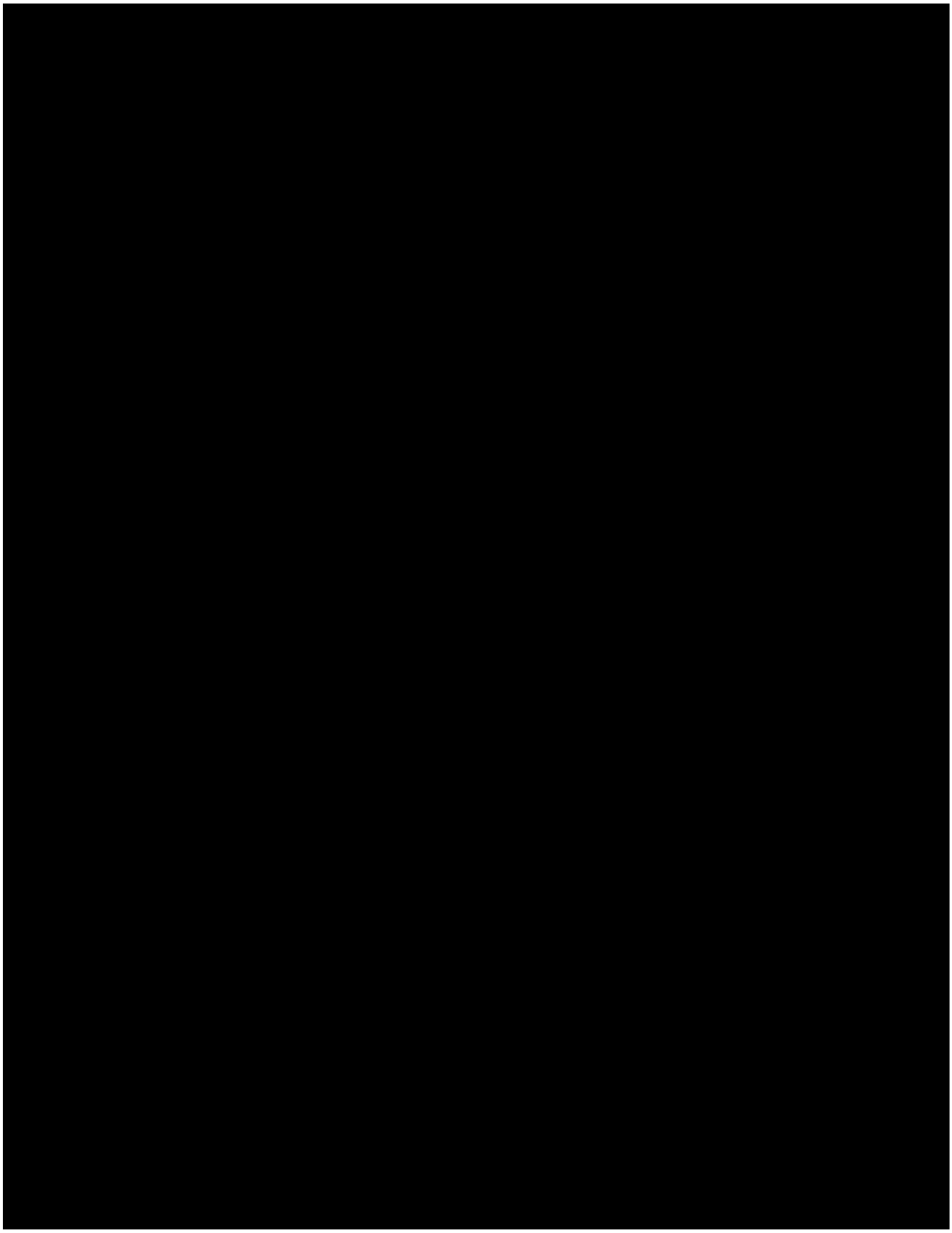

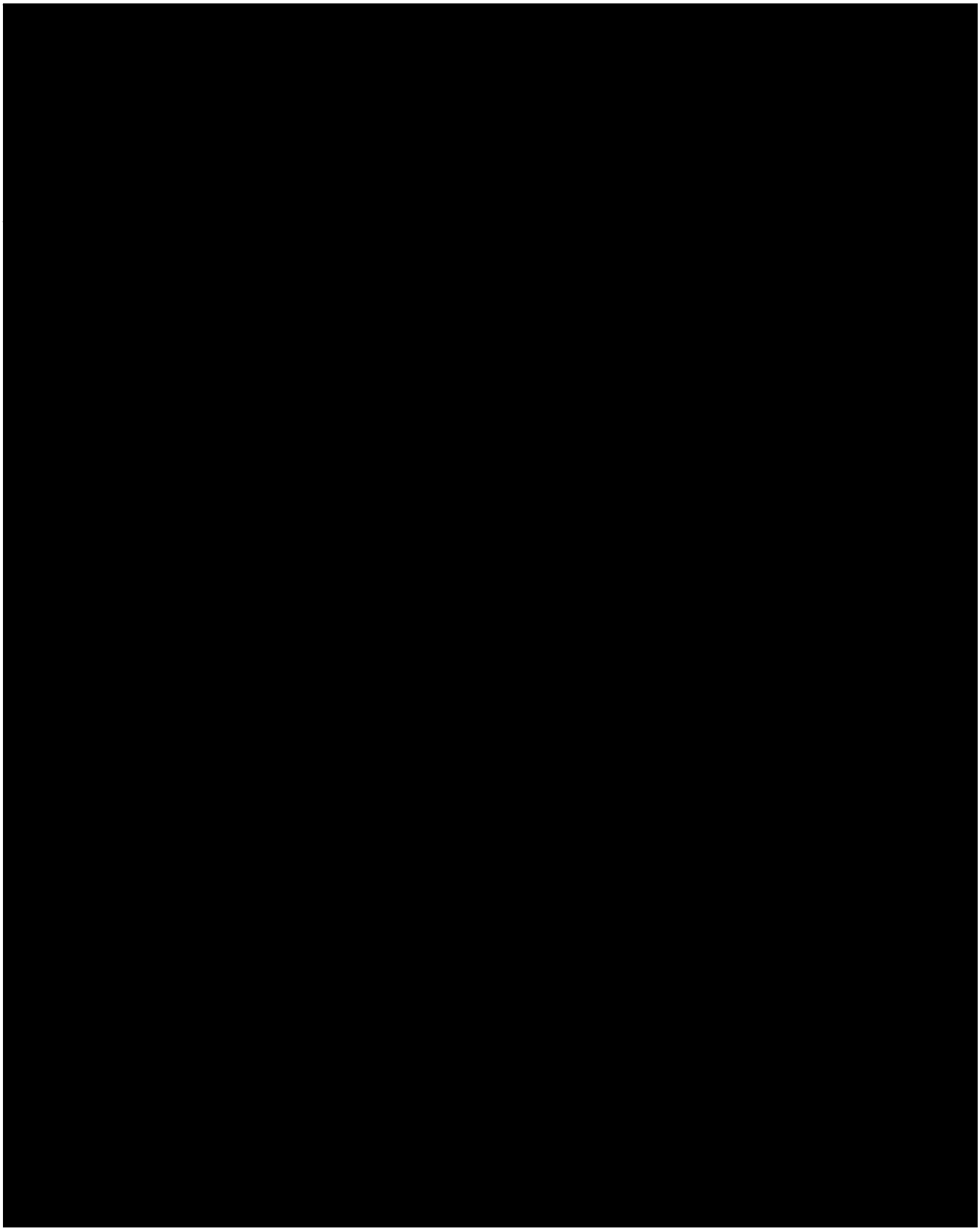

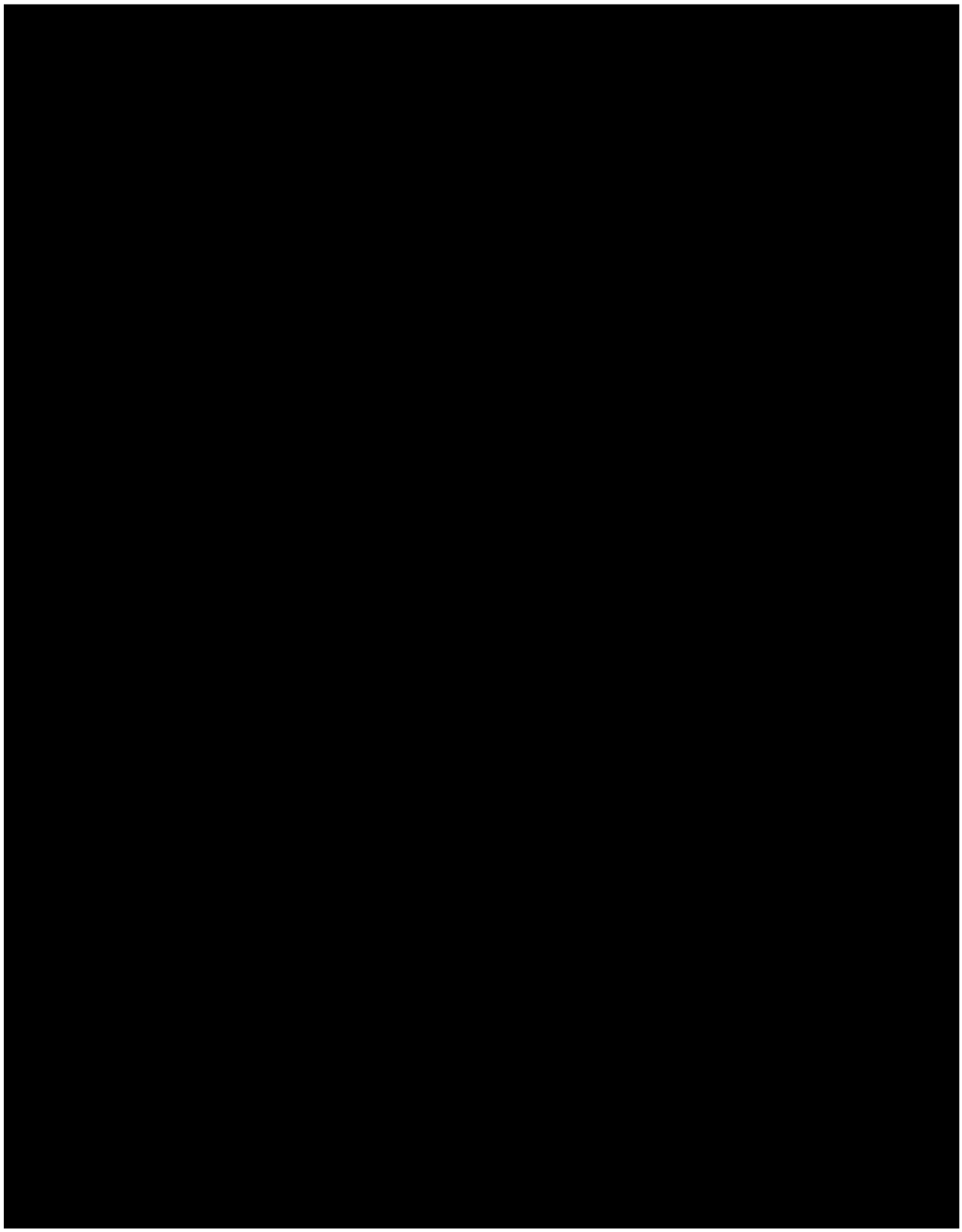

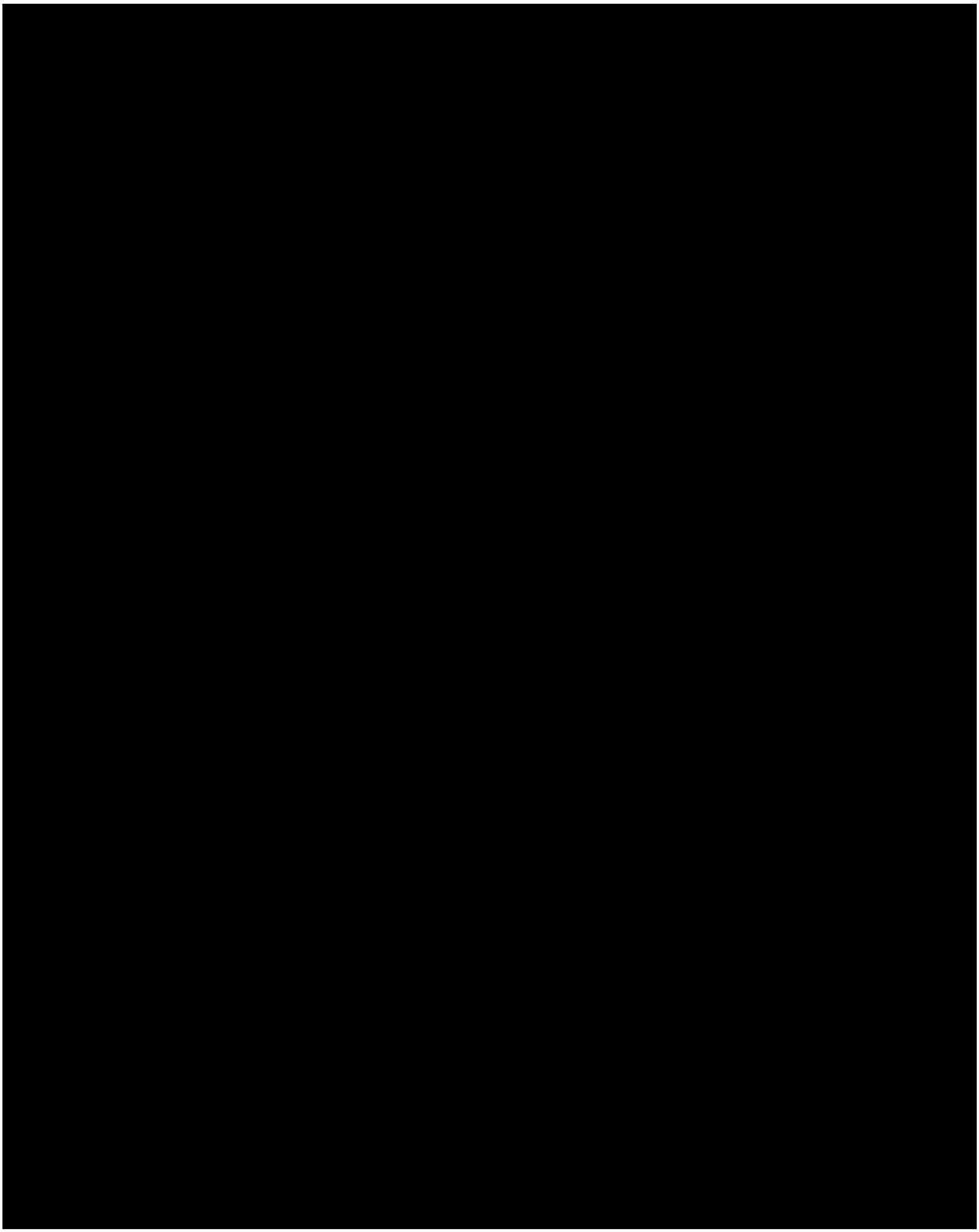

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Impurities (HPLC) [Known Impurities] (UTW-11-0327)	Compound	Specifications	
	1AU90	Not more than 0.4 %	ND
	2AU90	Not more than 0.1 %	ND
	97W86	Not more than 0.2 %	ND
	3AU90	Not more than 0.5 %	< 0.05 % w/w
	Treprostinil Methyl Ester	Not more than 0.2 %	ND
	Treprostinil Ethyl Ester	Not more than 0.5 %	ND
	750W93	Not more than 0.5 %	ND
	751W93	Not more than 0.3 %	ND
Impurities (HPLC) [Unidentified Impurities] (UTW-11-0327)	Not more than 0.2 % AUC each		0.07 % AUC (RRT 0.26)
Impurities (HPLC) [Total Related Substances] (UTW-11-0327)	Not more than 1.5 %		0.1 % w/w

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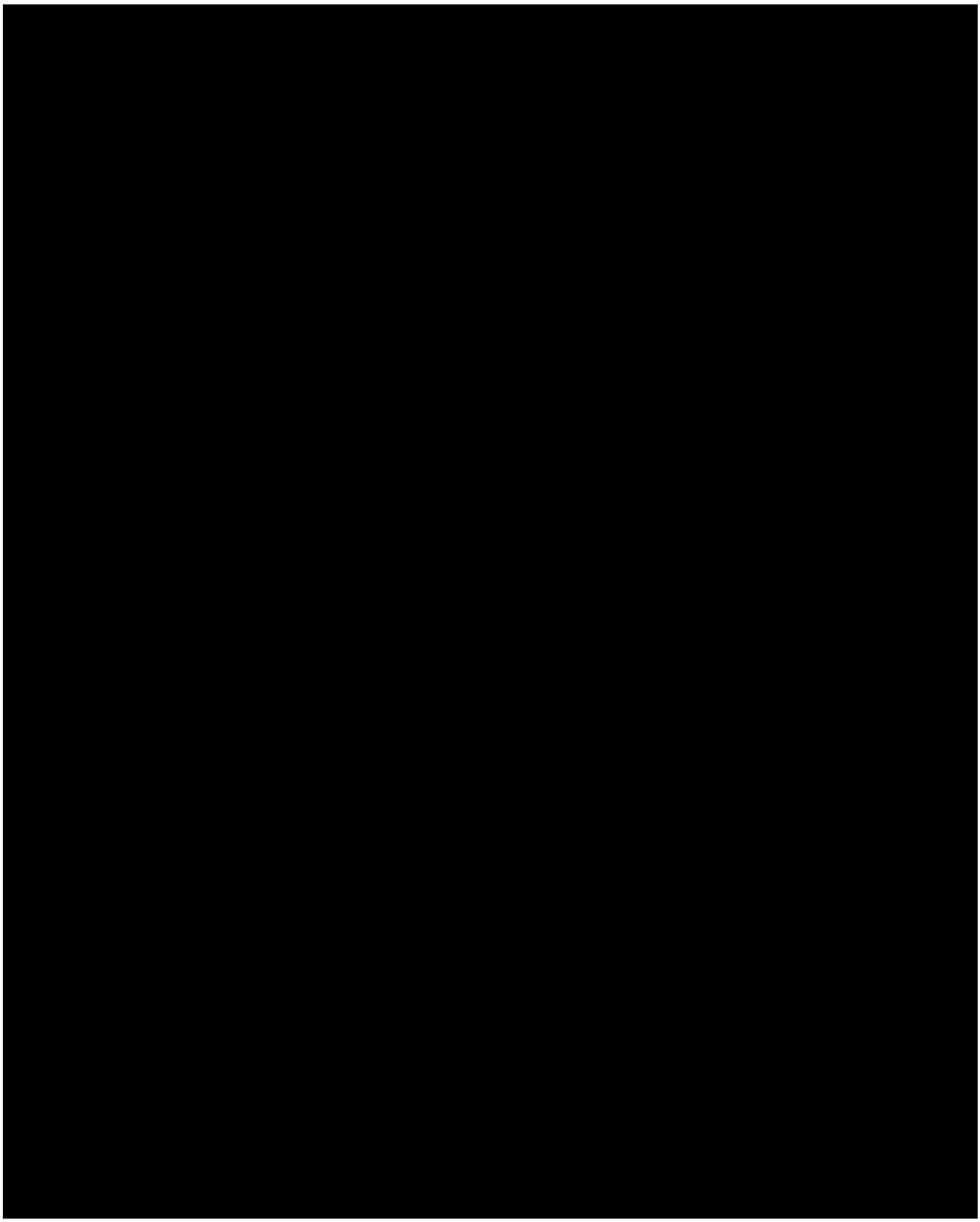

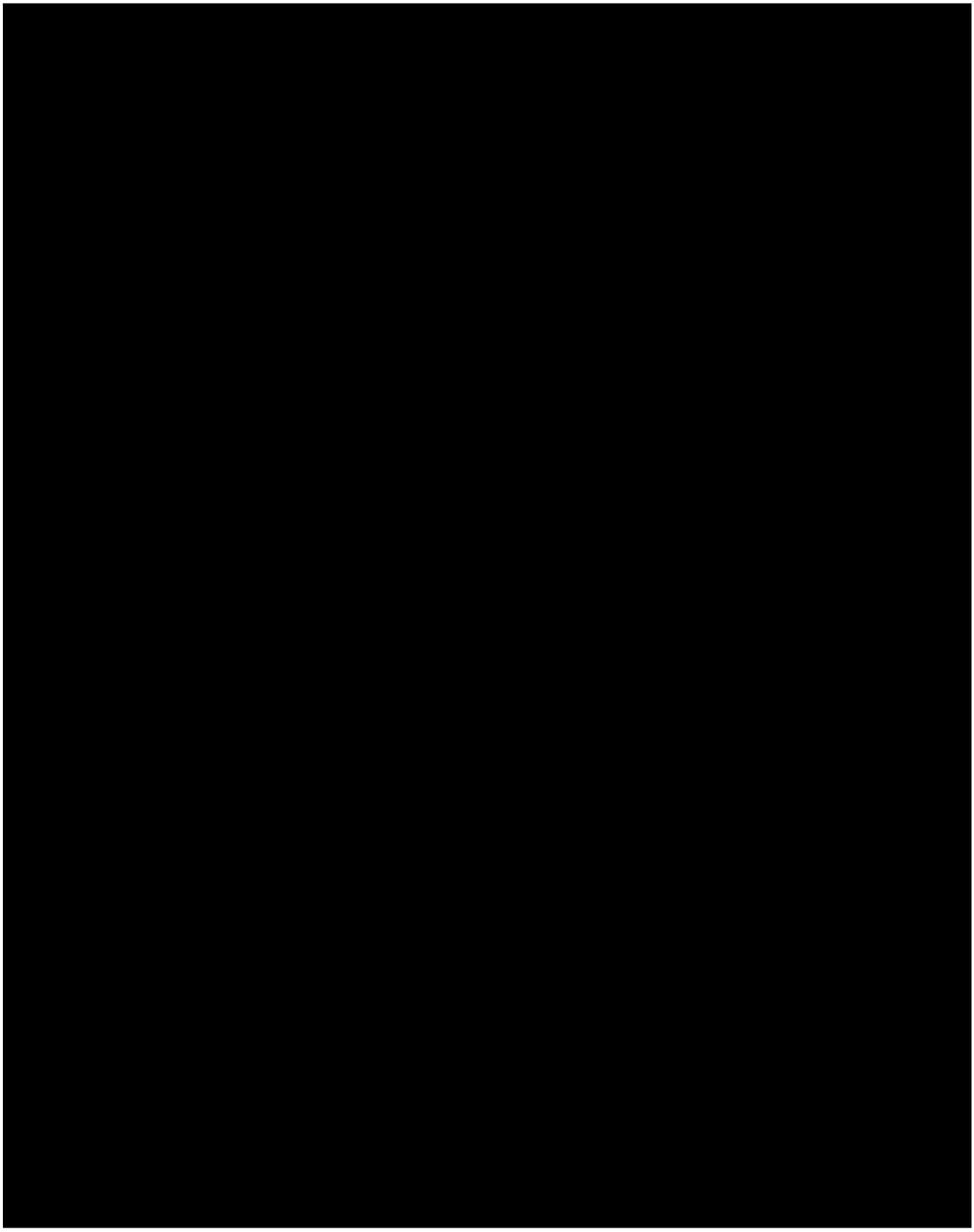

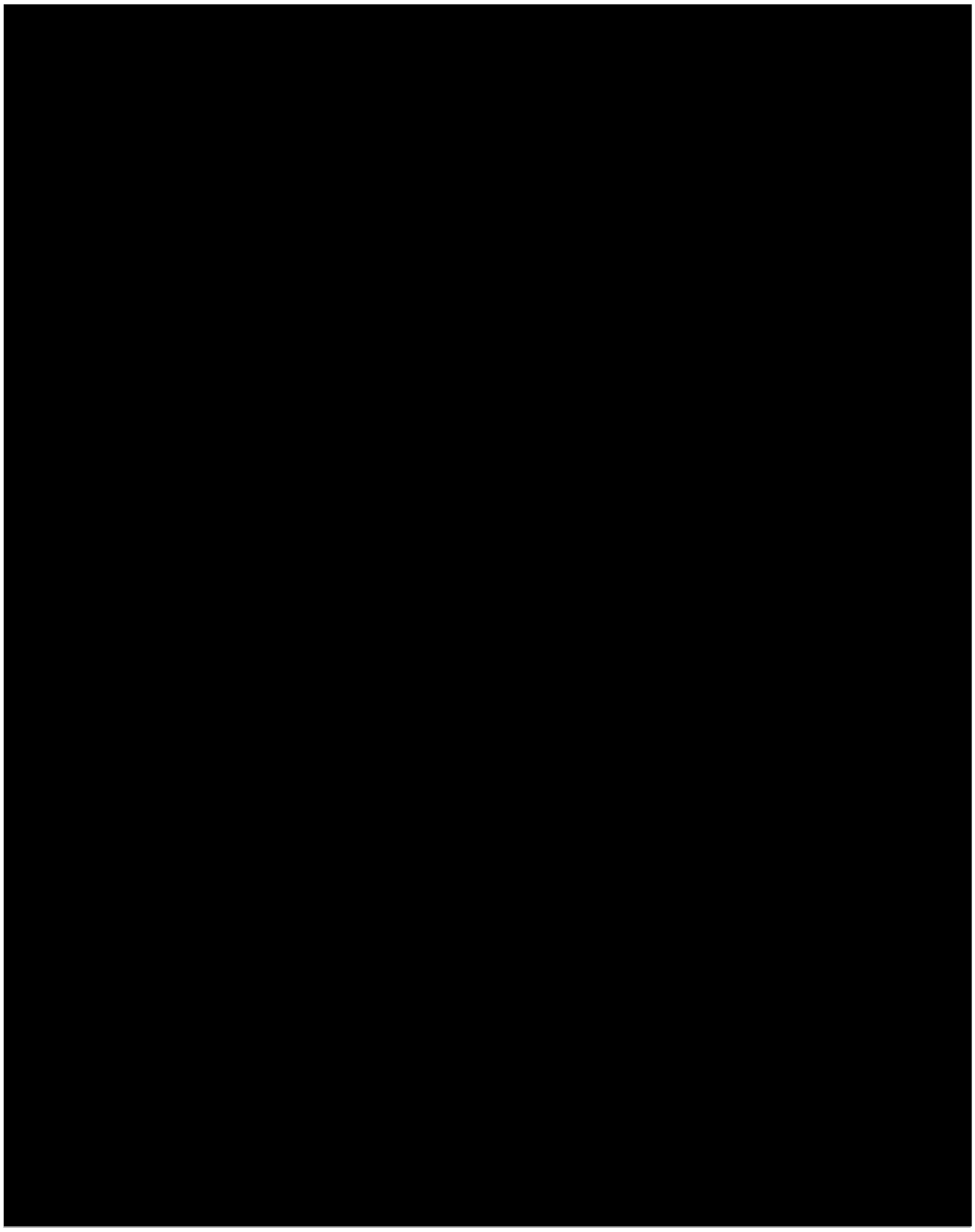

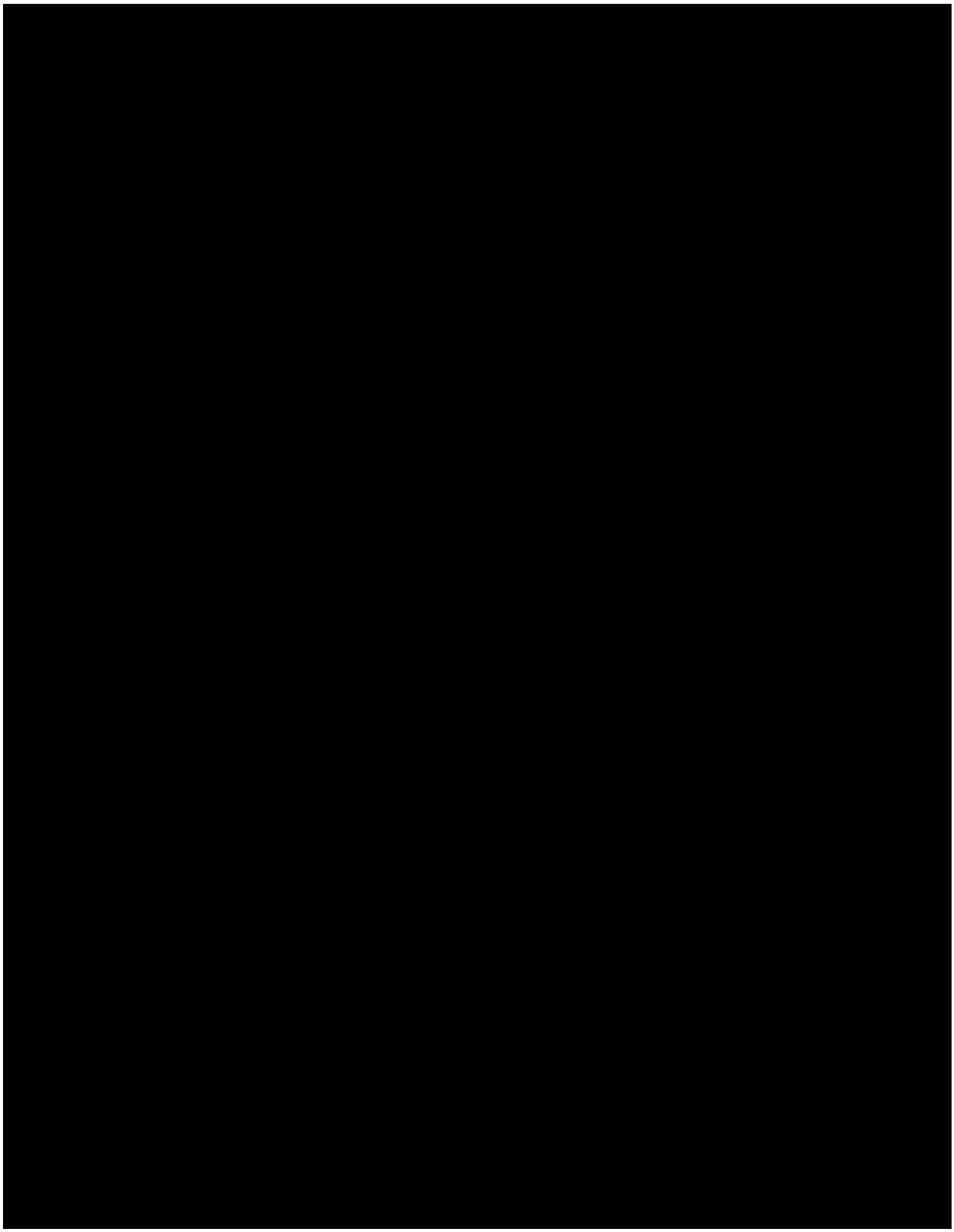

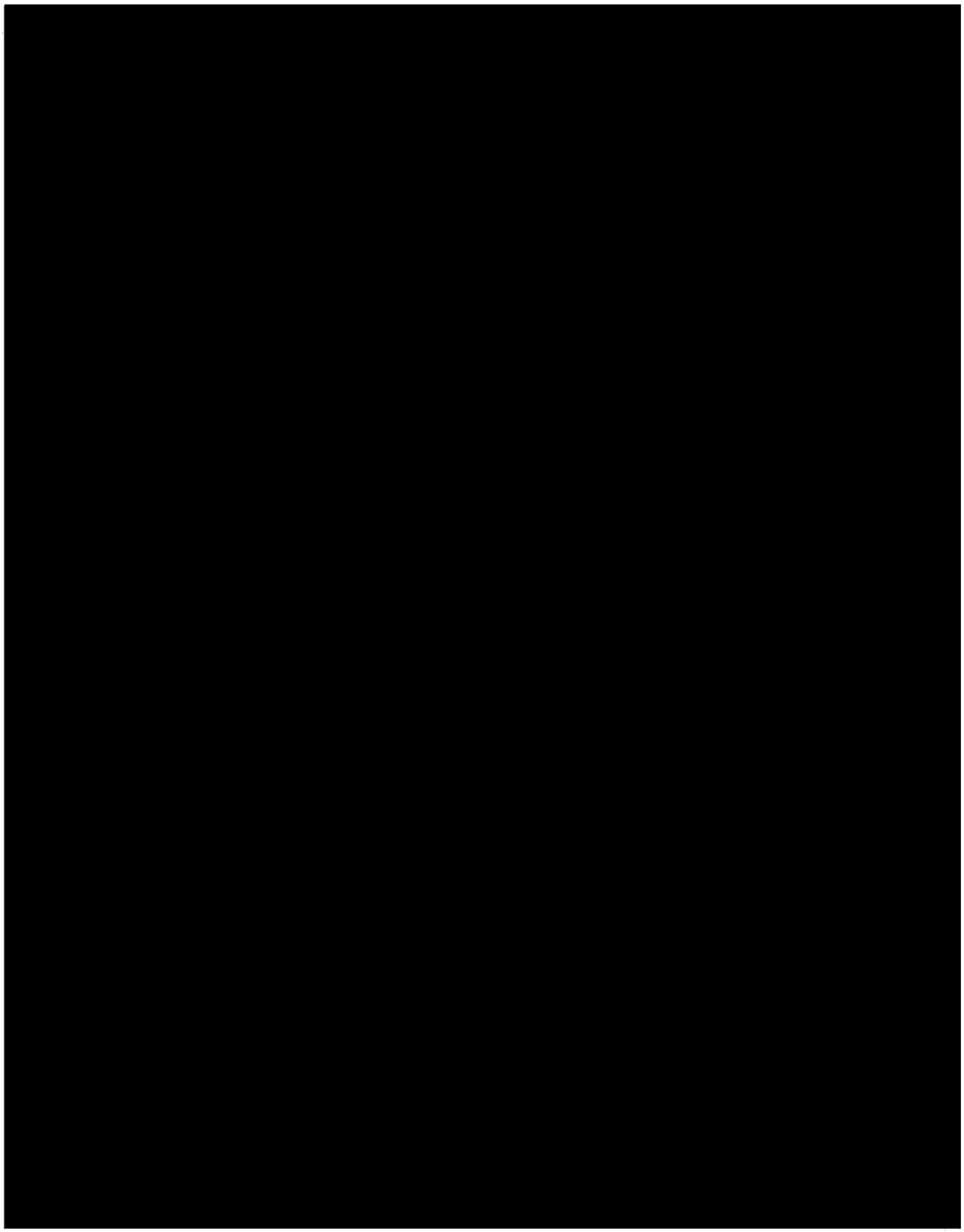

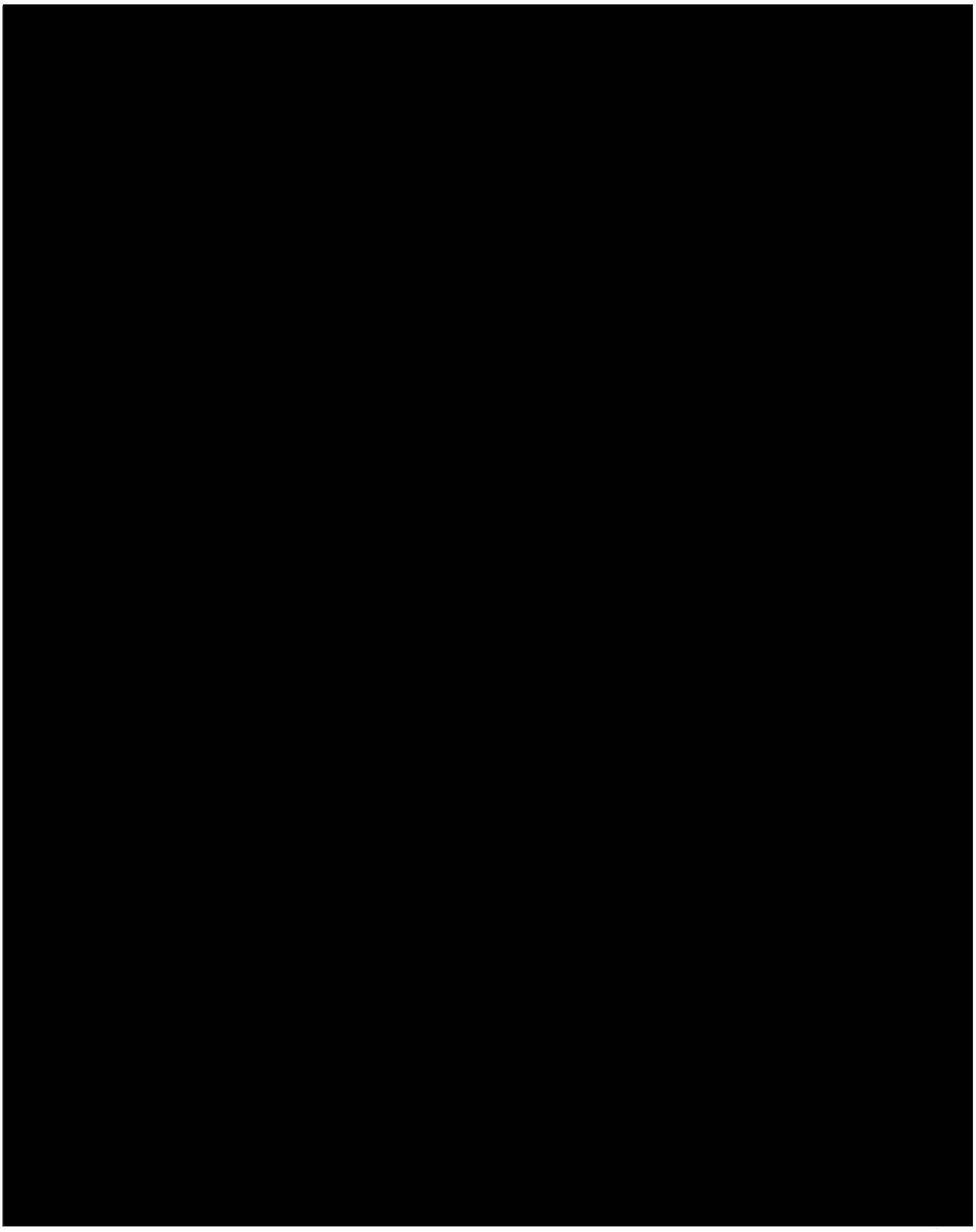

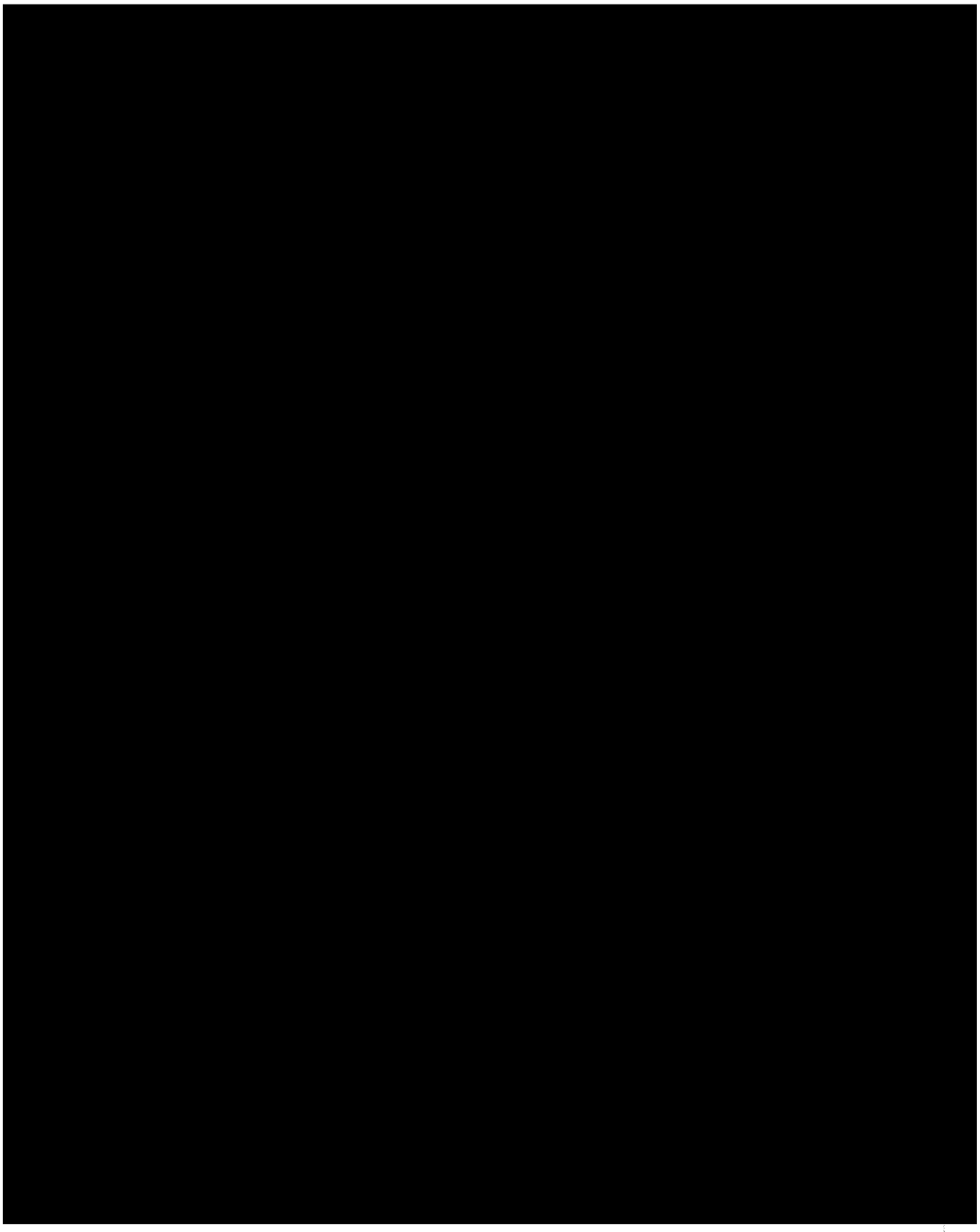

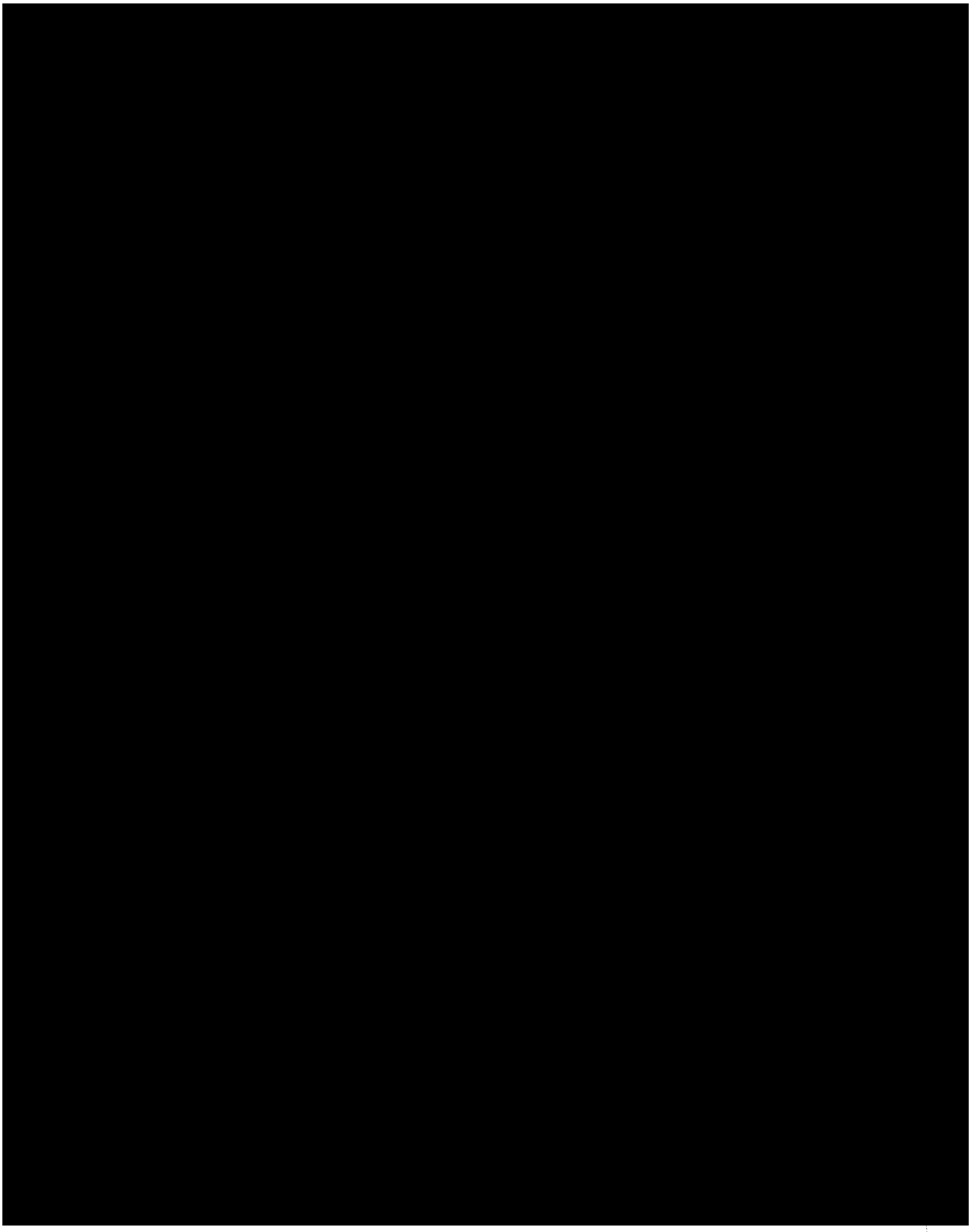

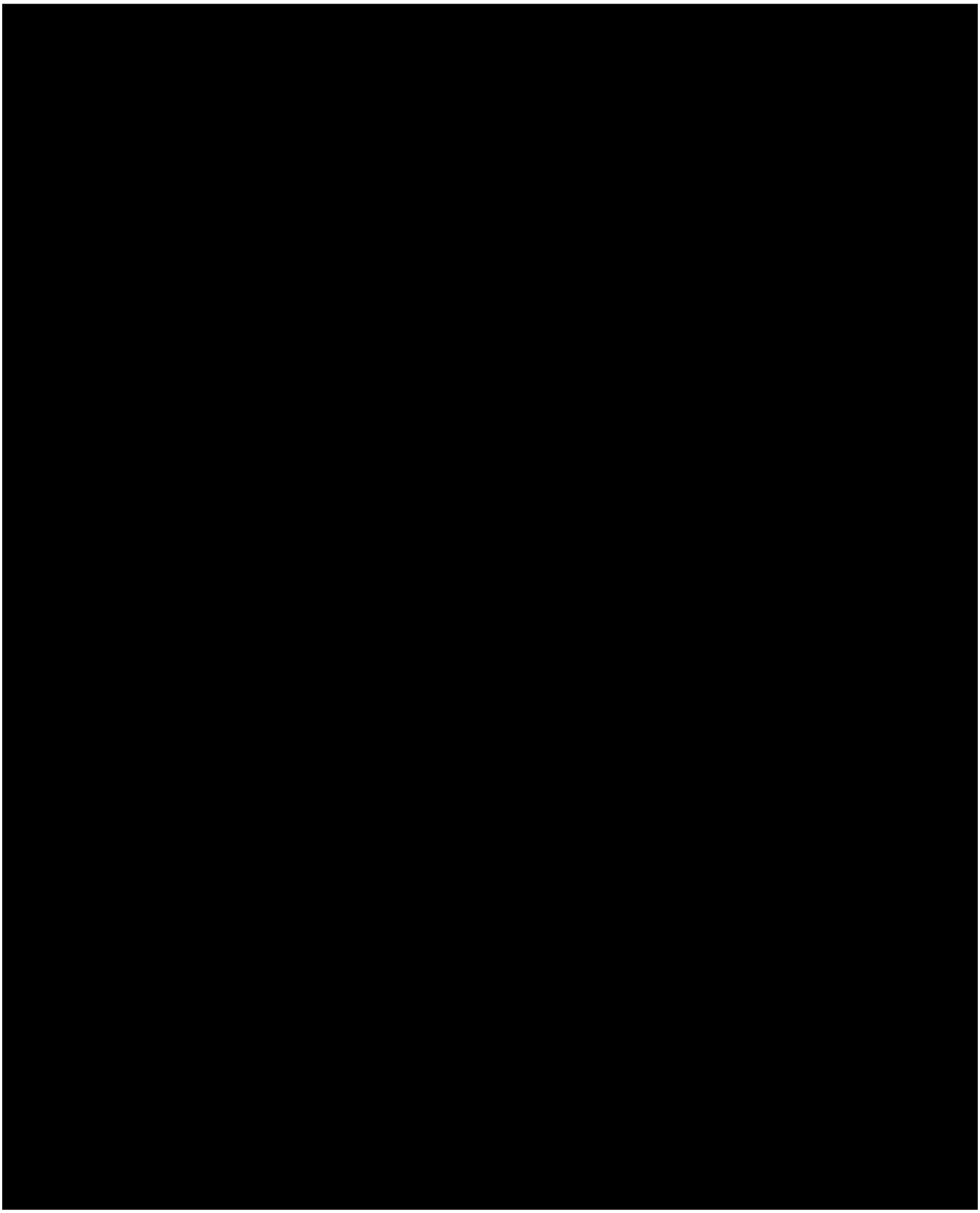

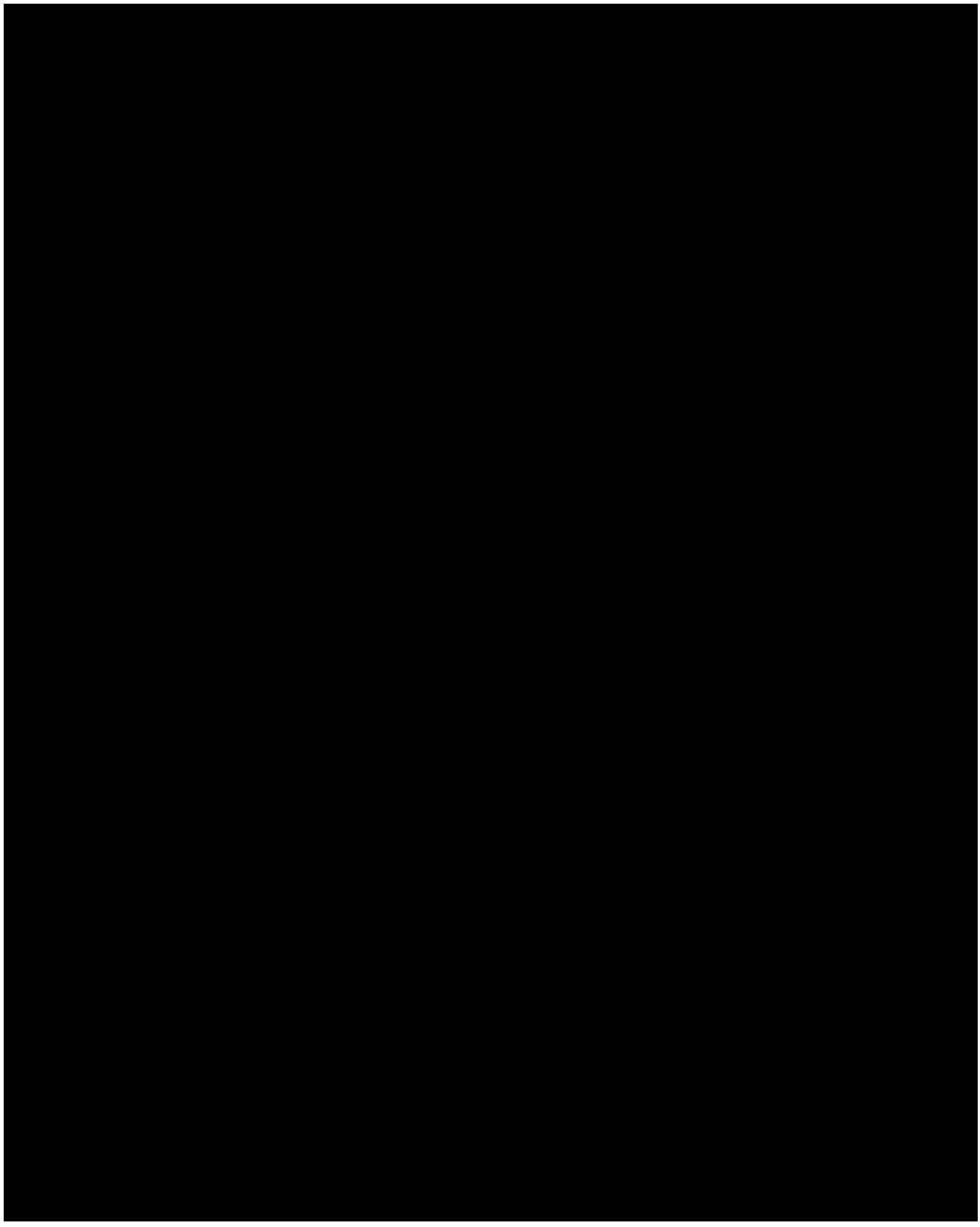

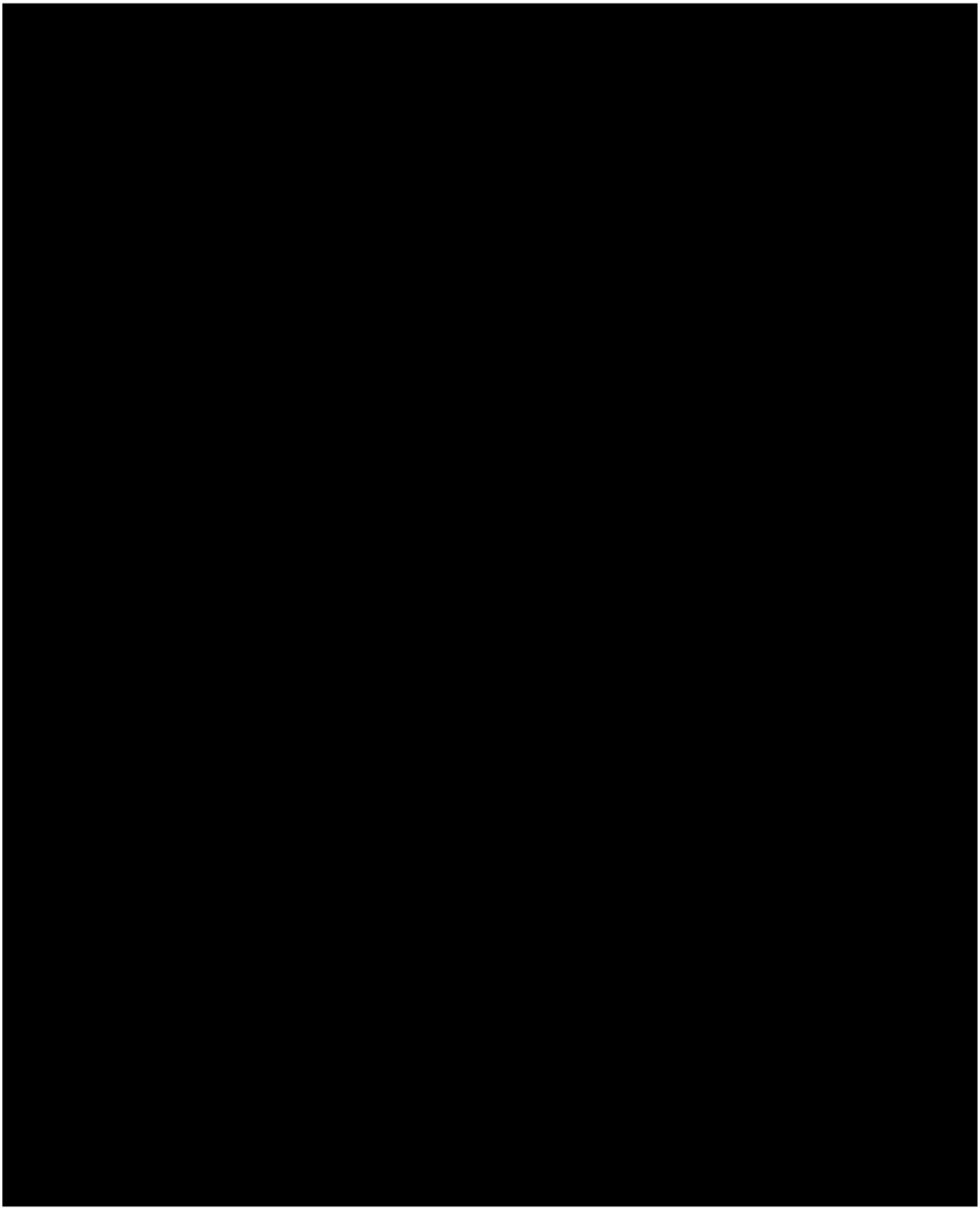

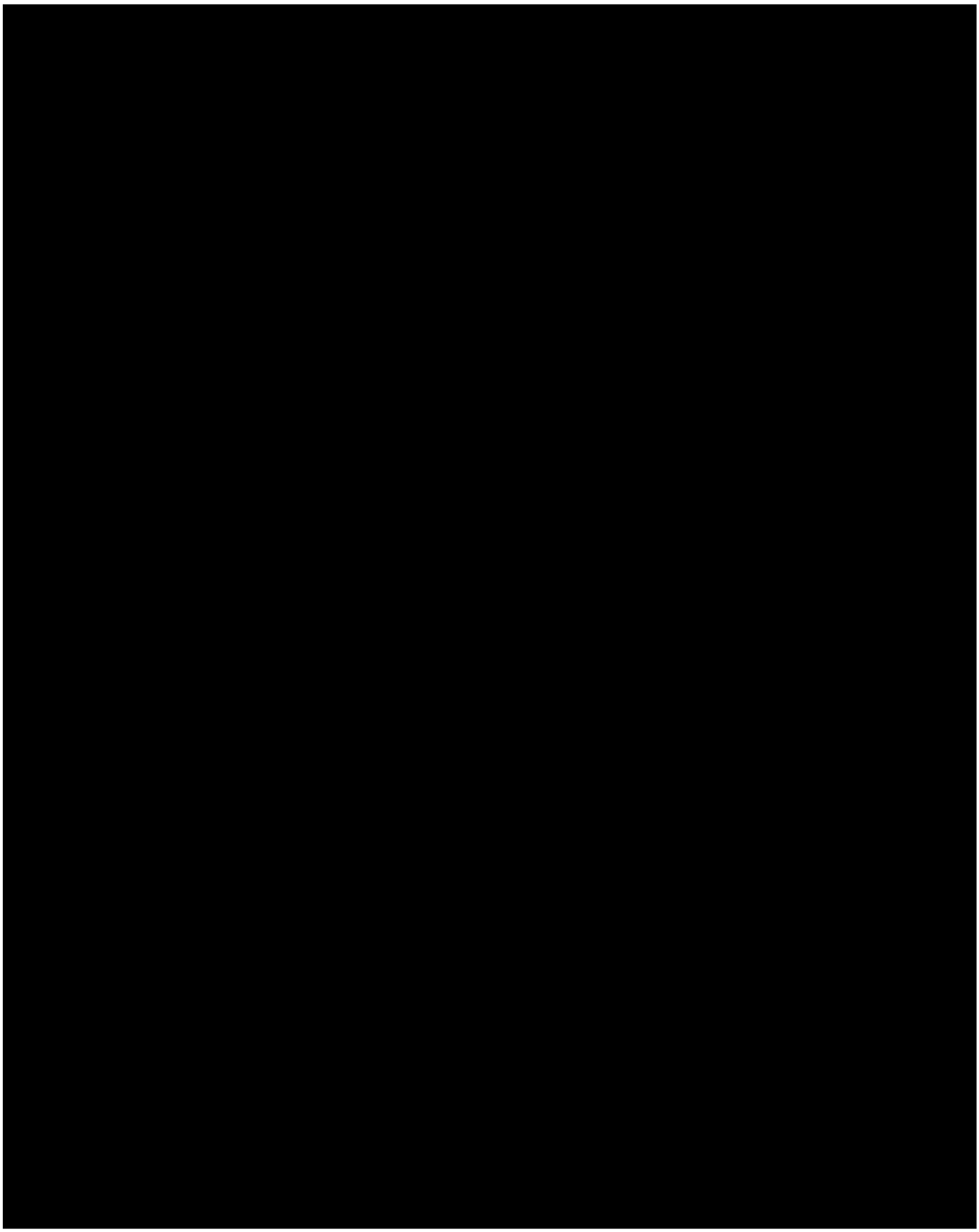

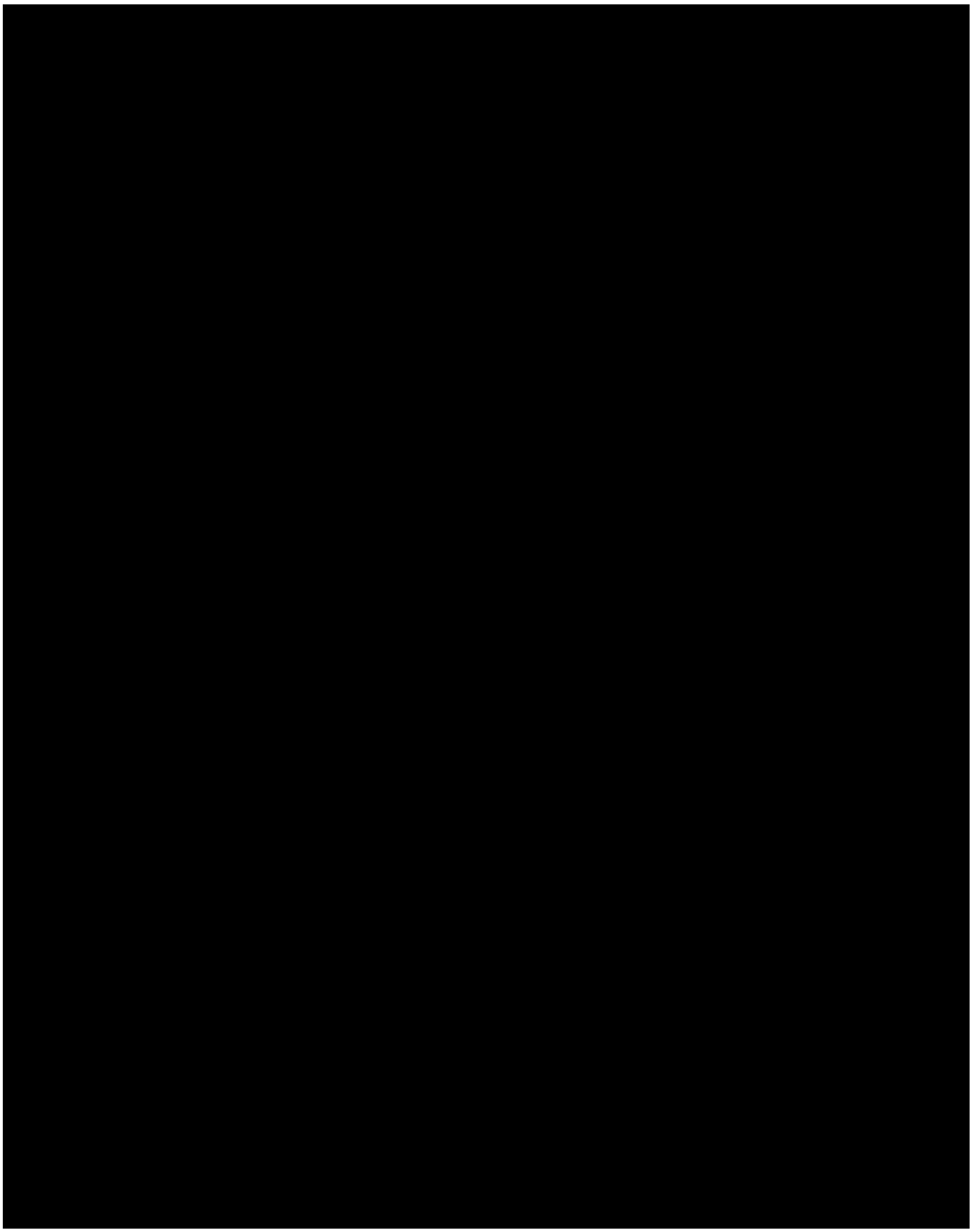

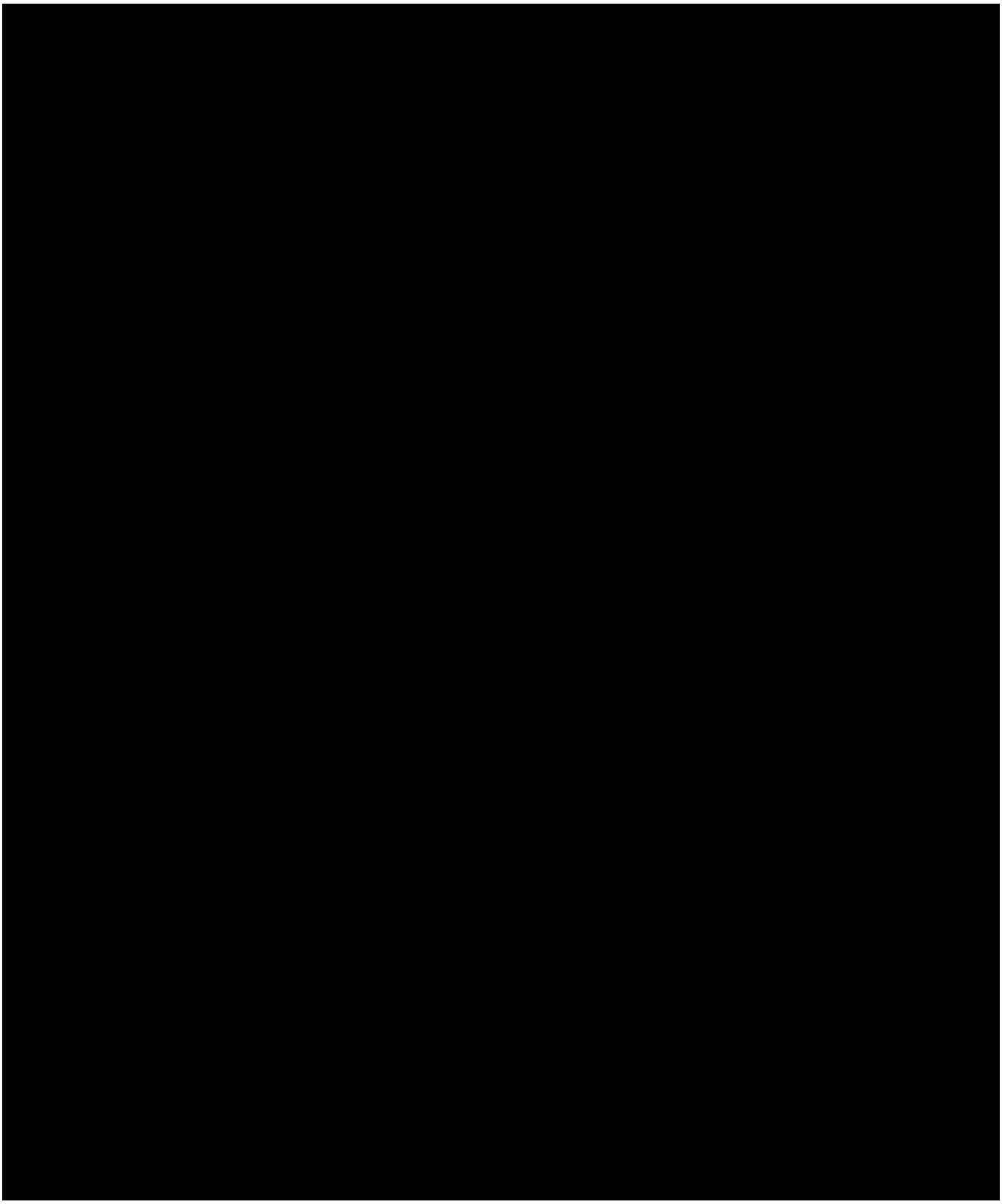

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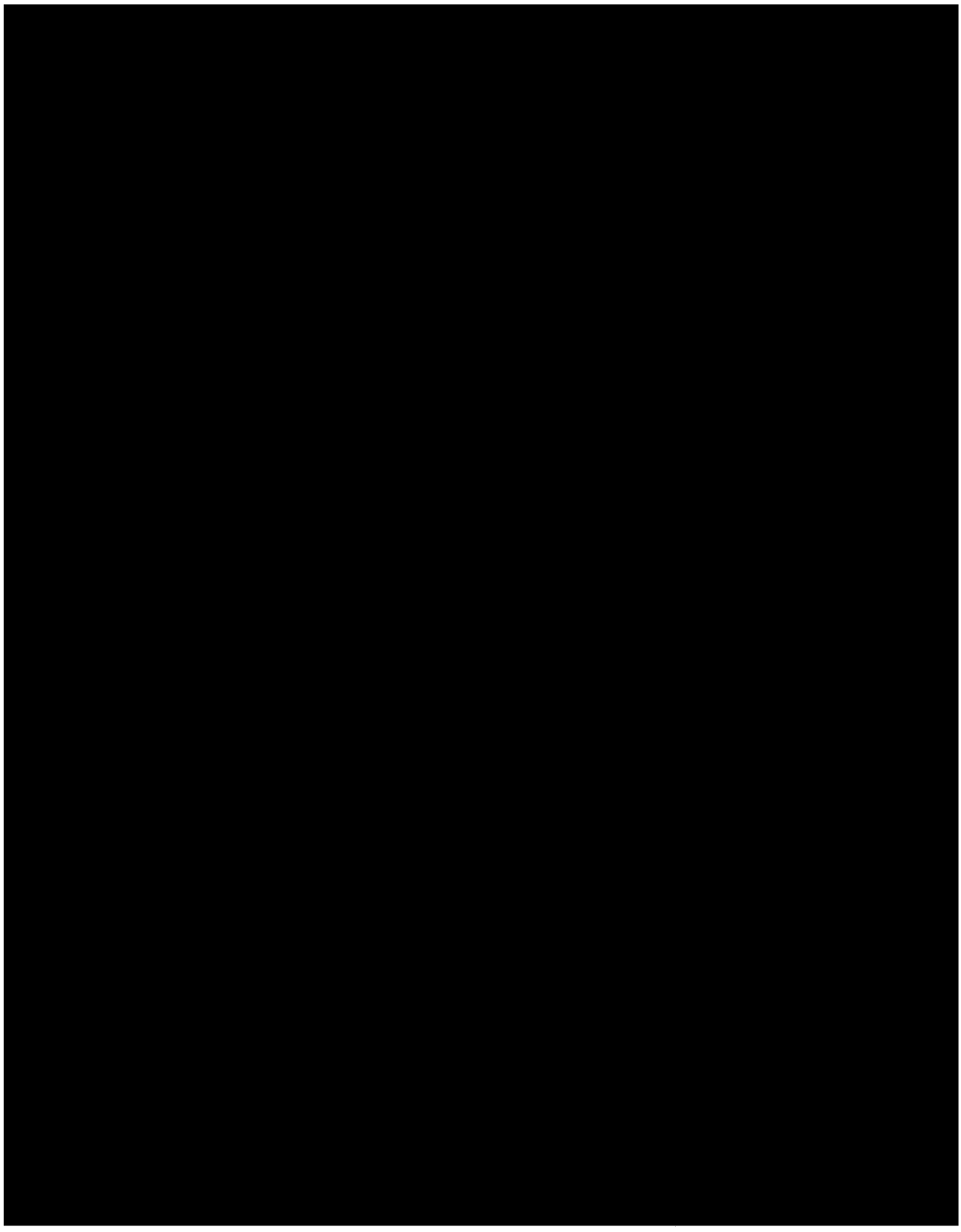

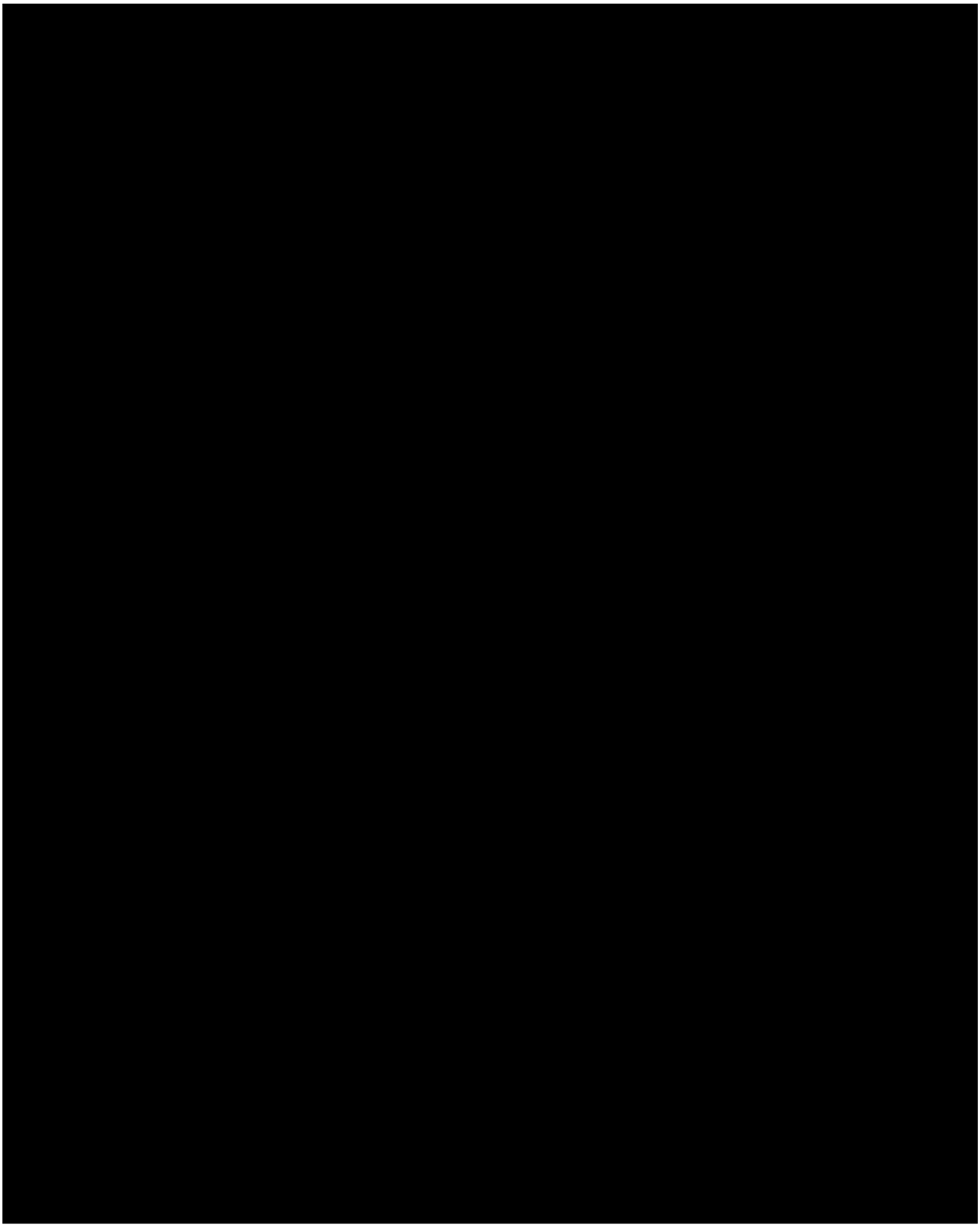

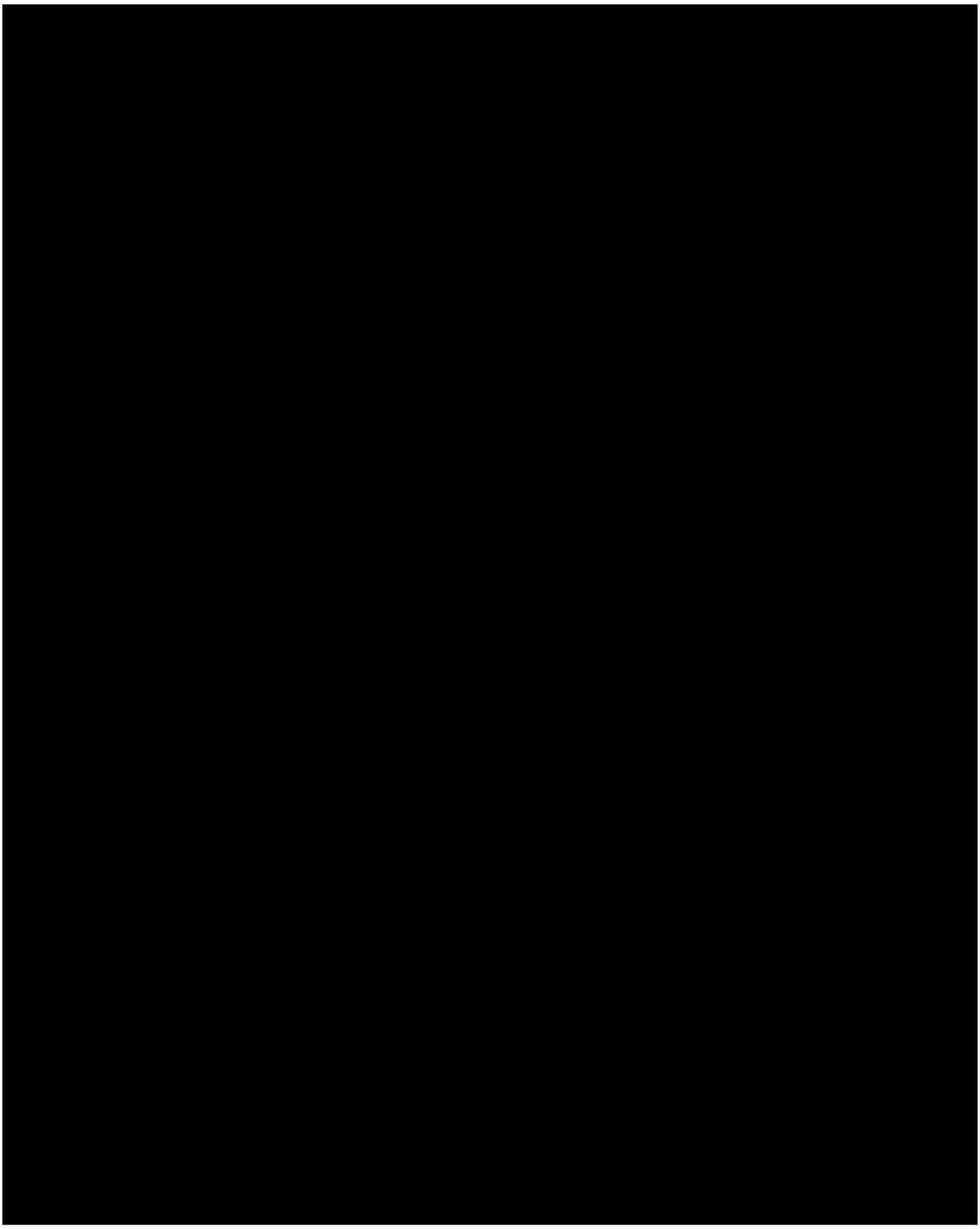

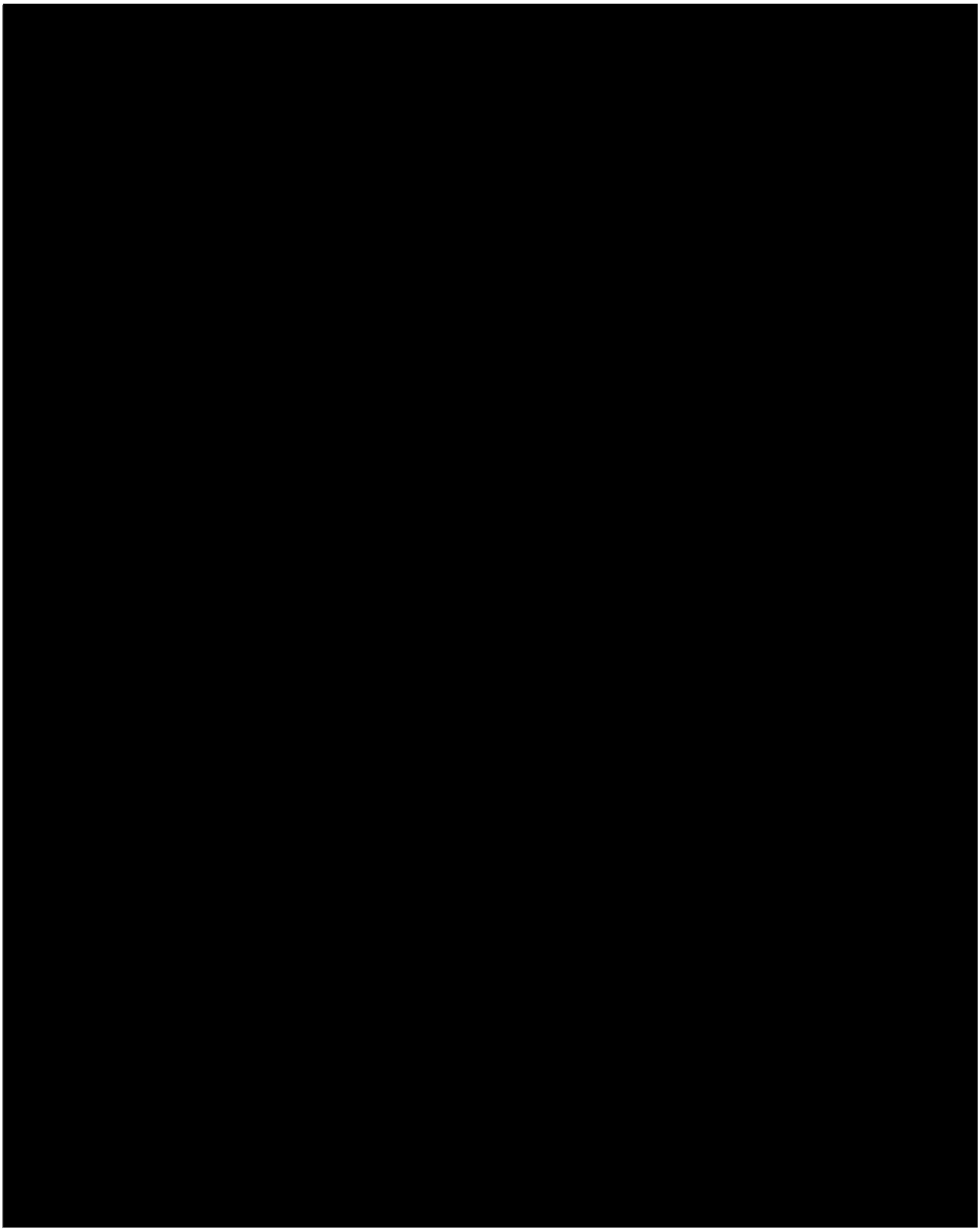

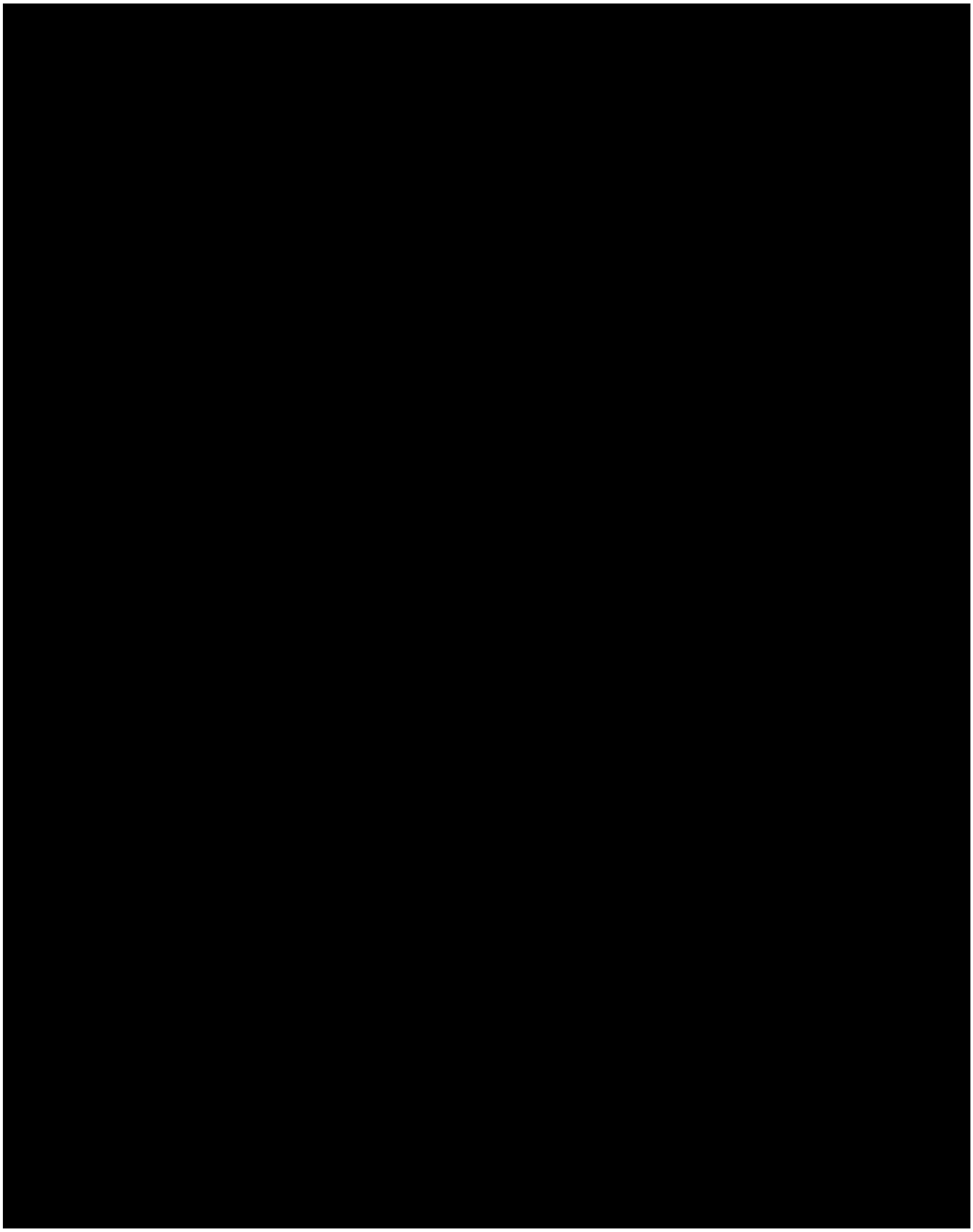

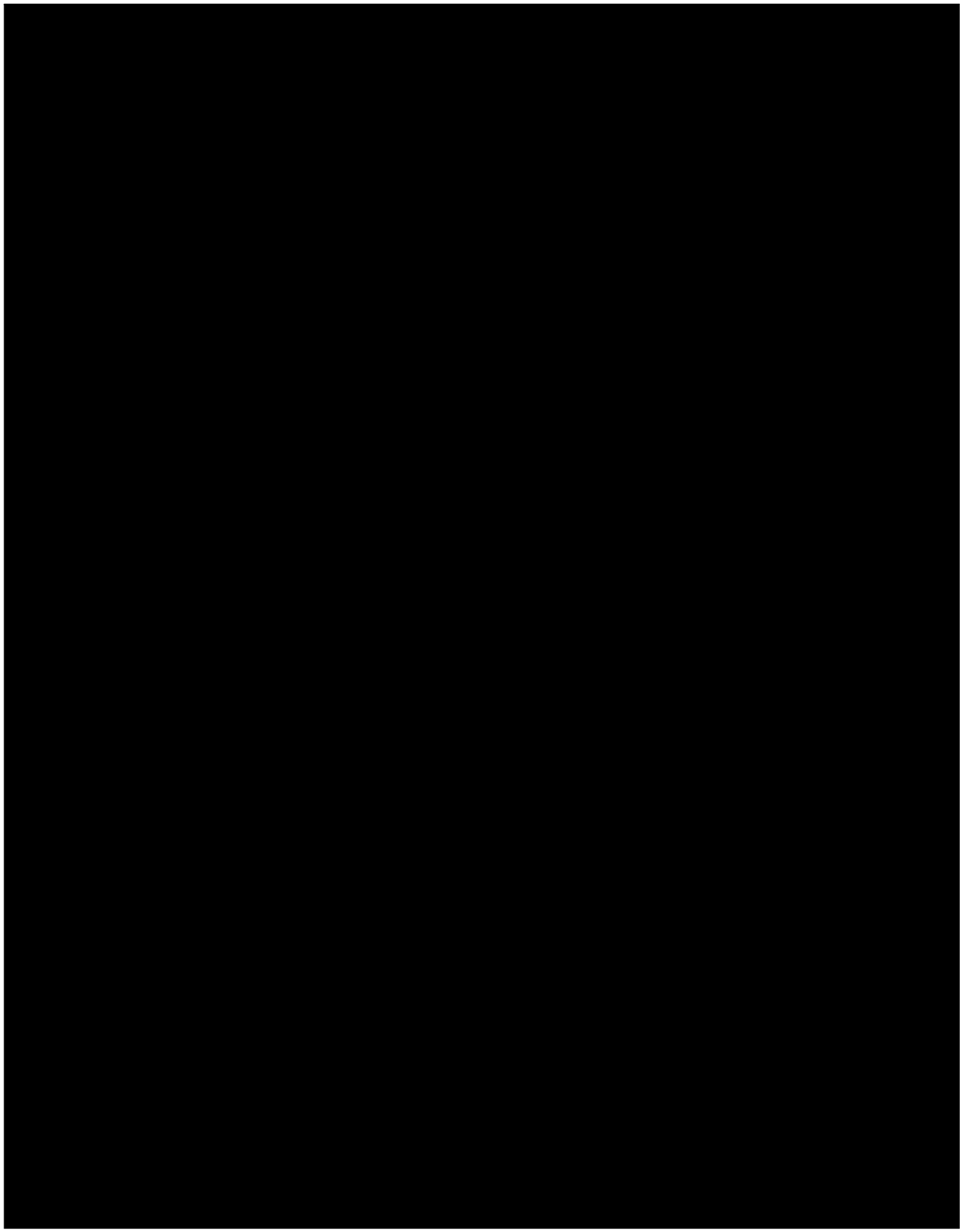

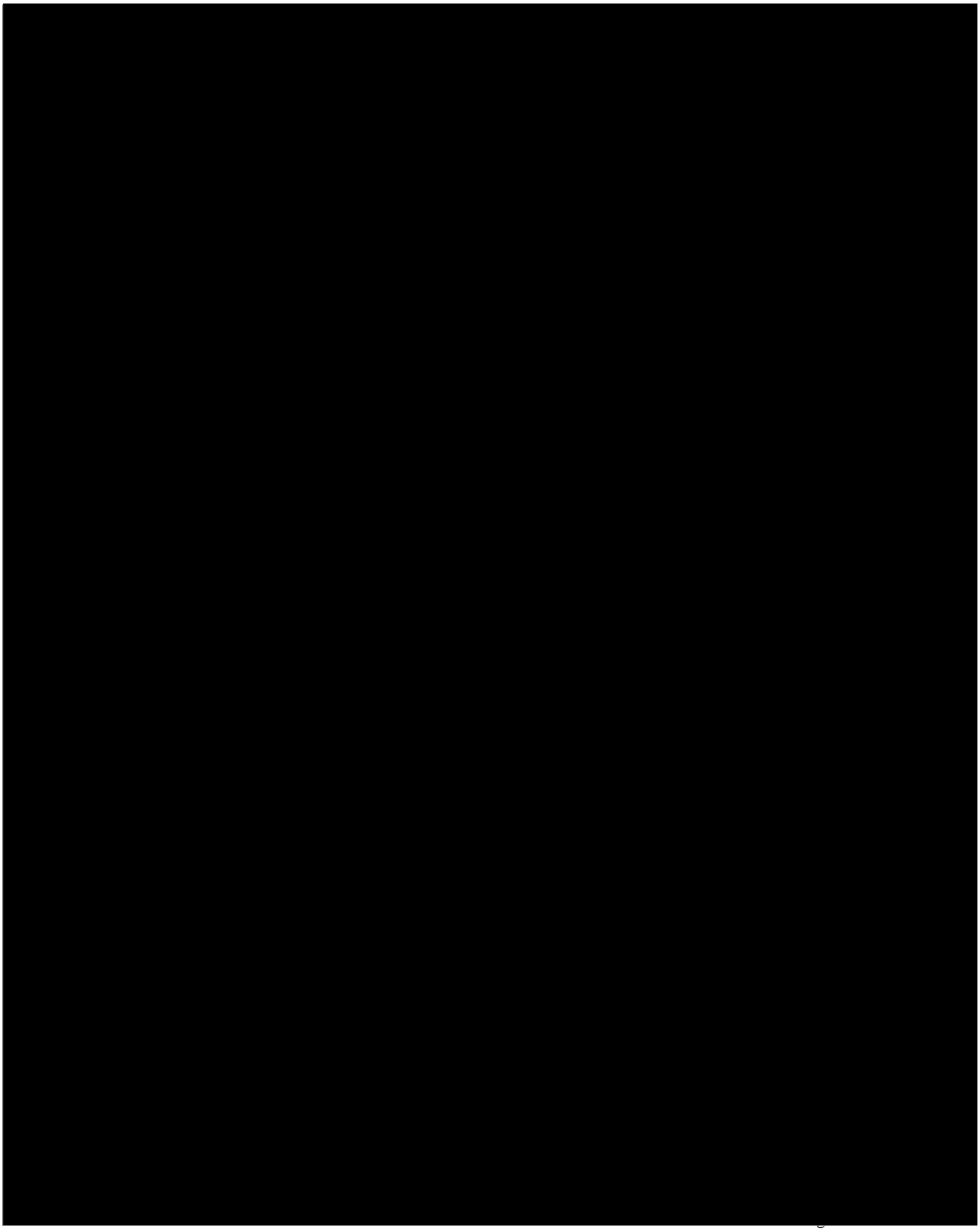

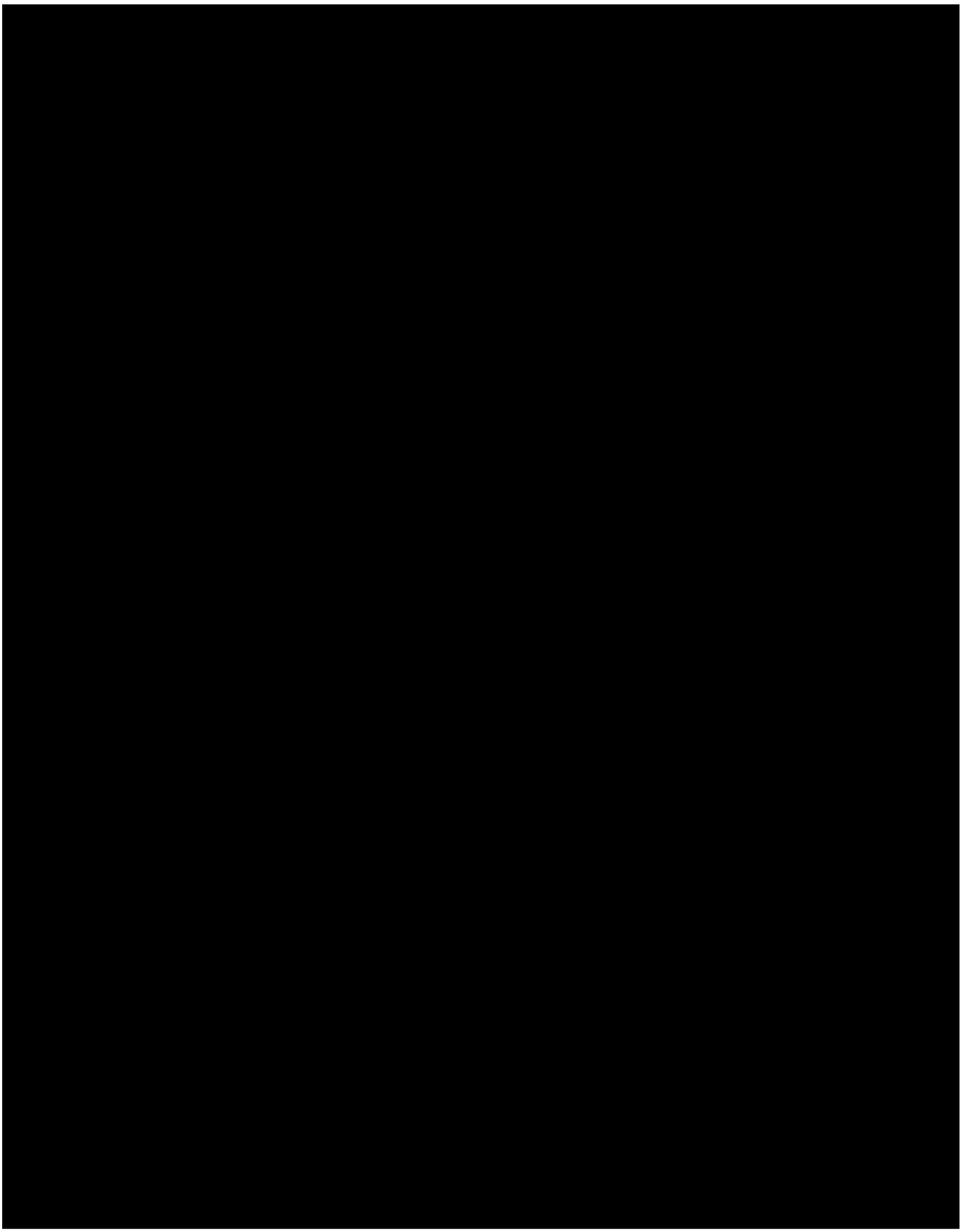

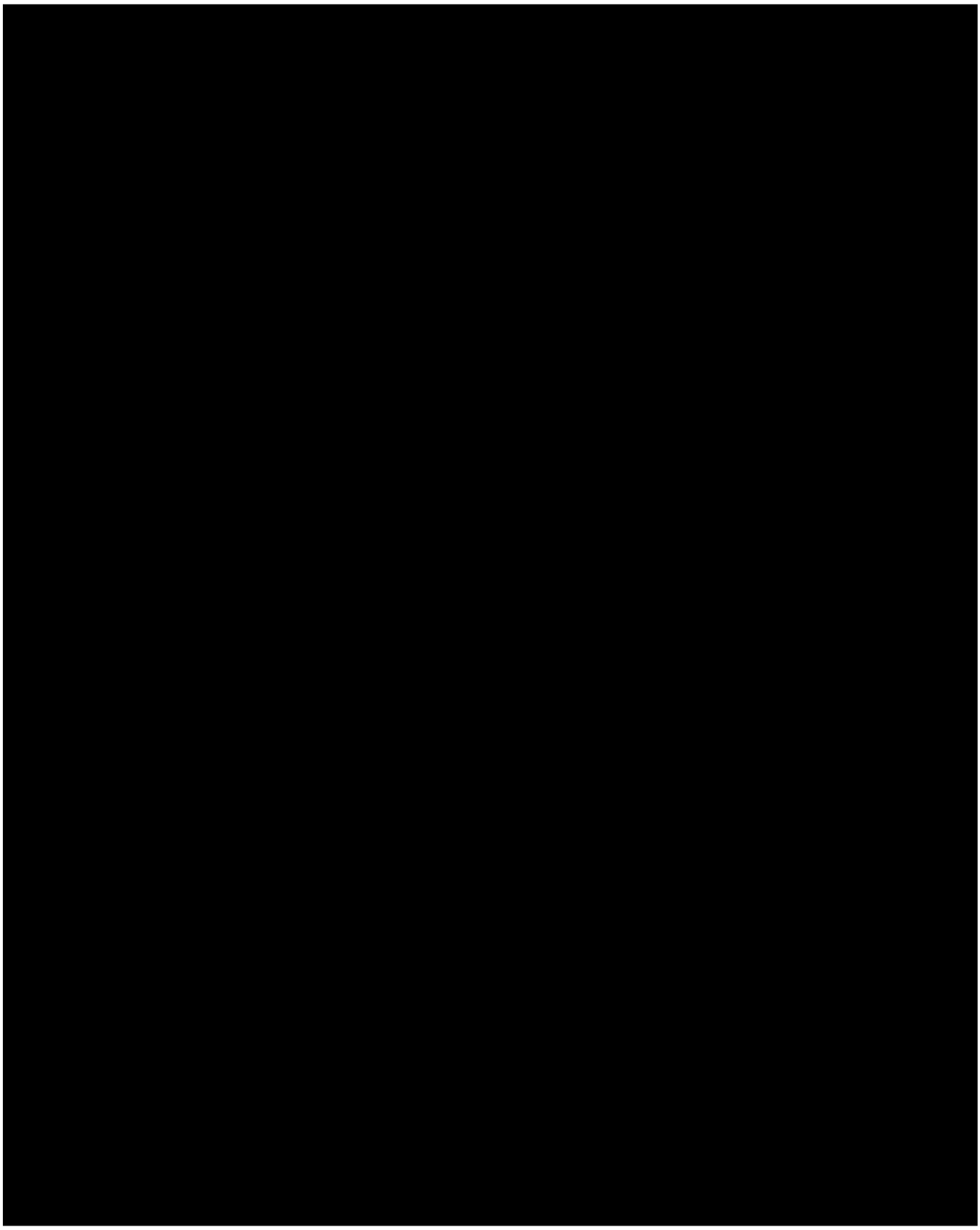

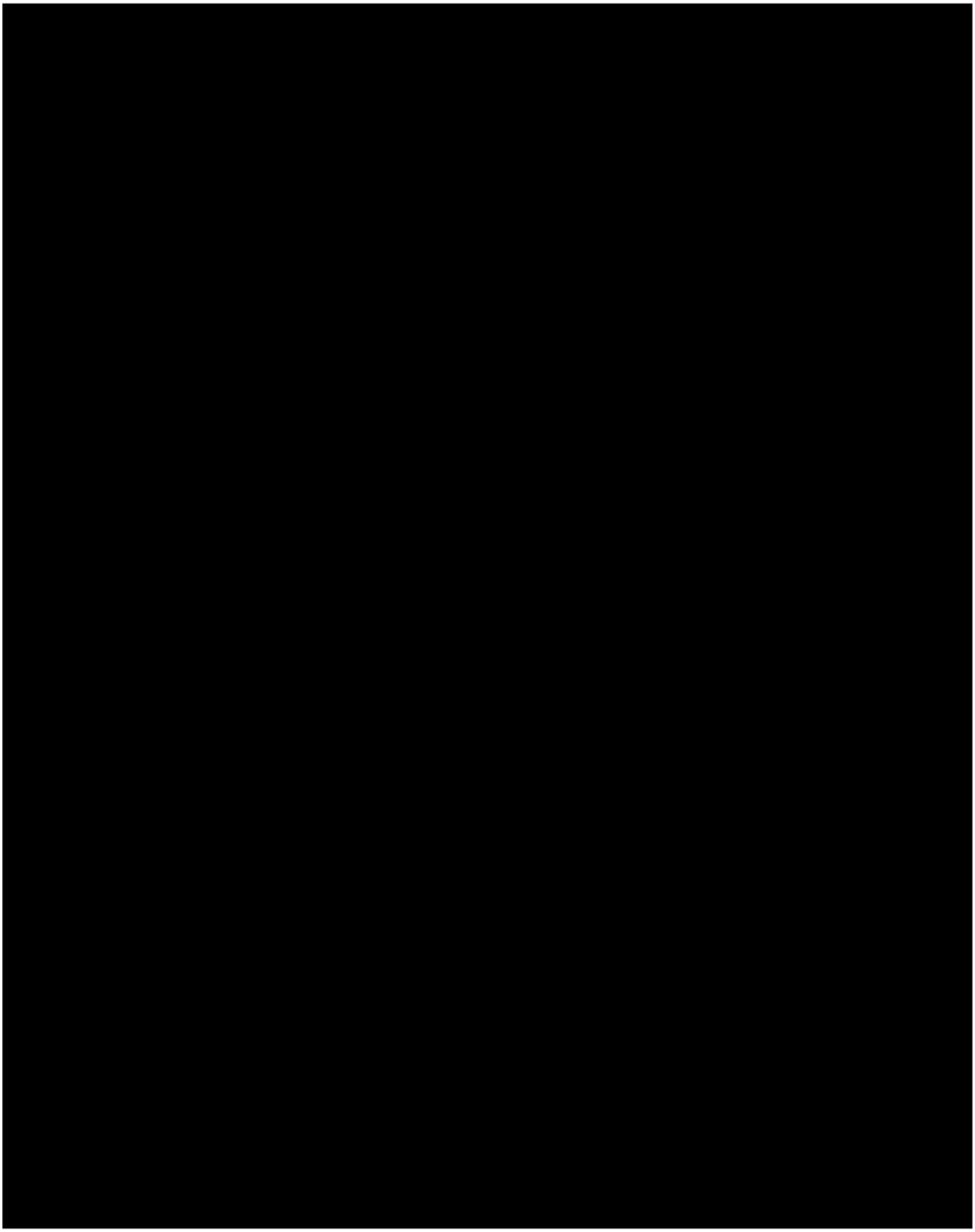

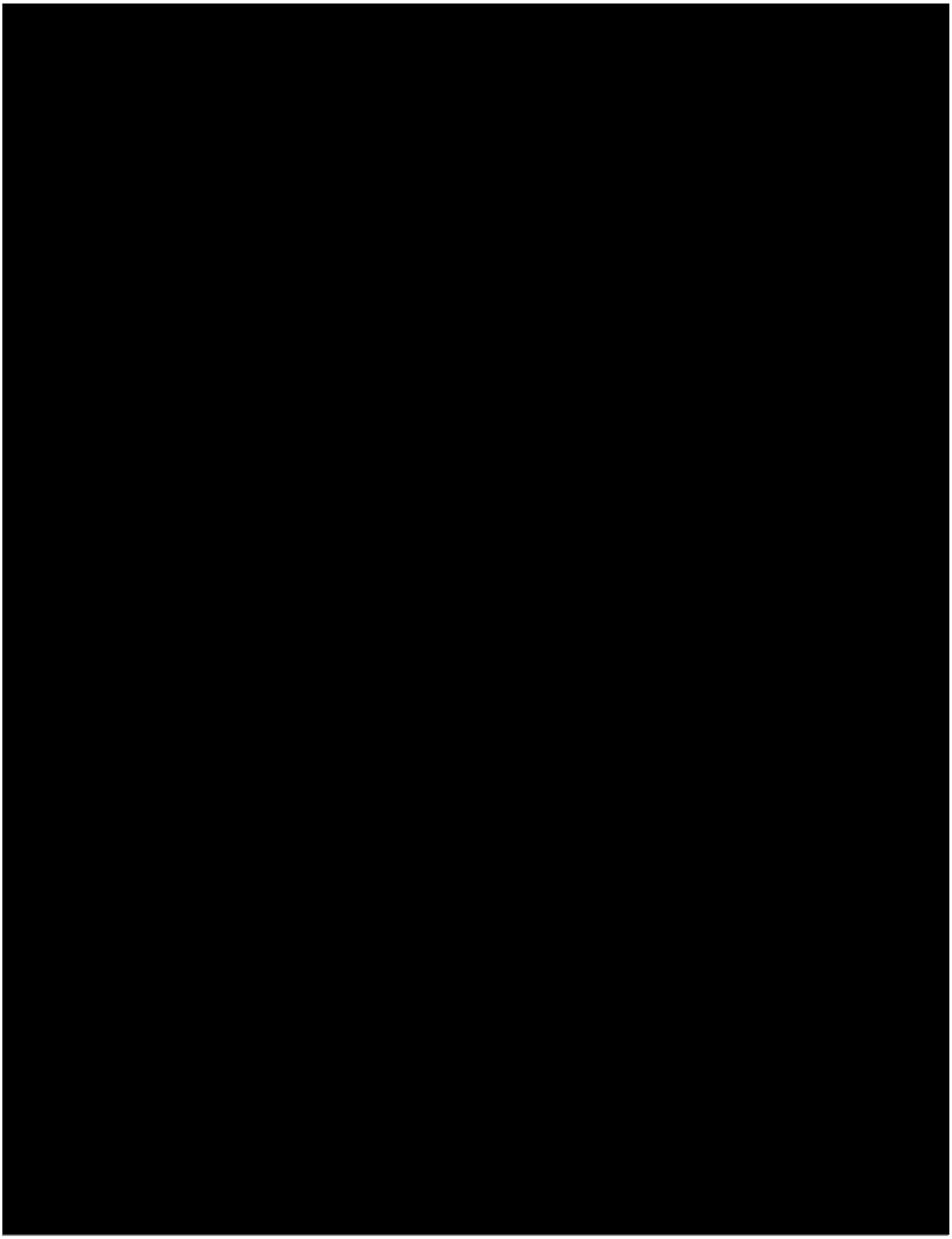

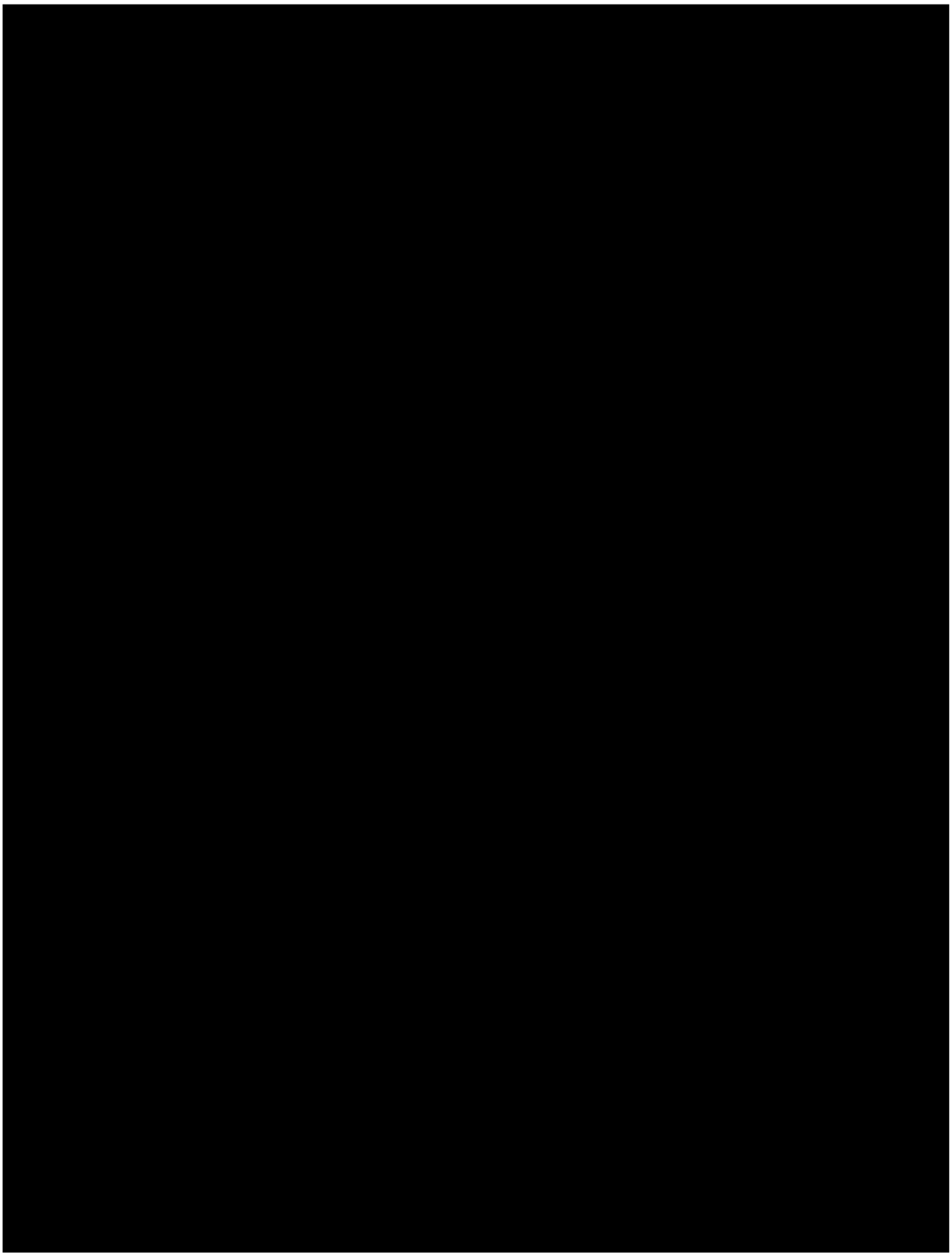

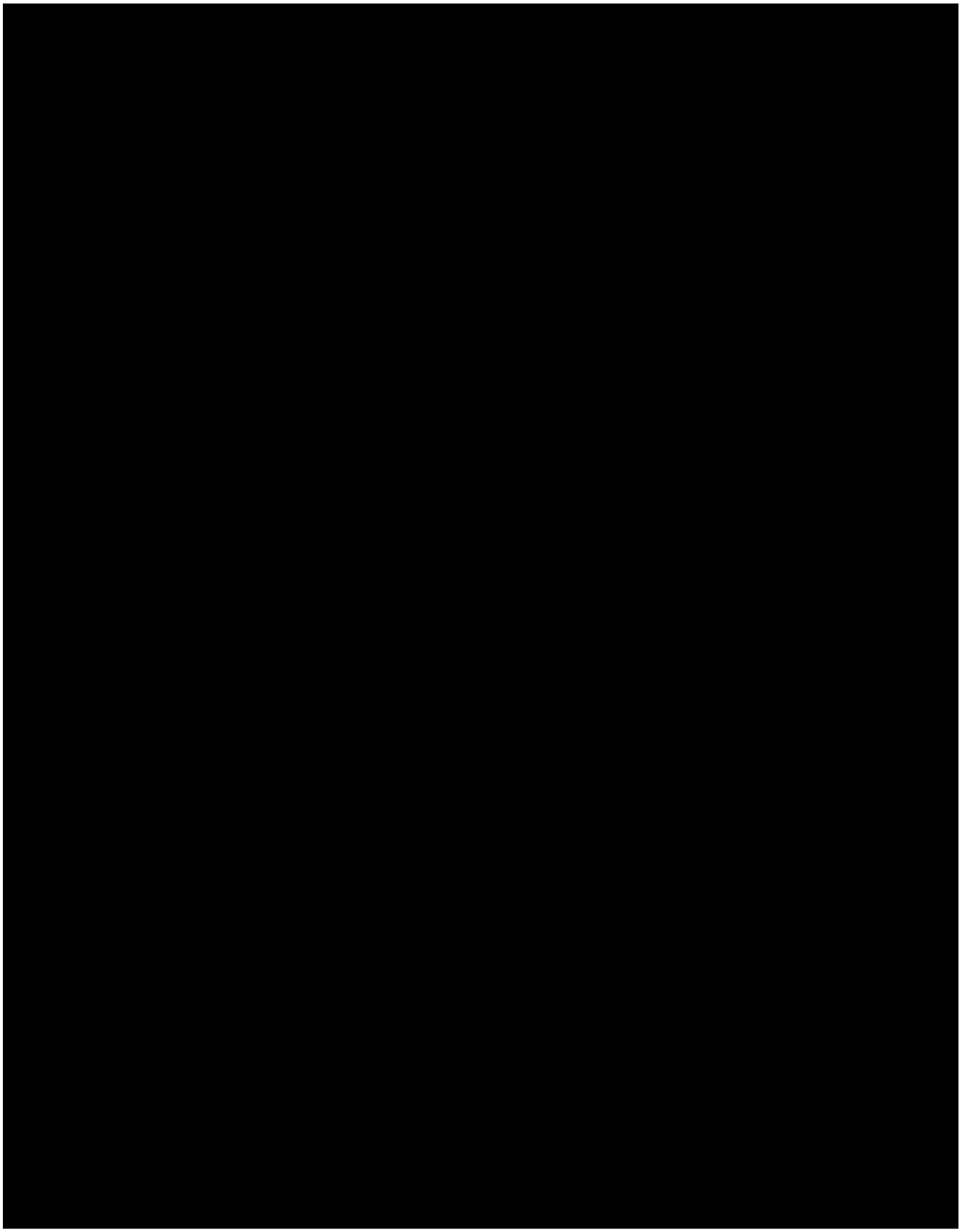

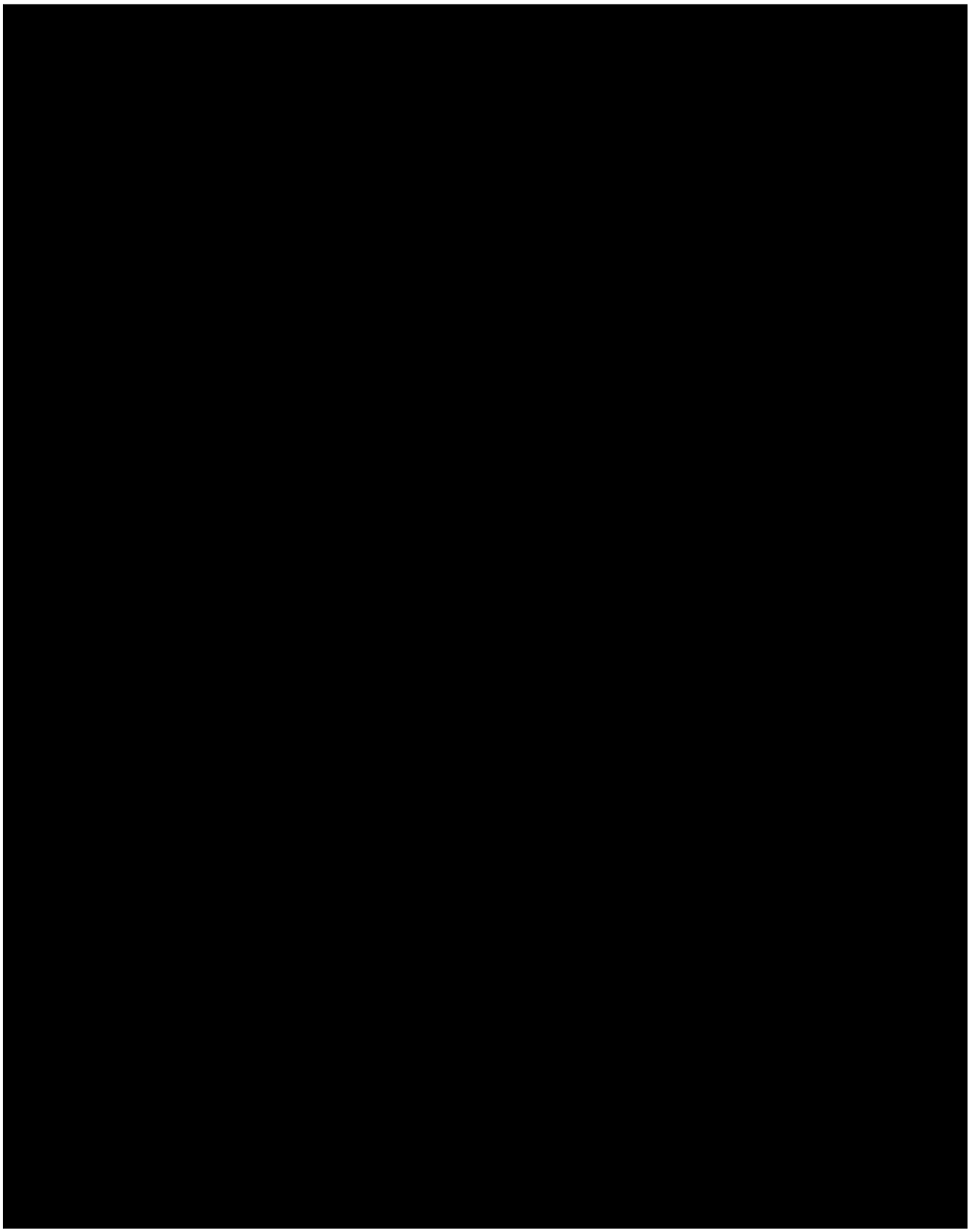

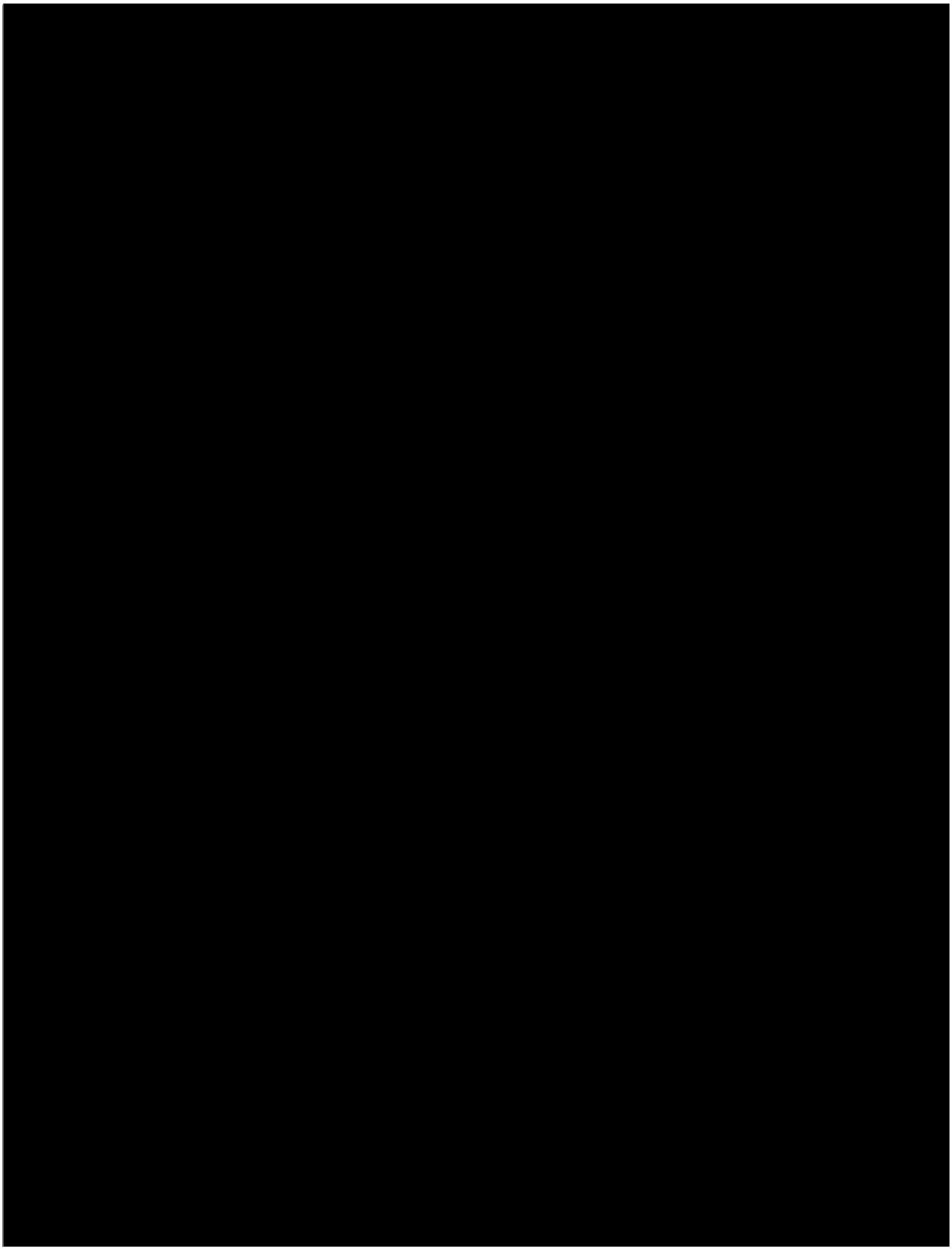

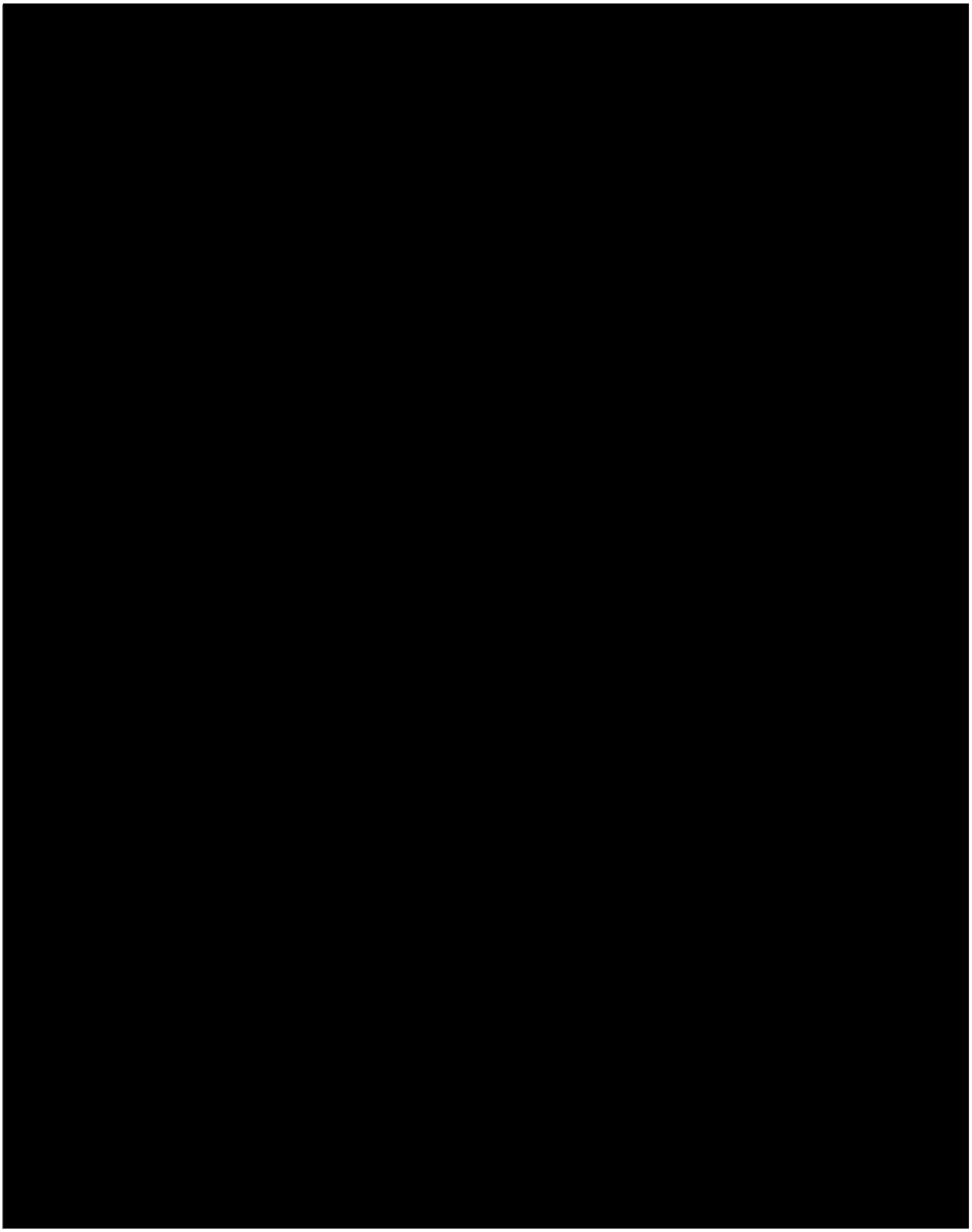

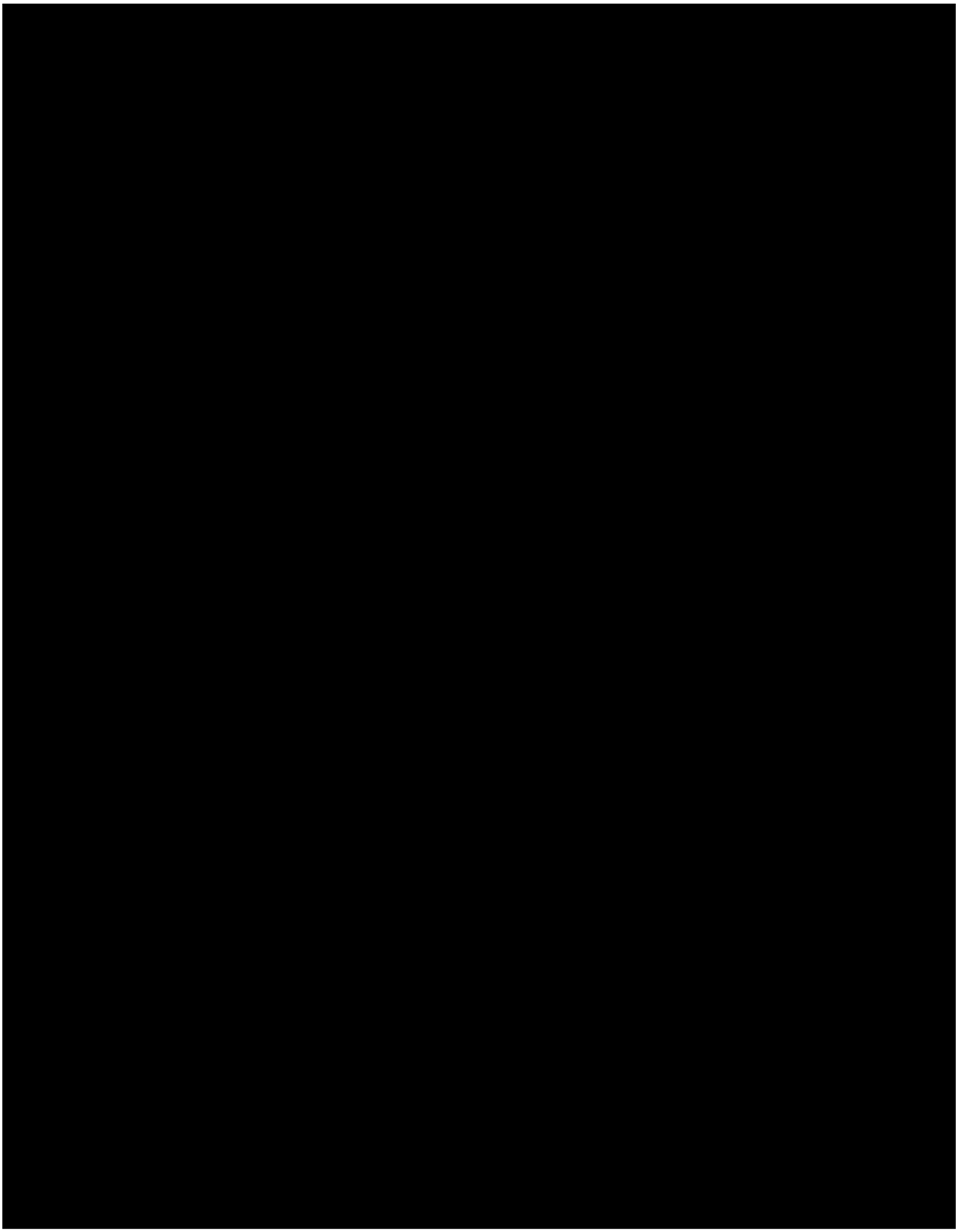

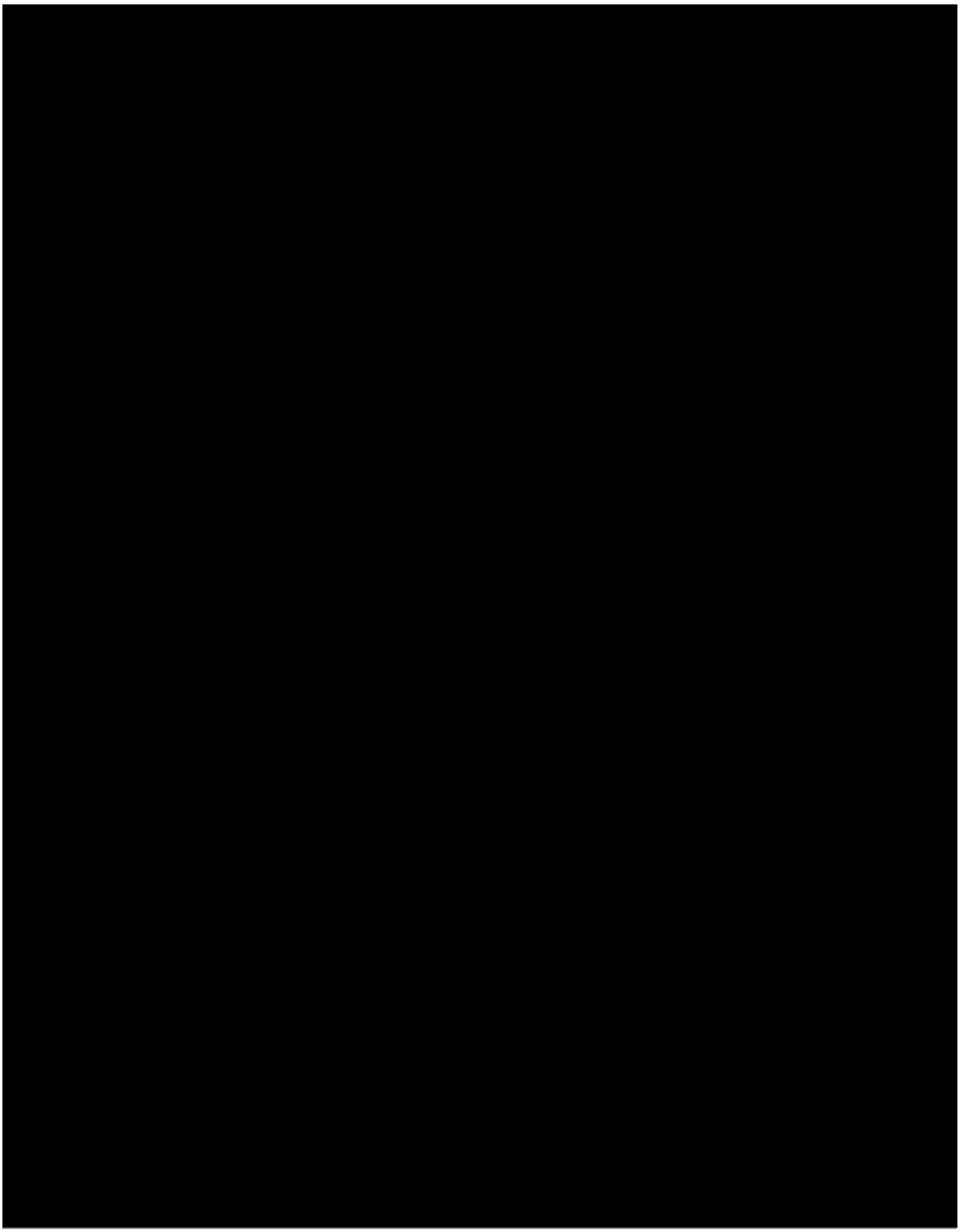

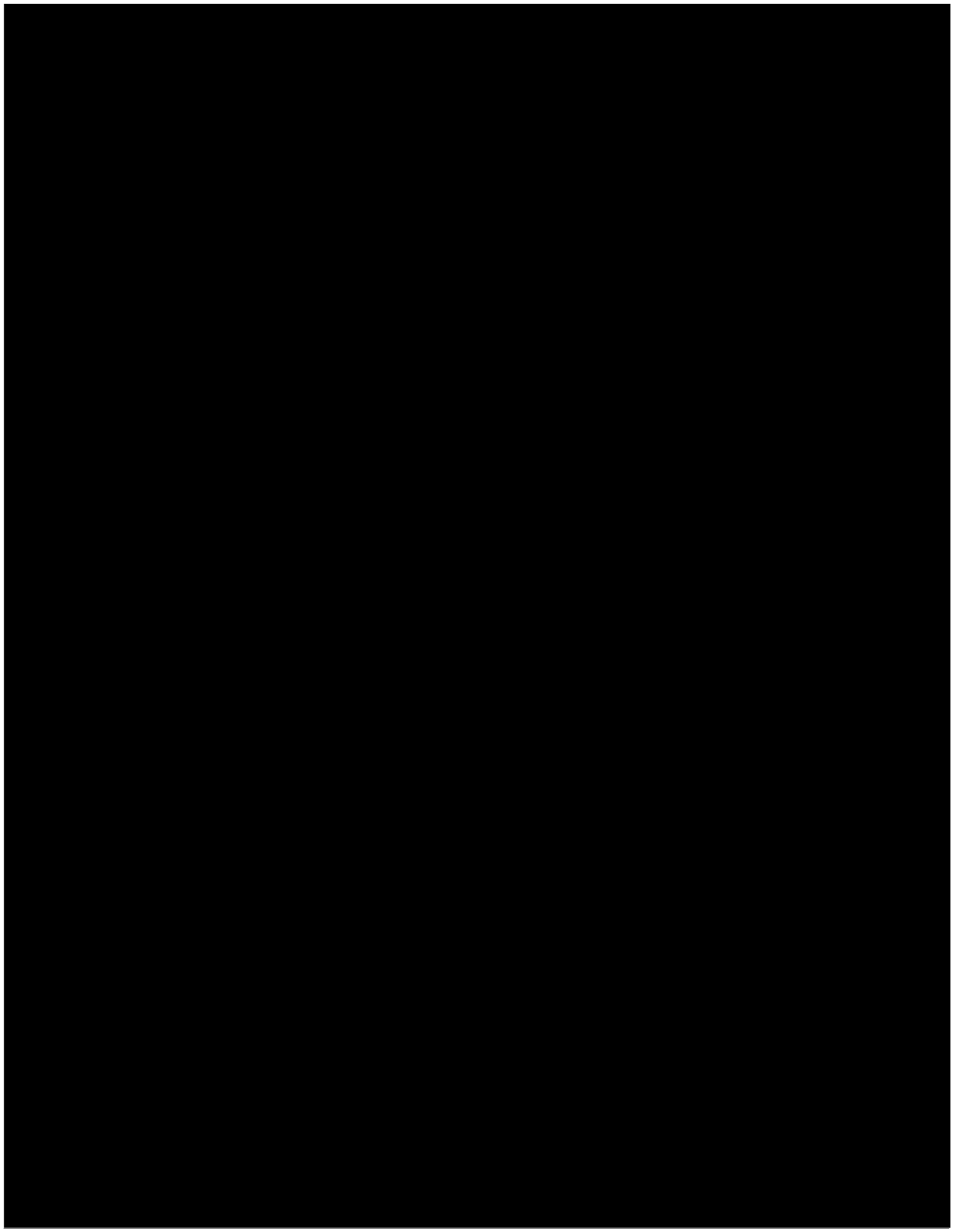

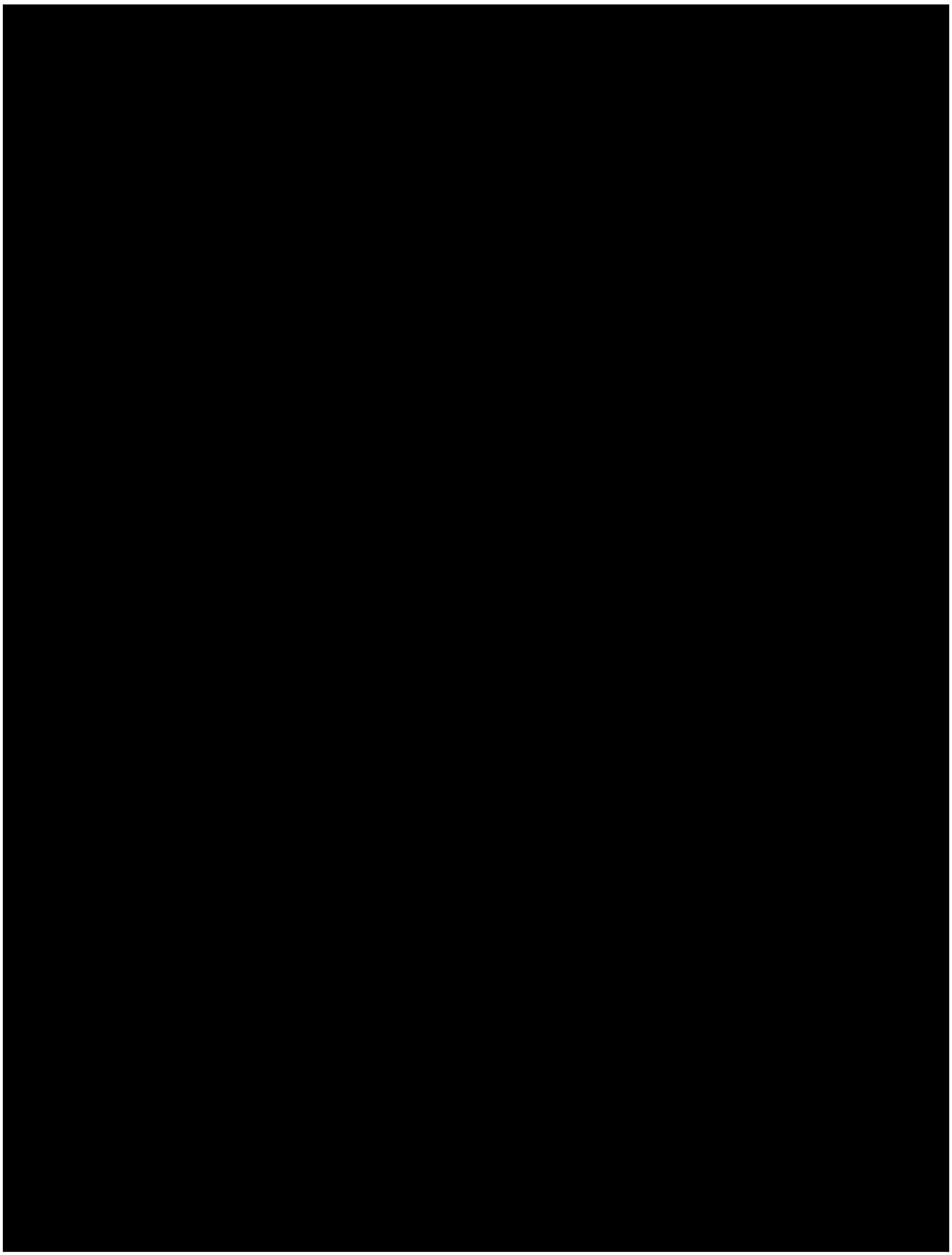

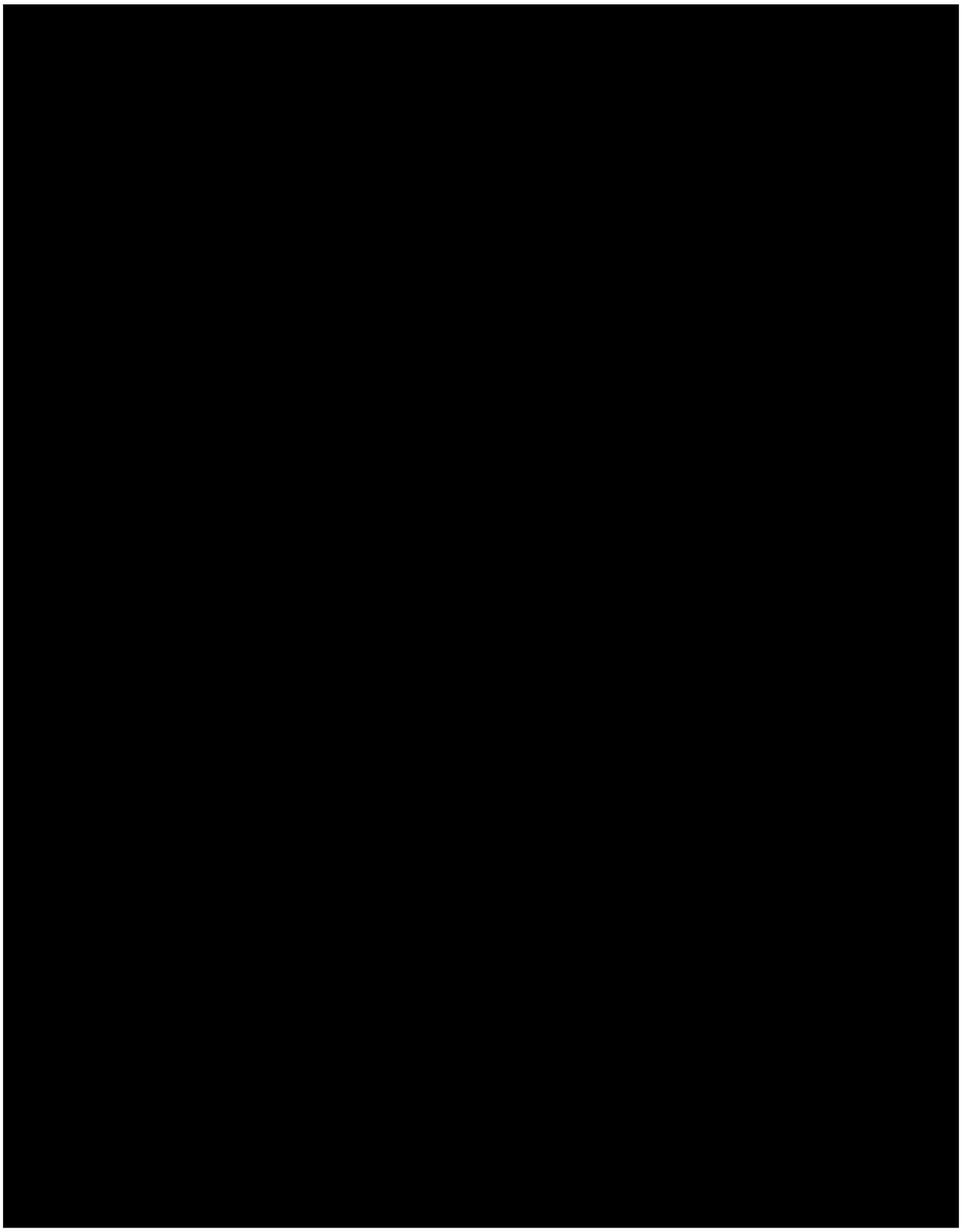

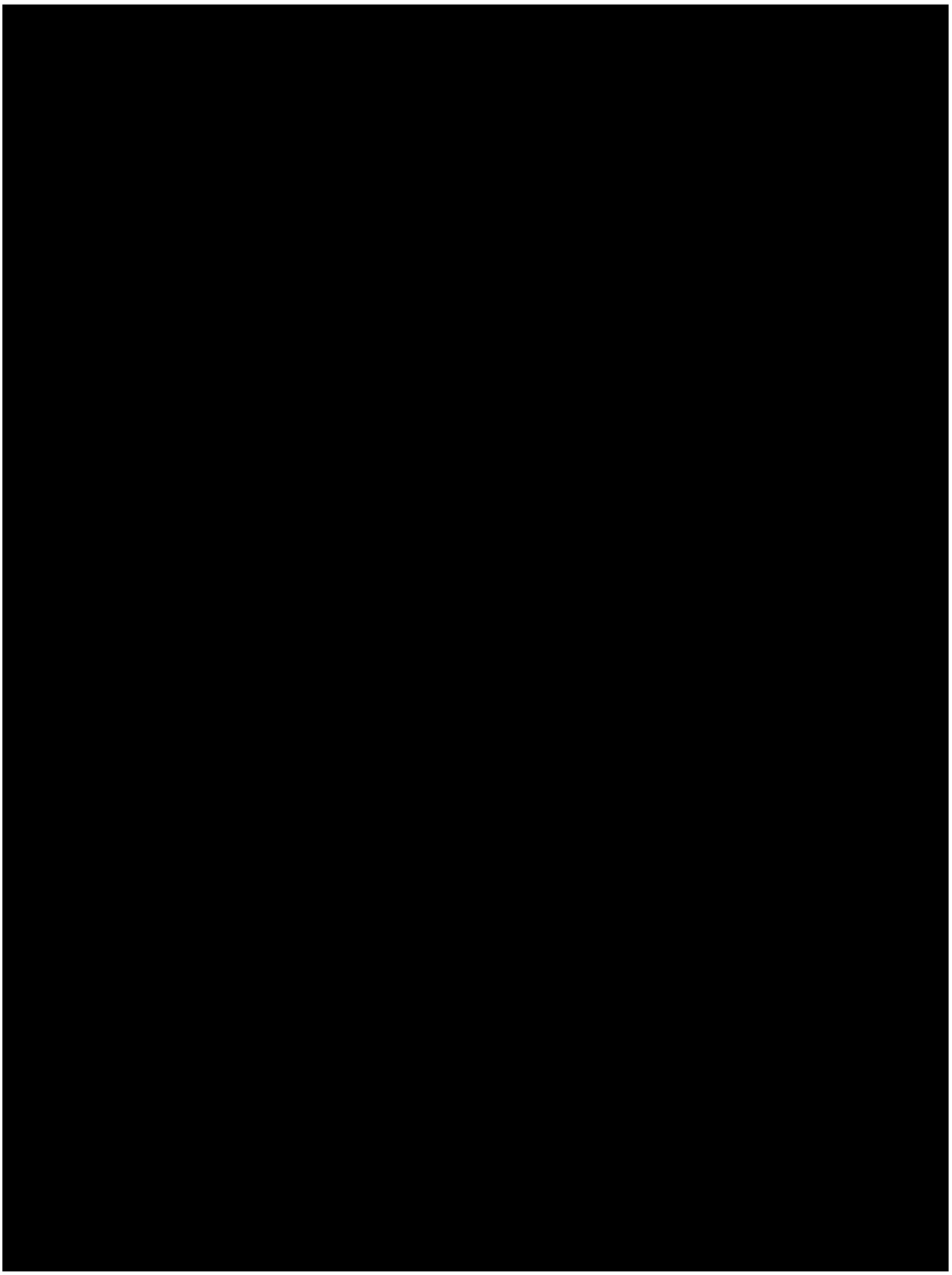

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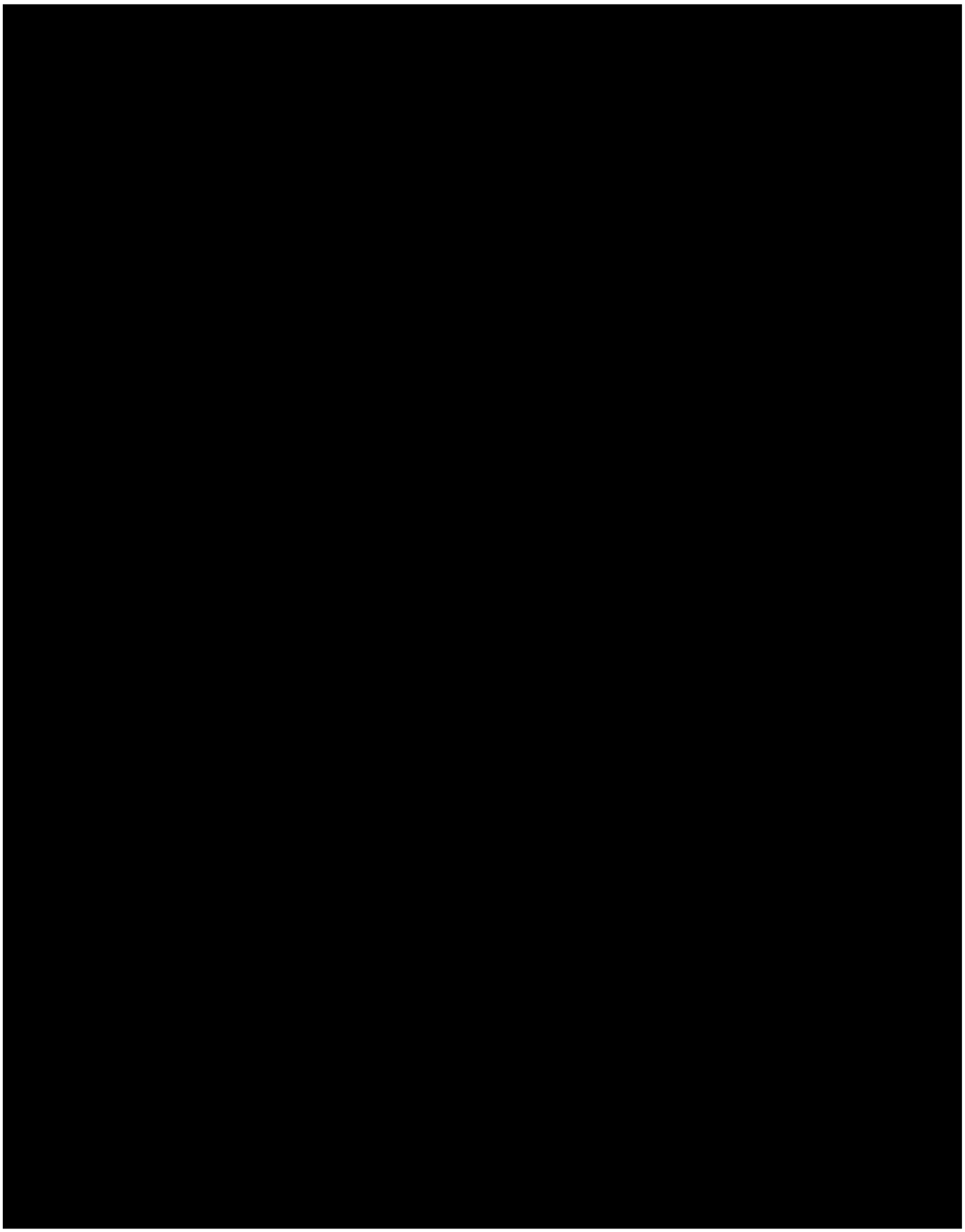

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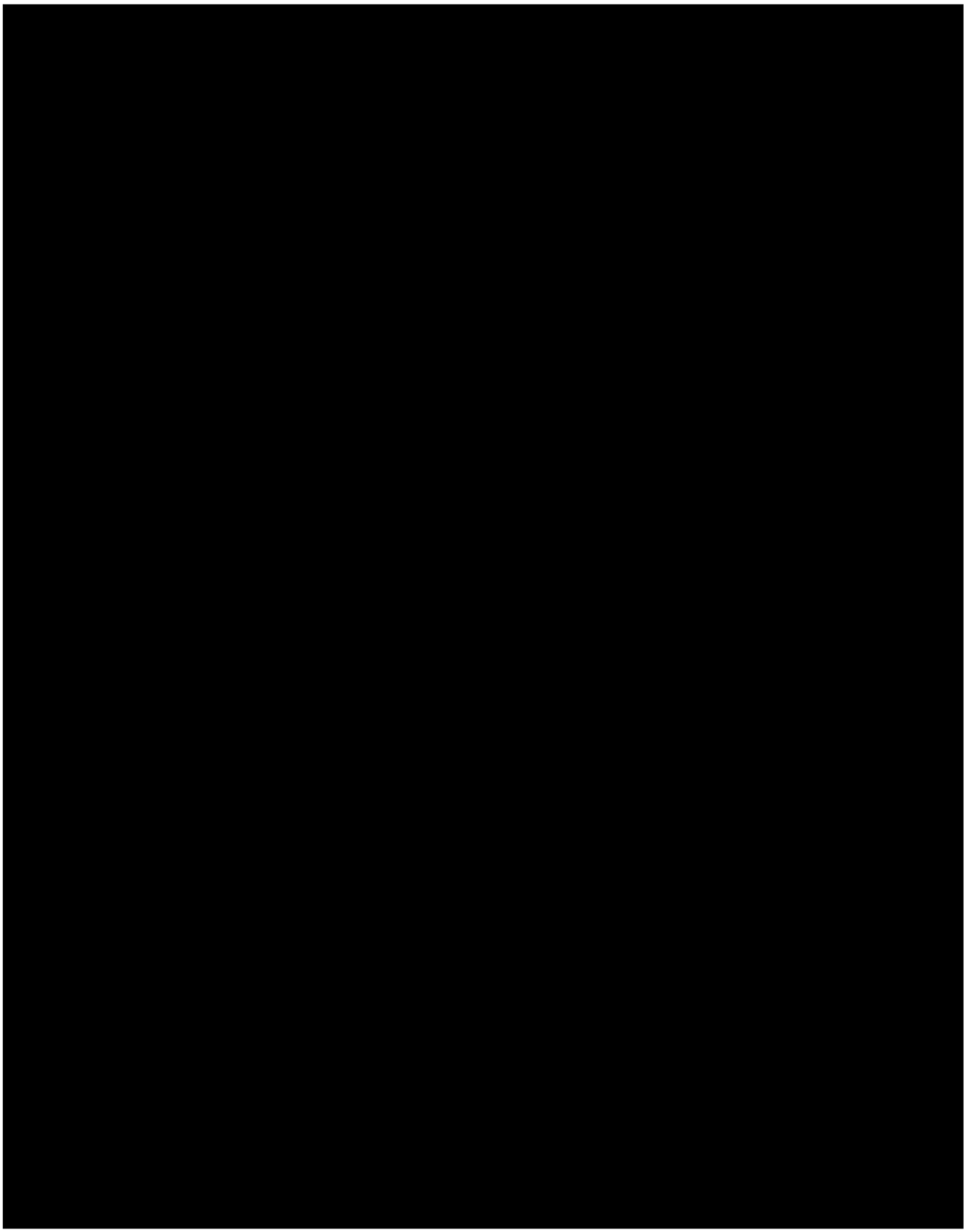

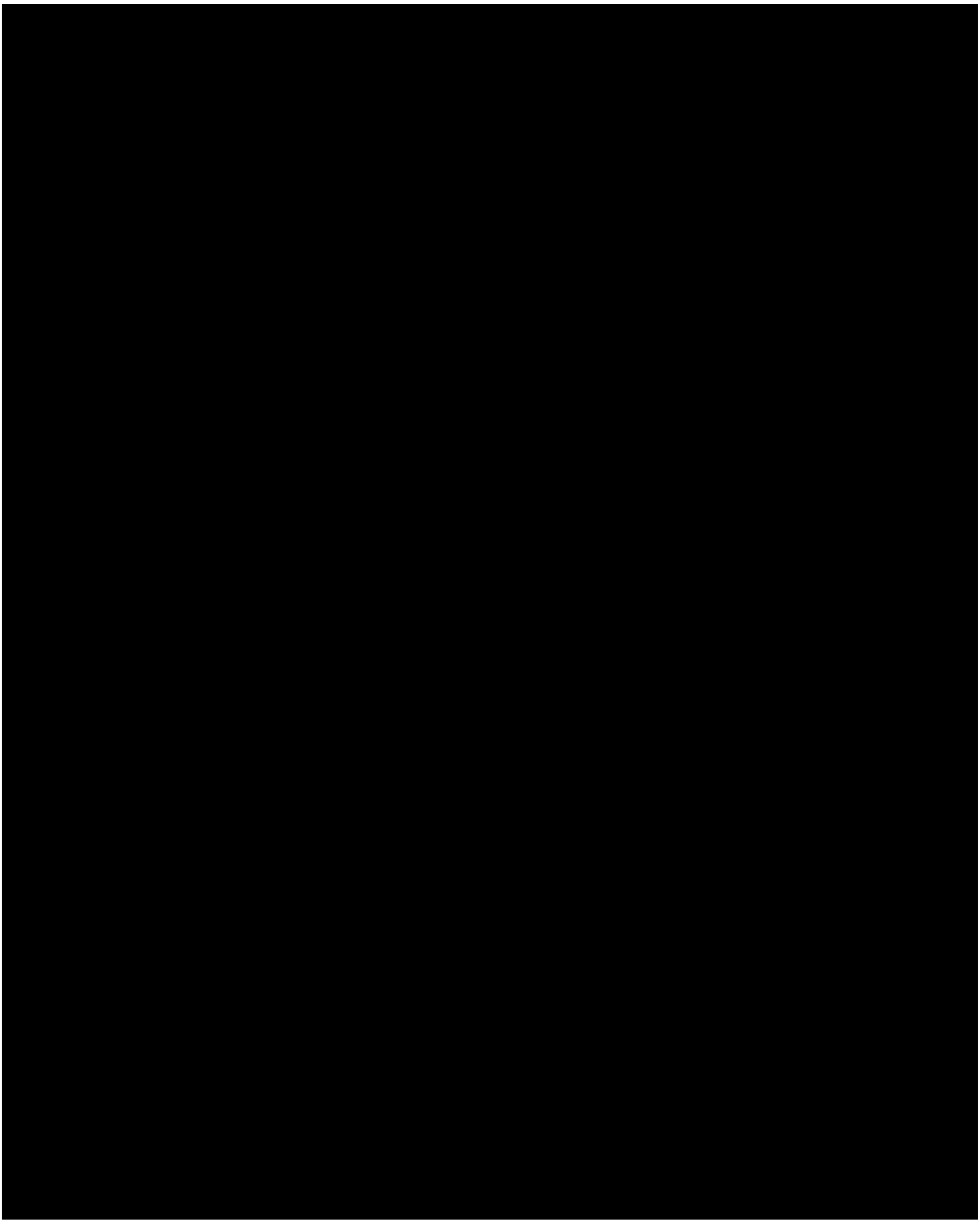

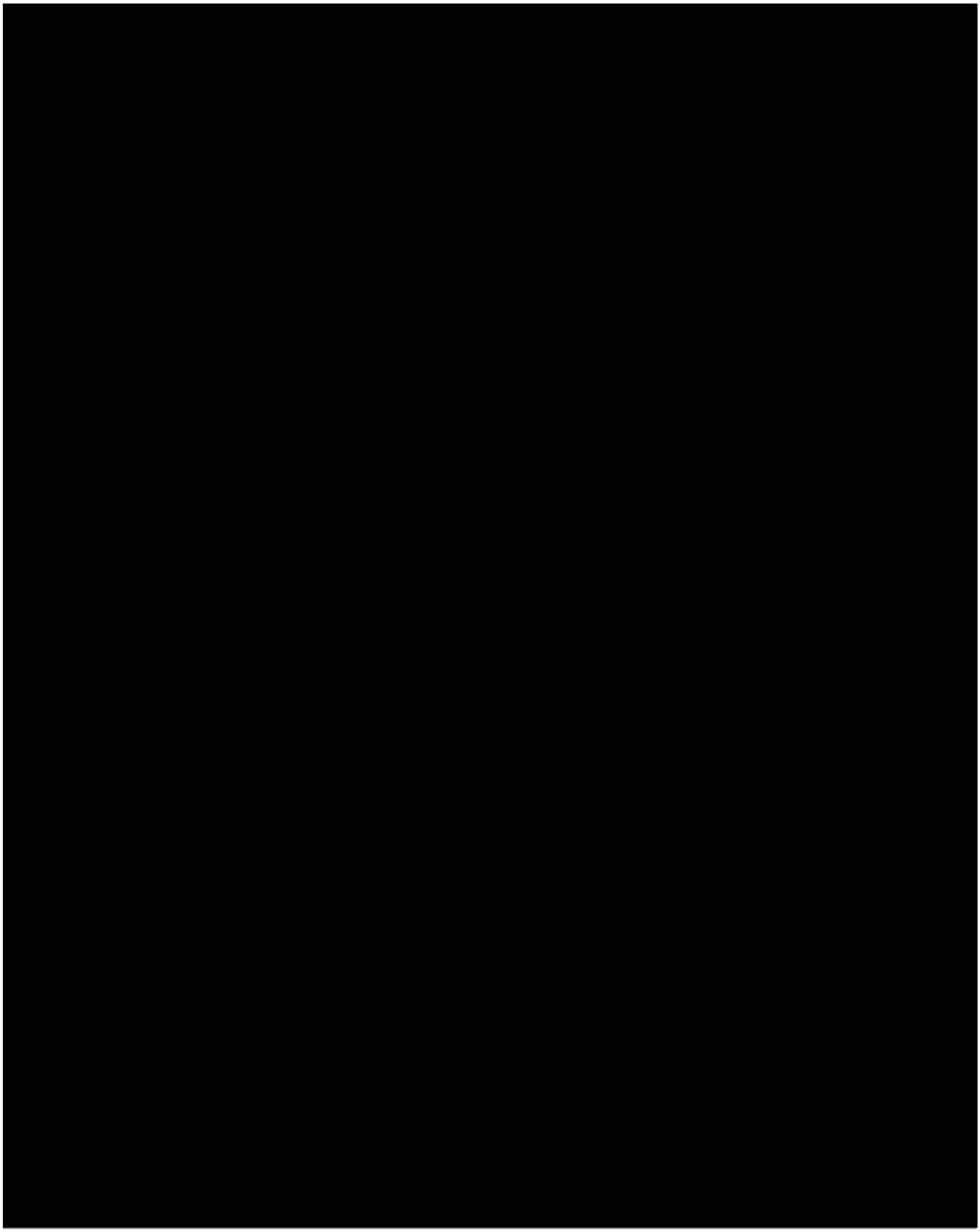

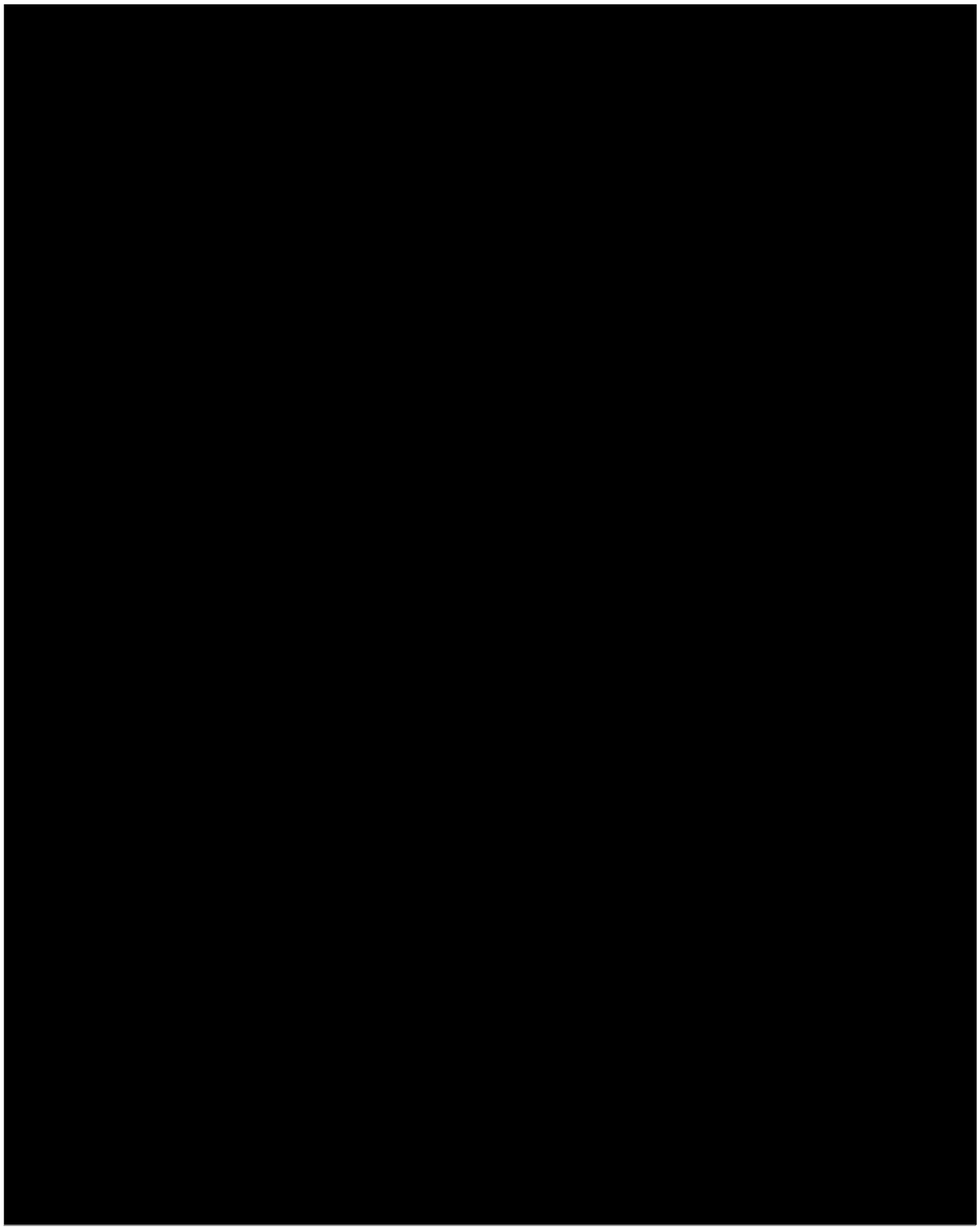

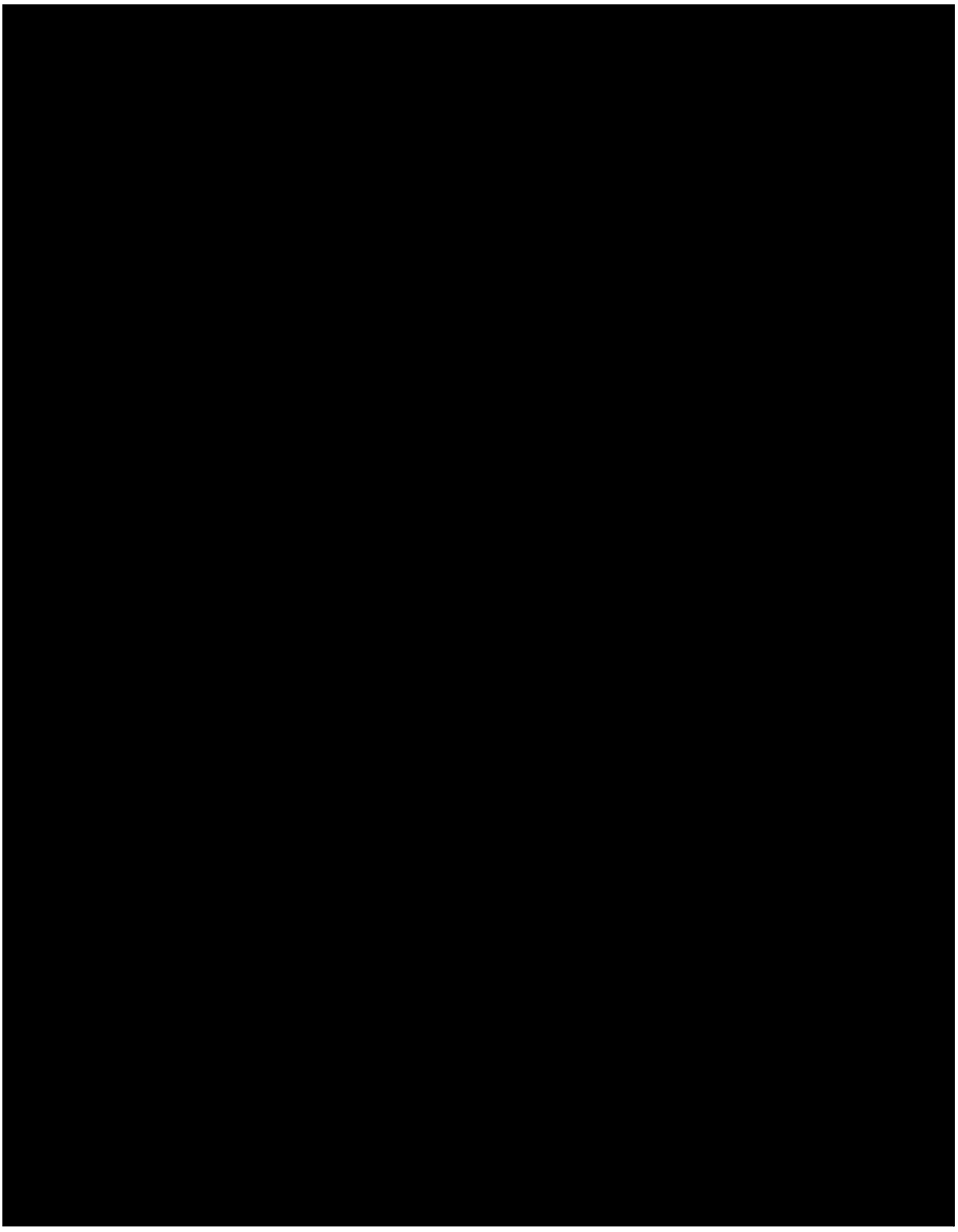

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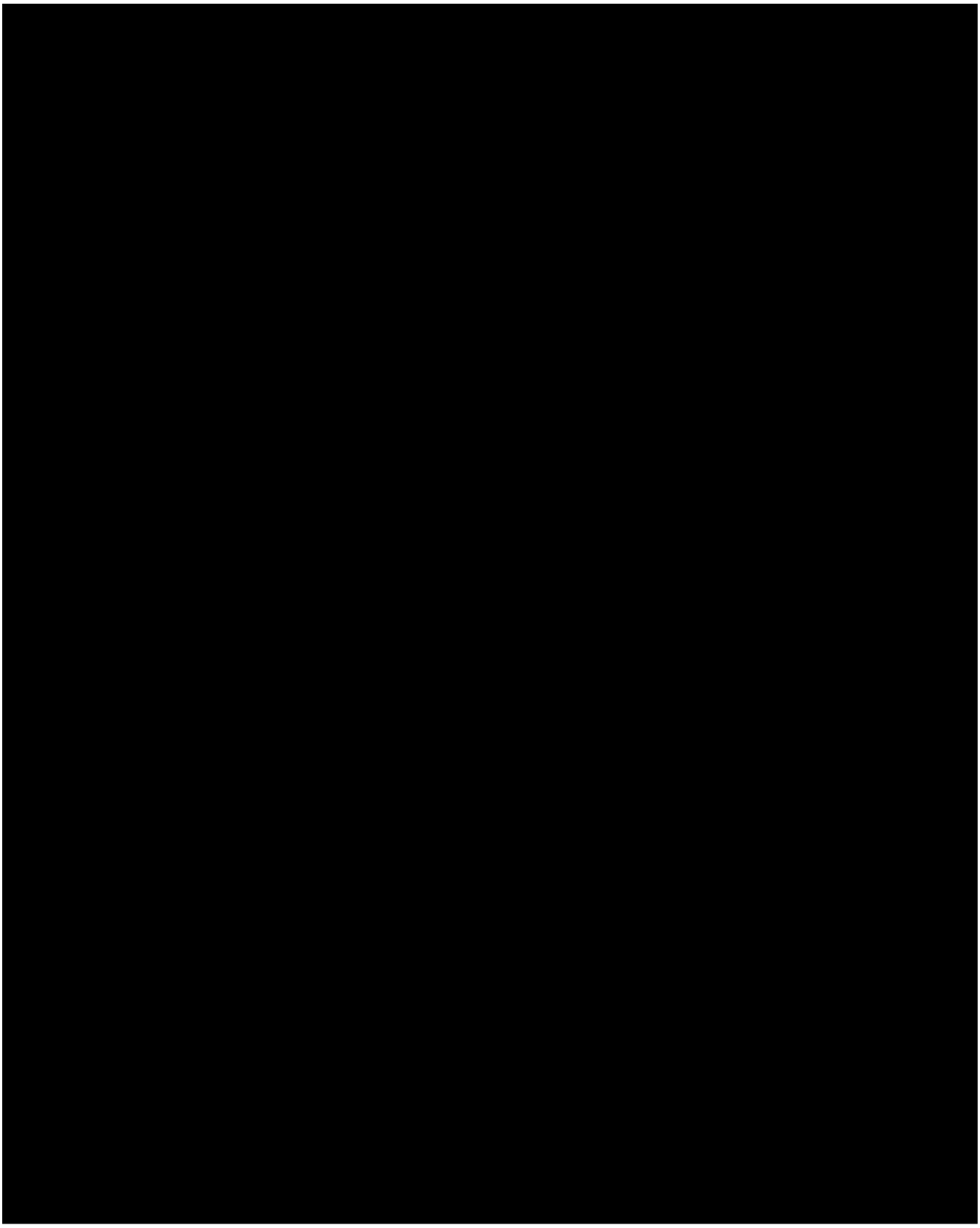

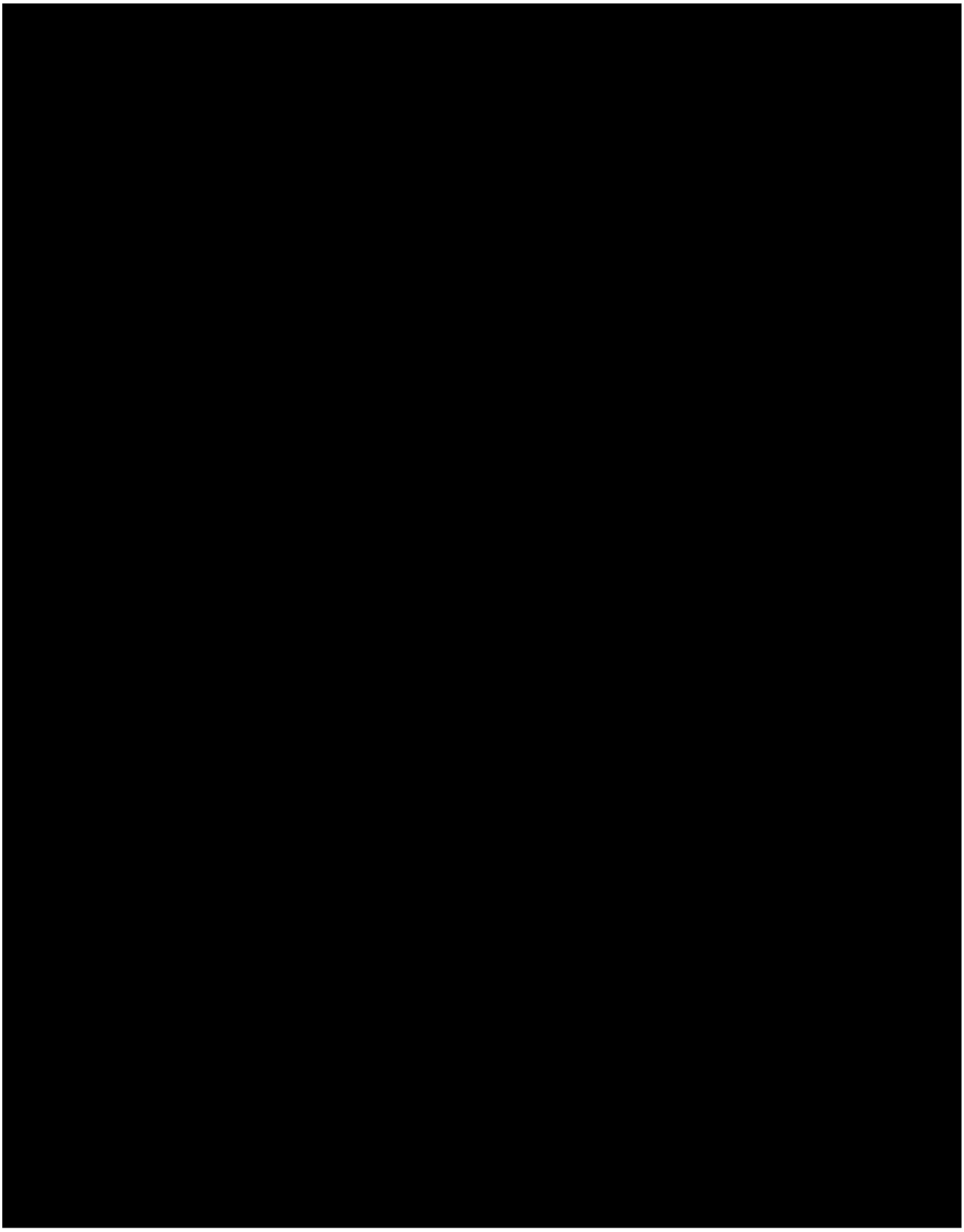

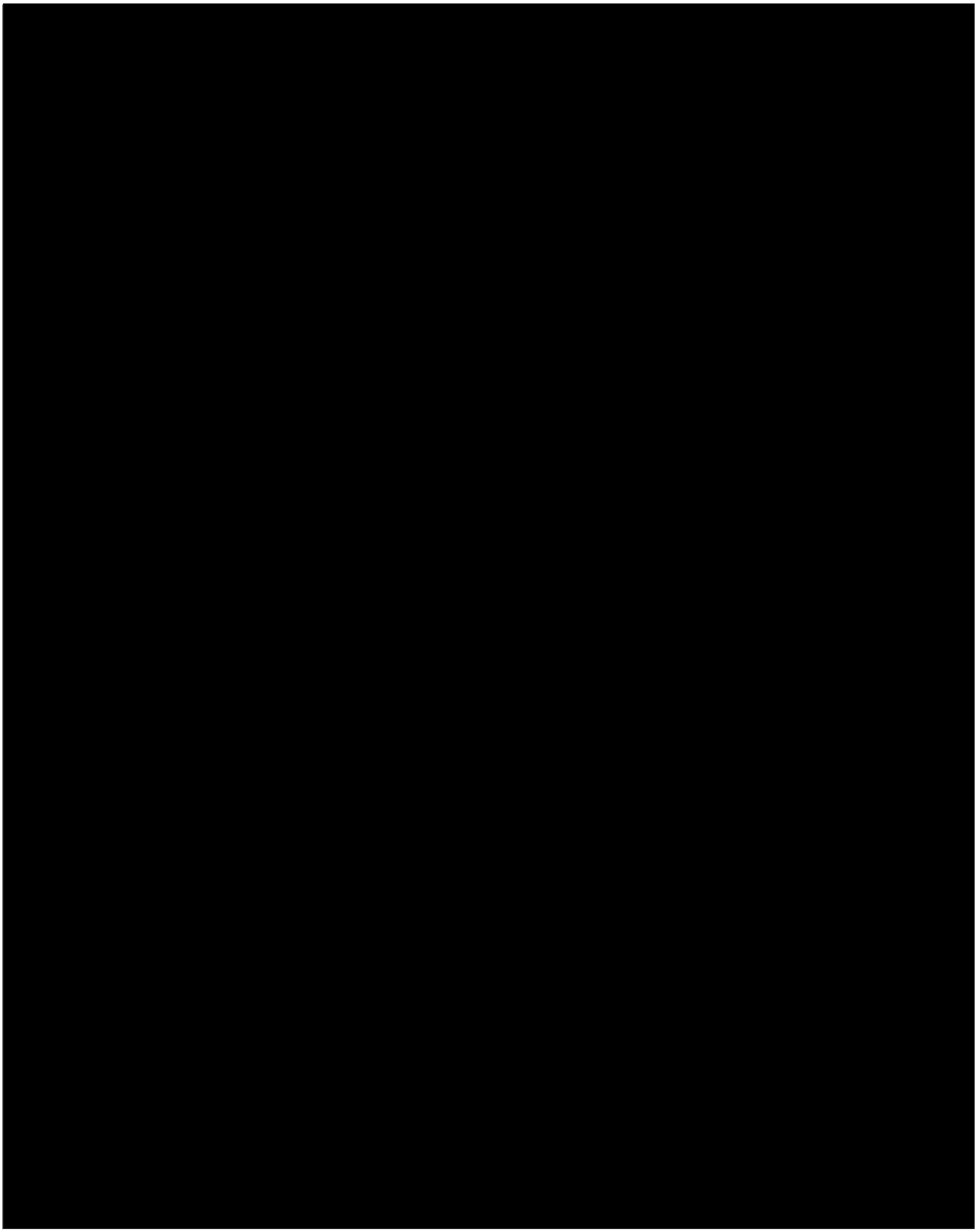

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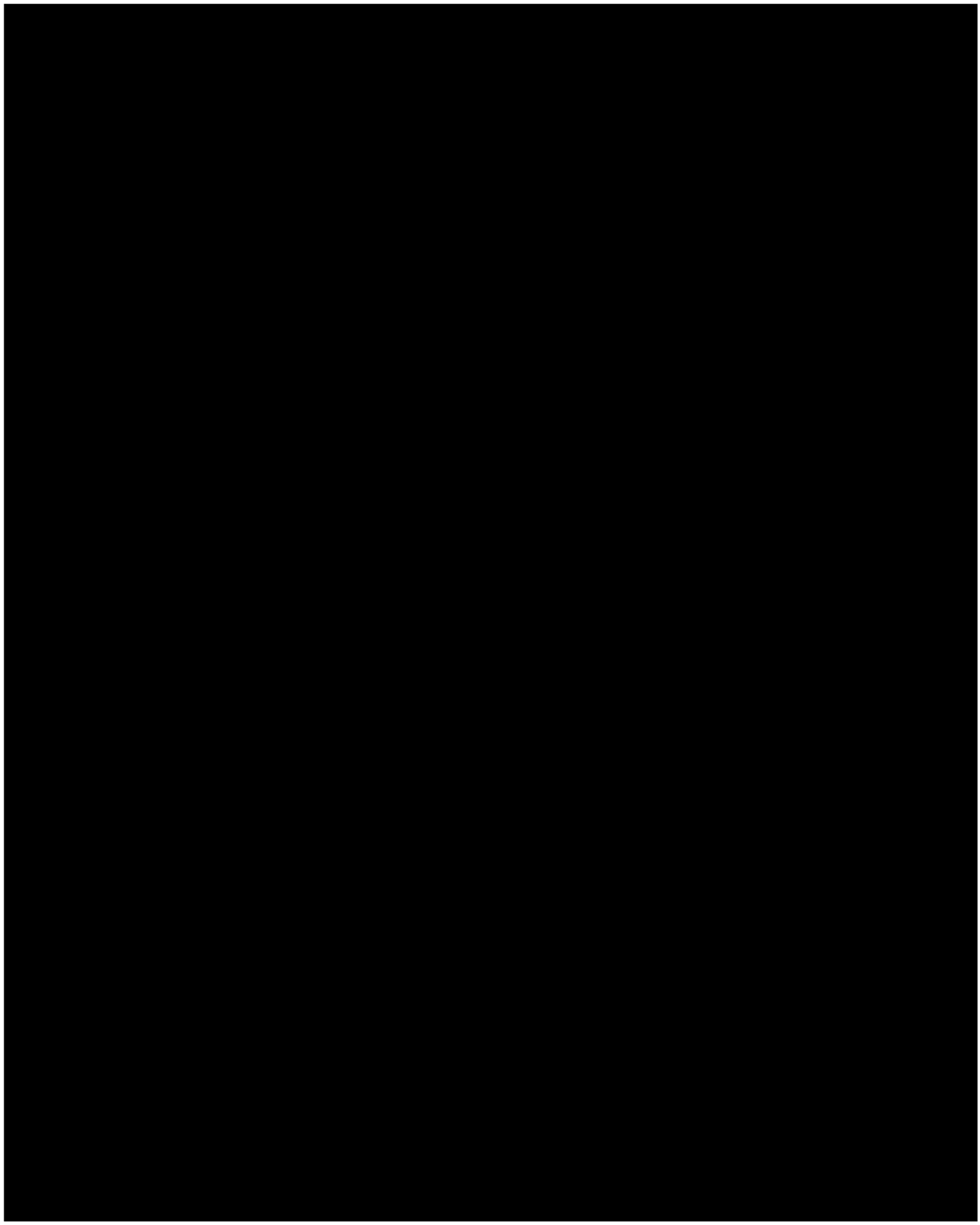

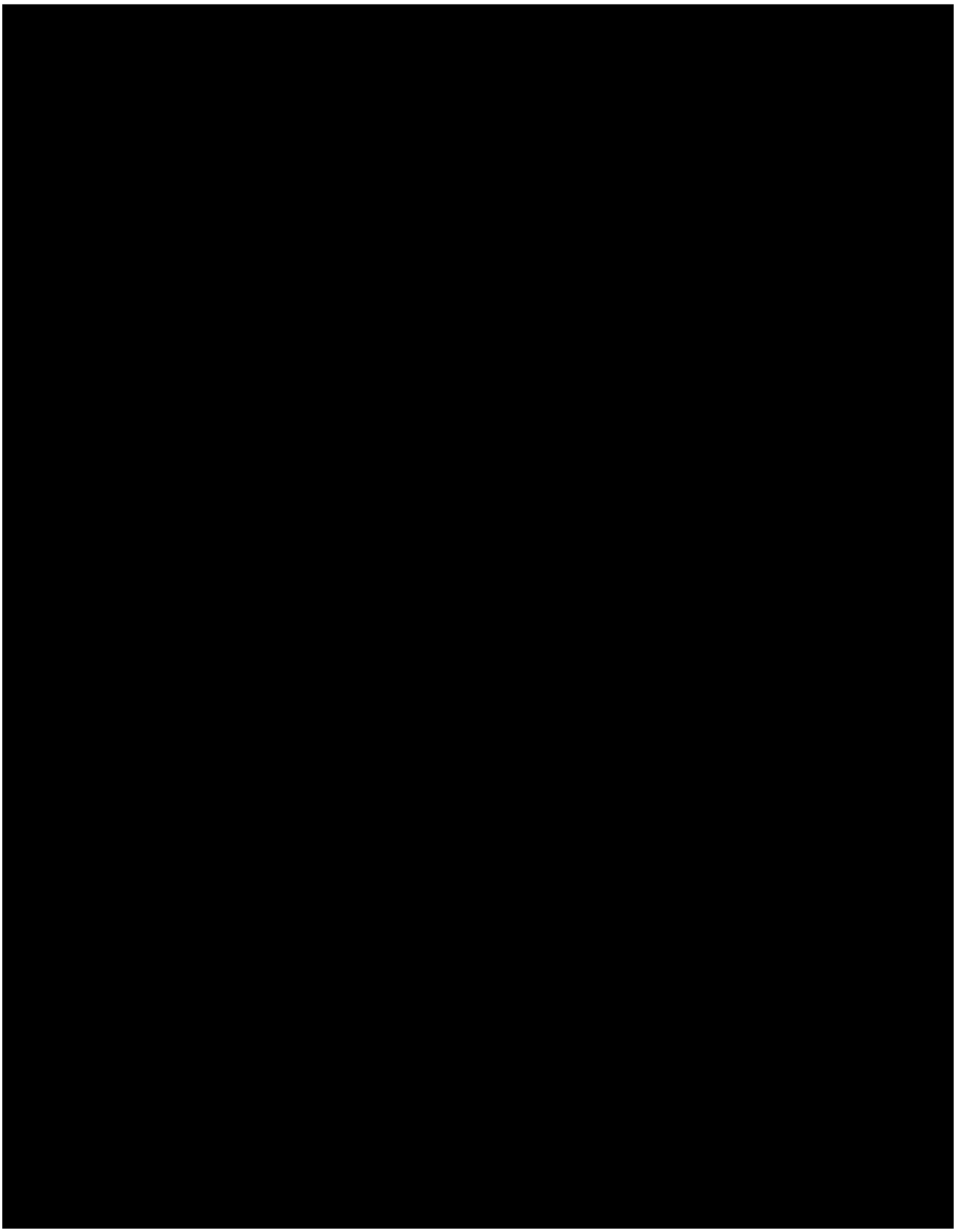

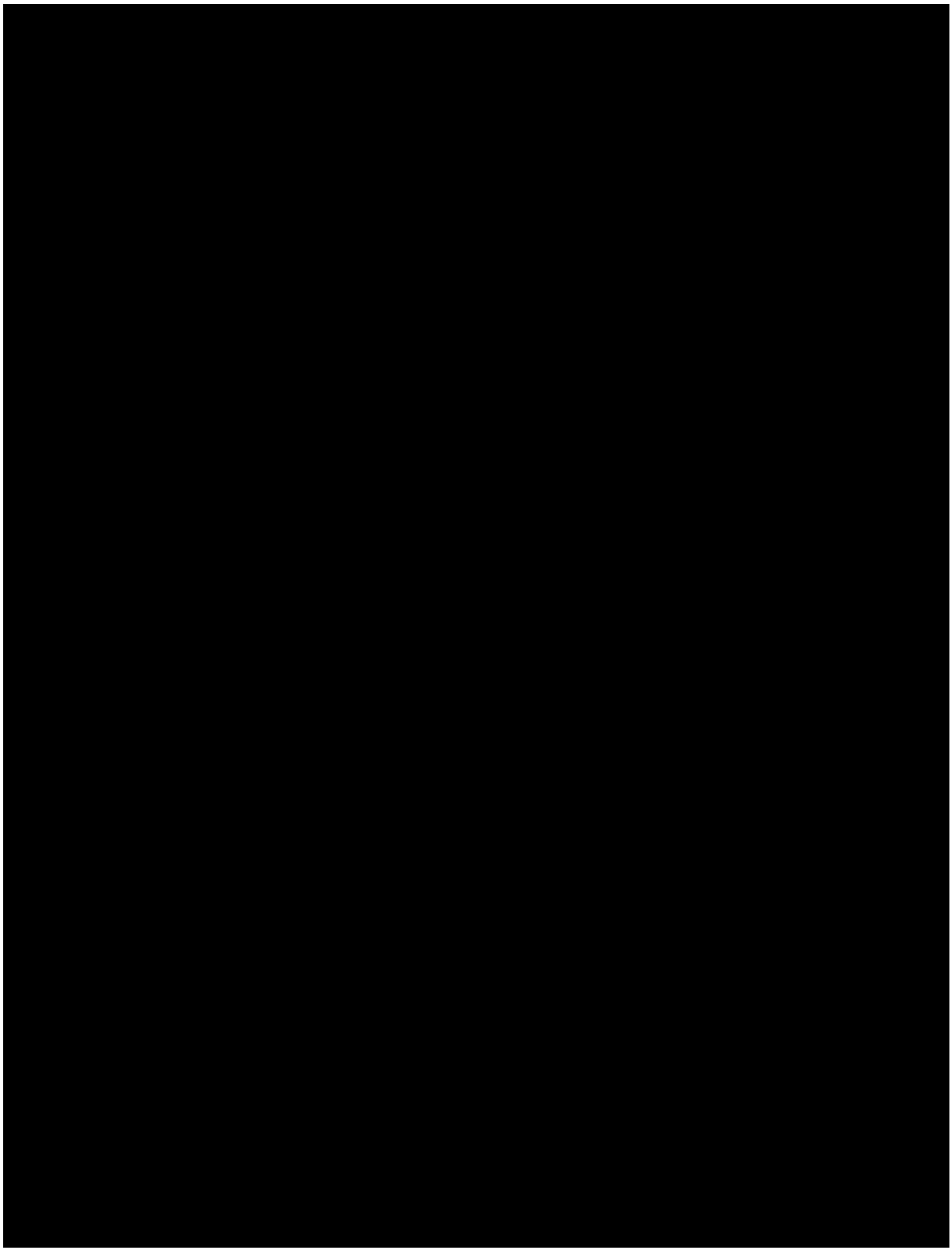

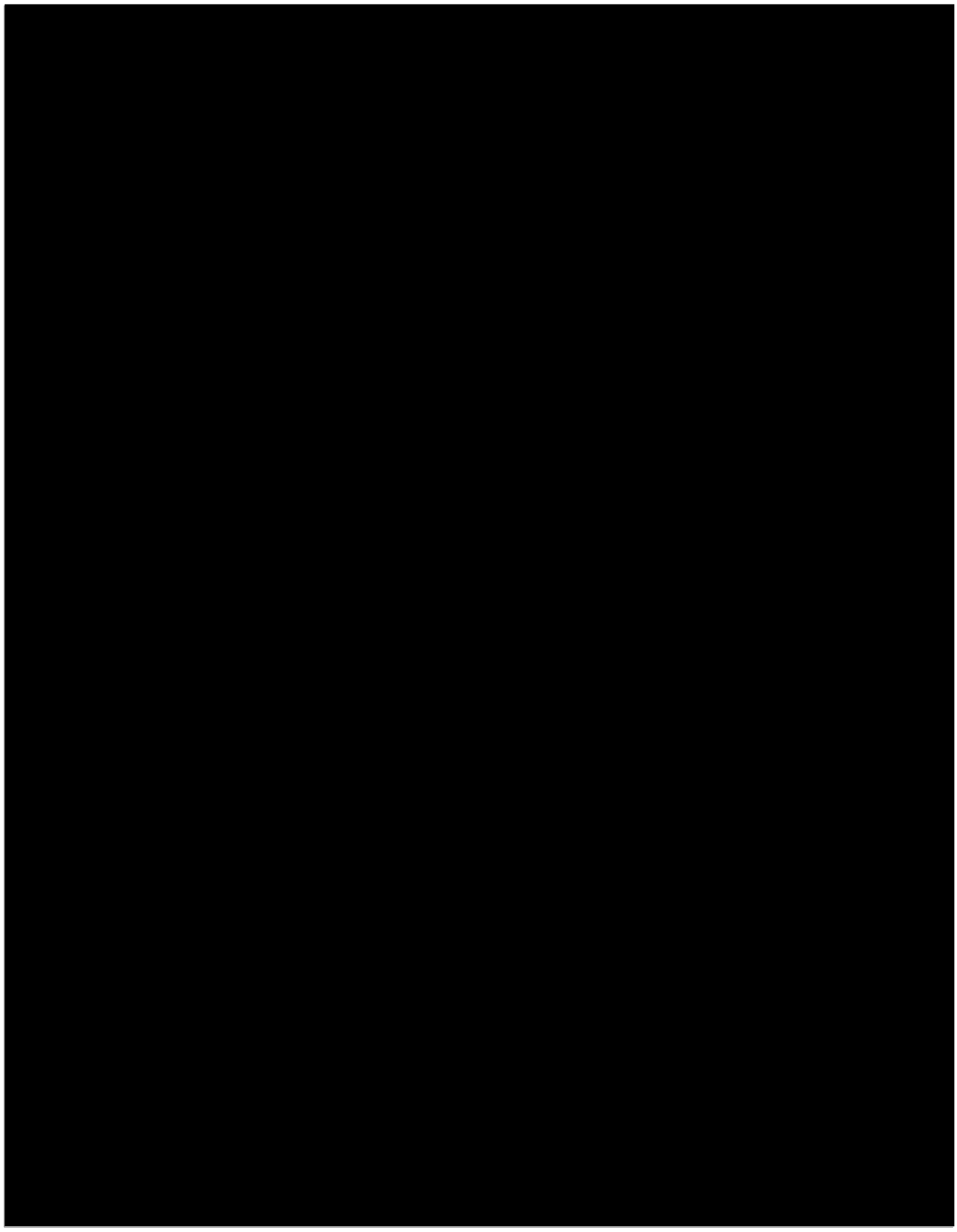

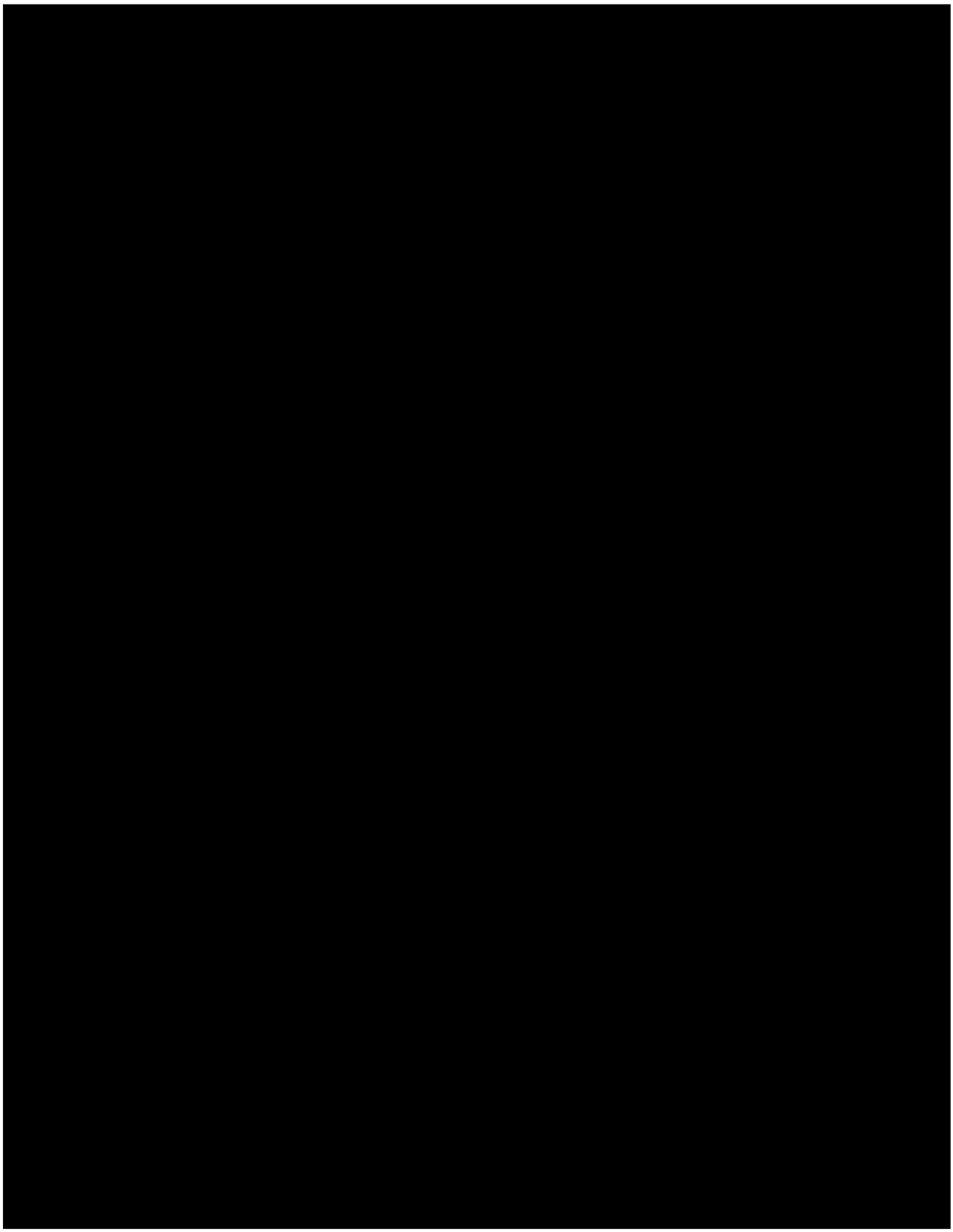

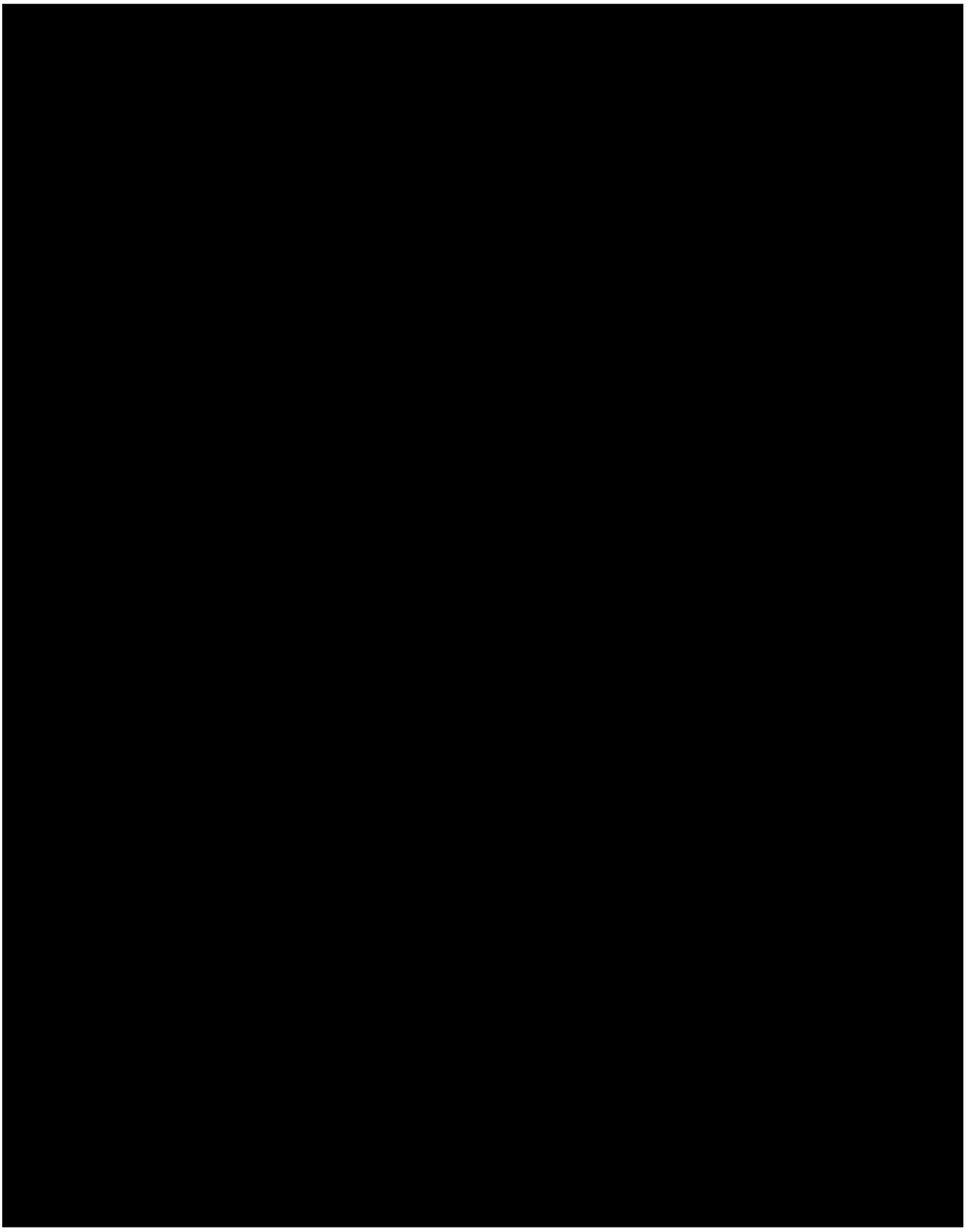

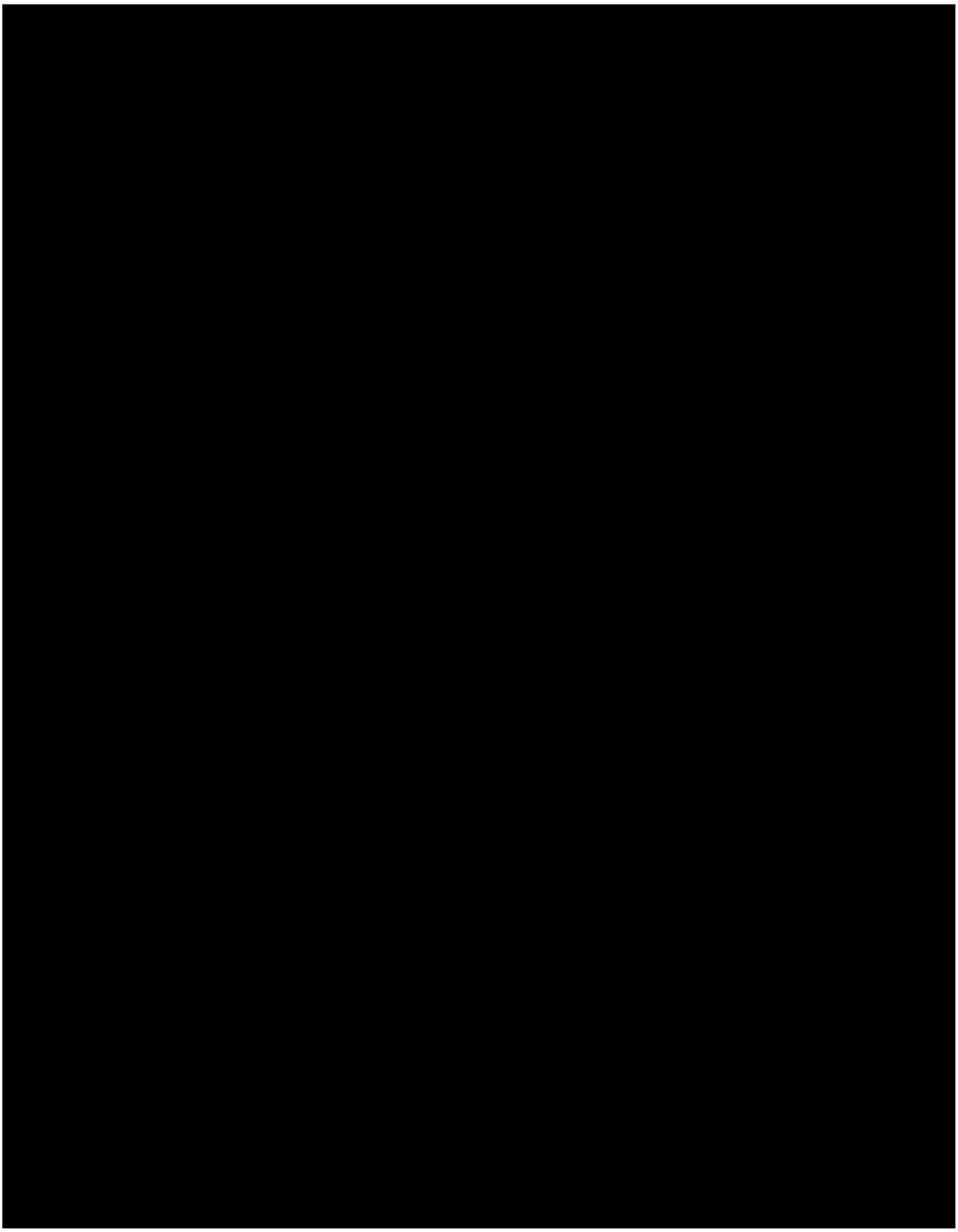

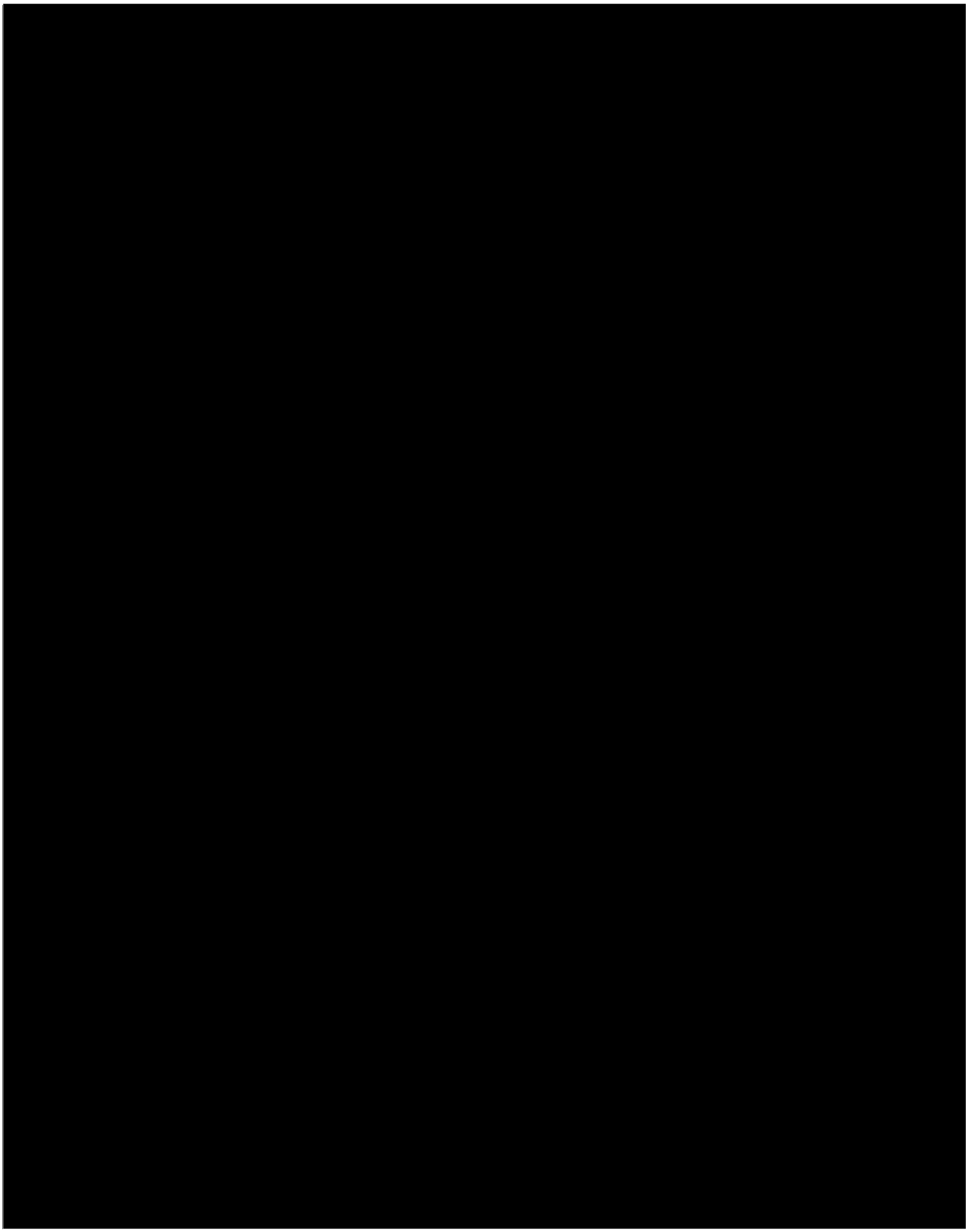

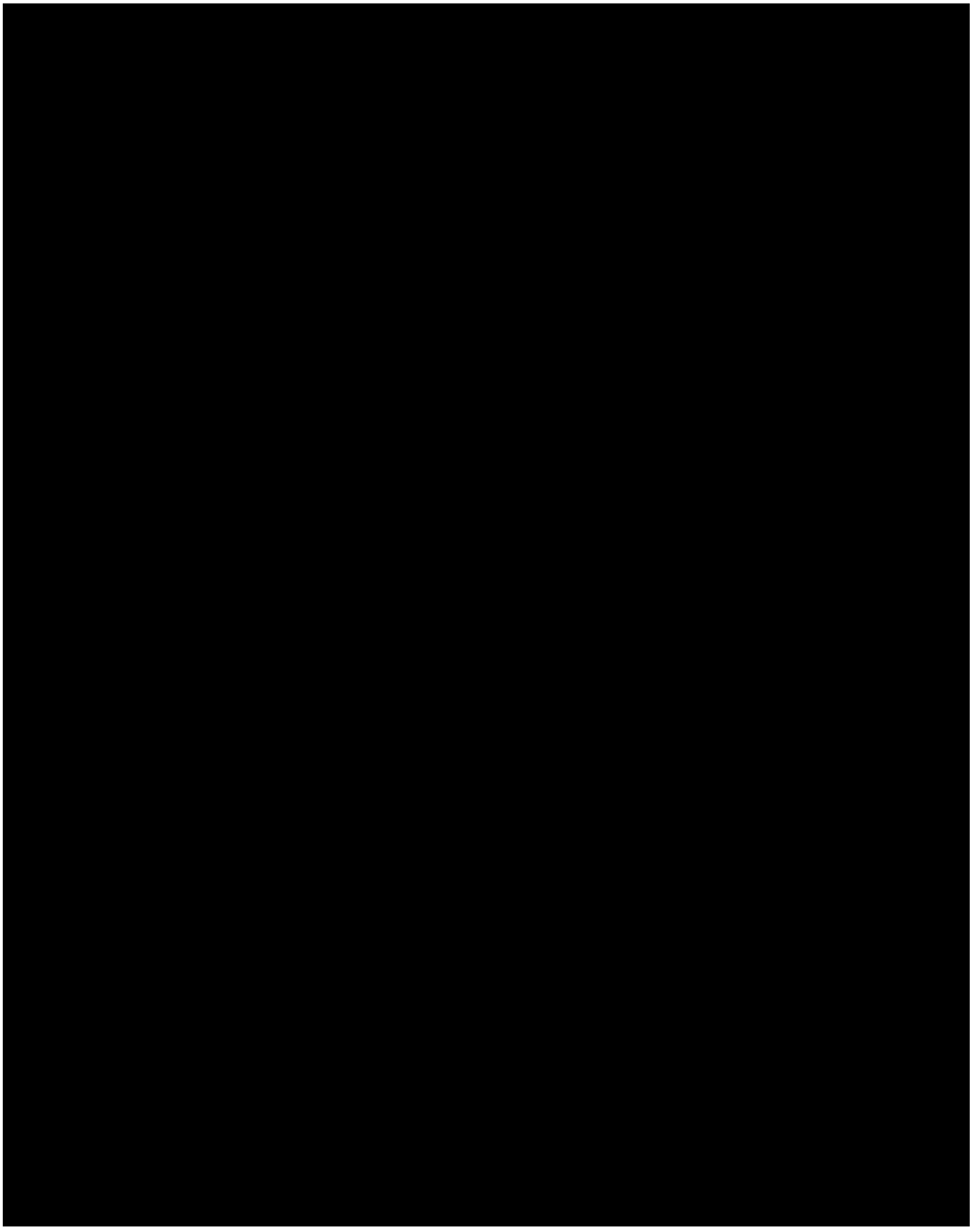

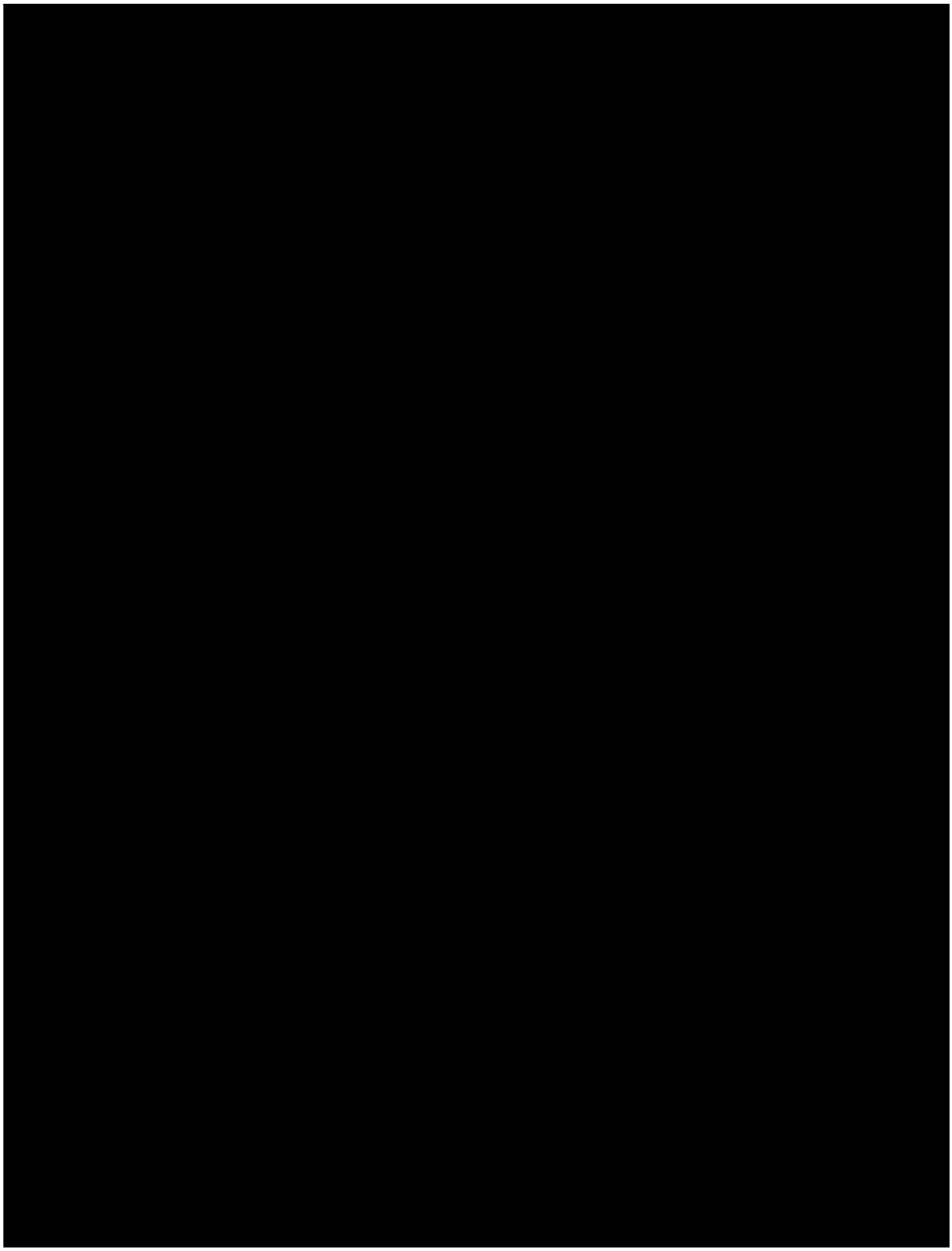

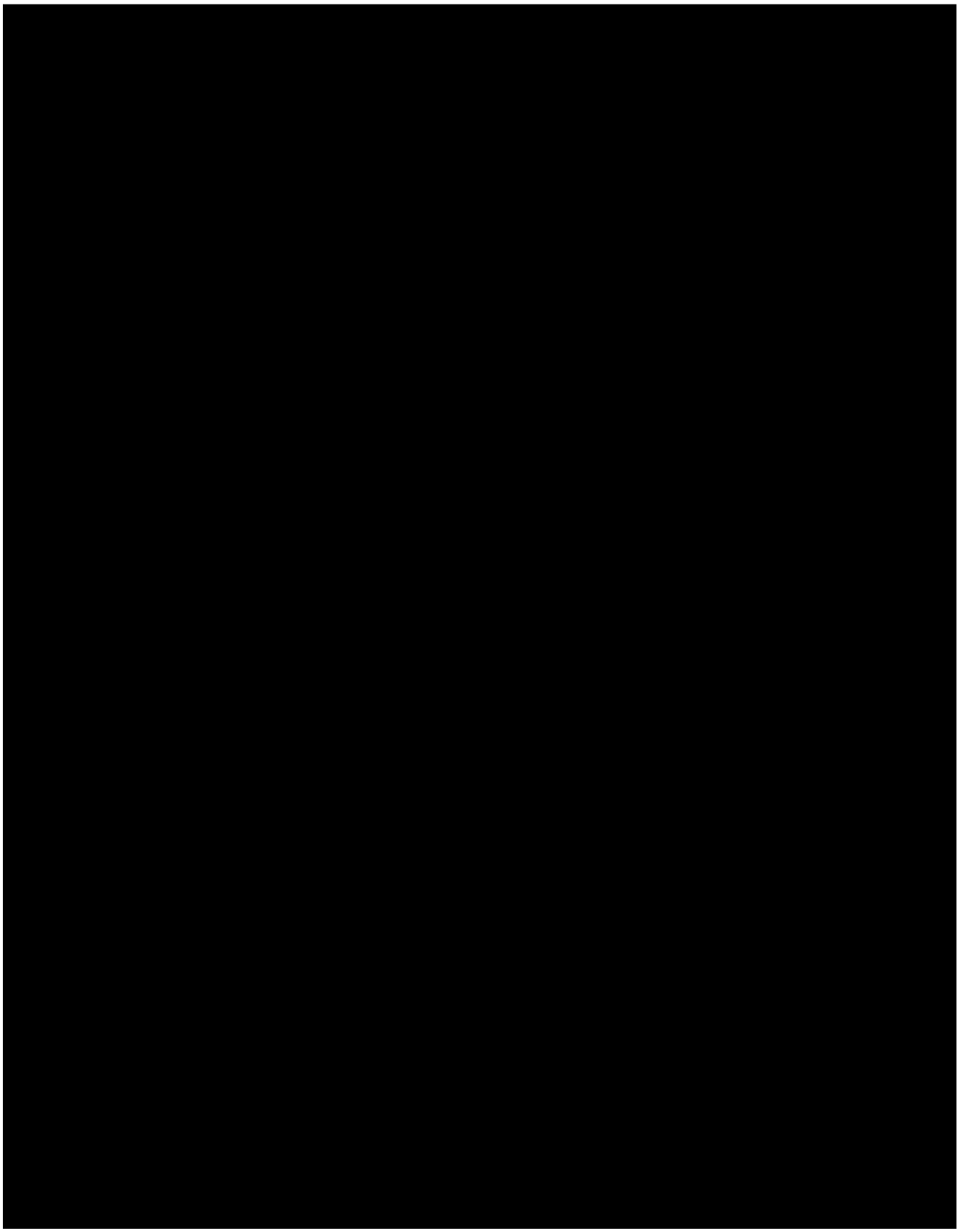

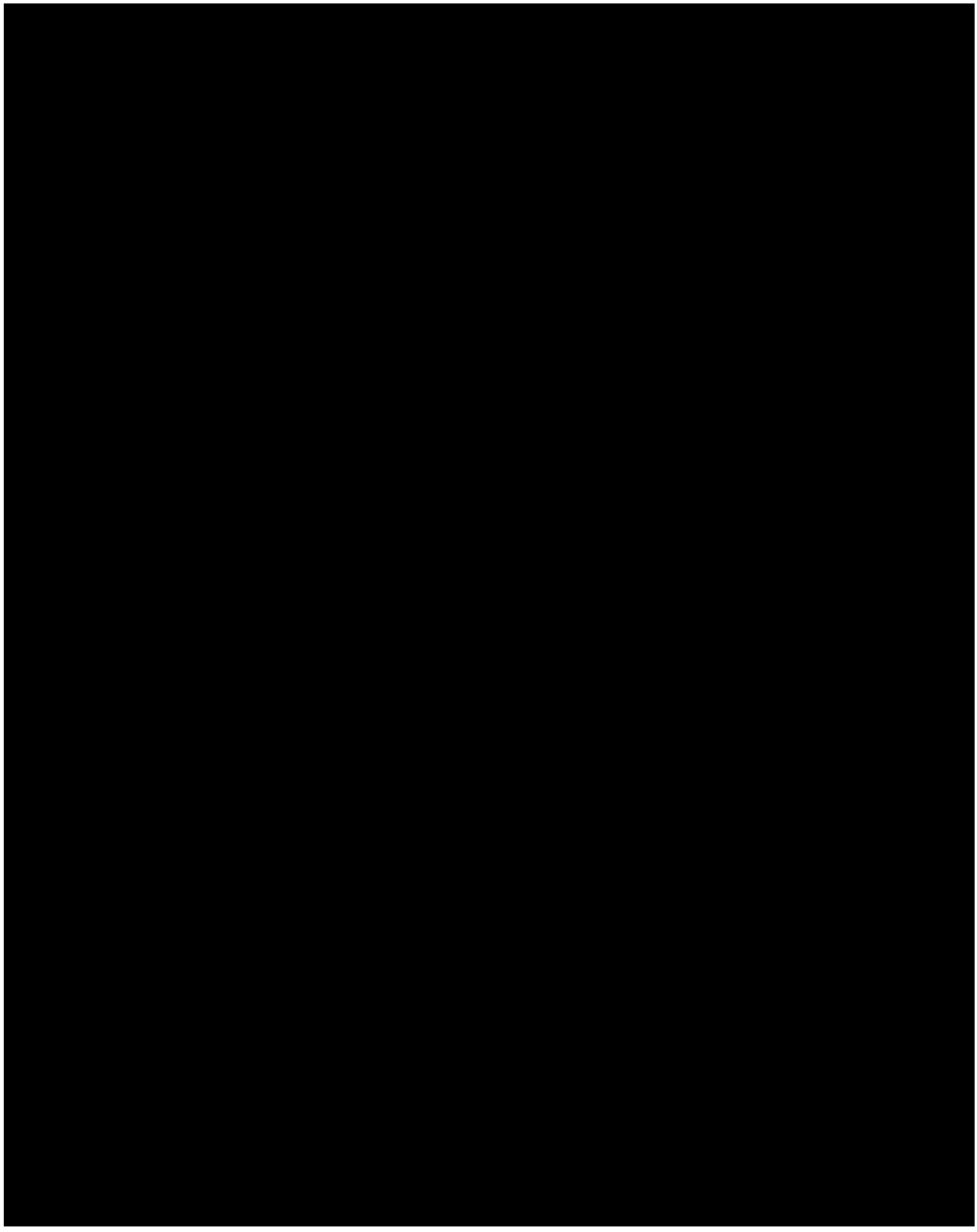

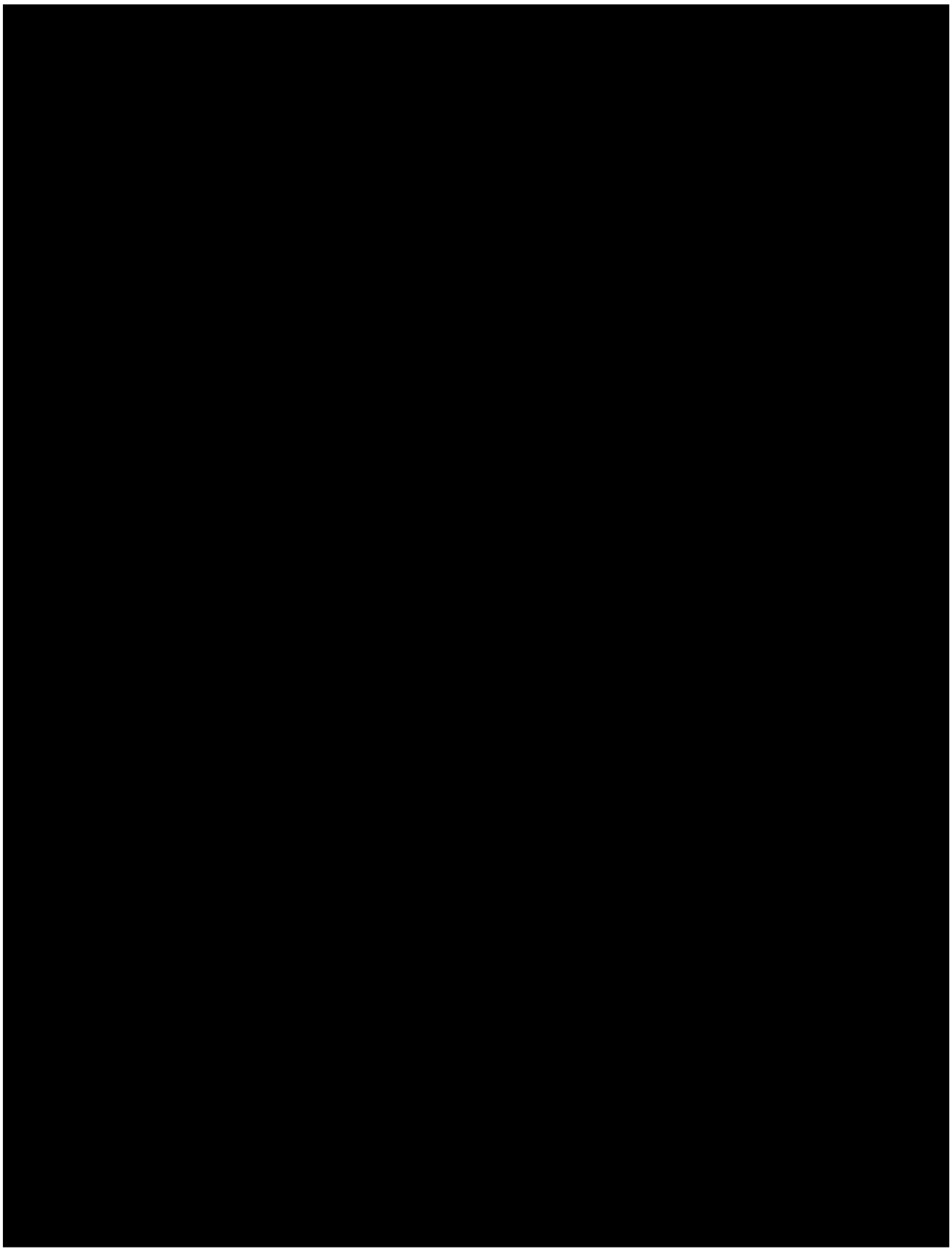

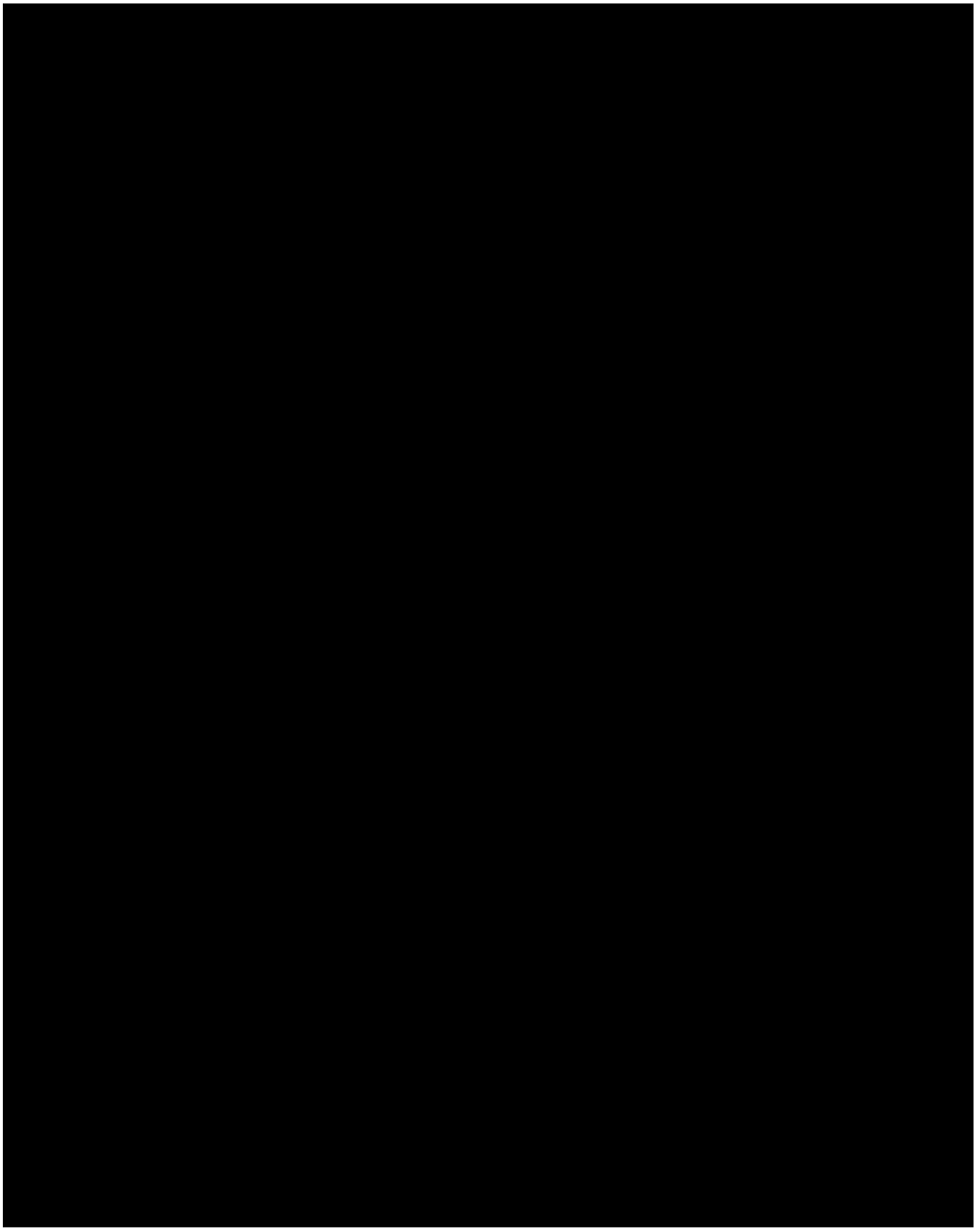

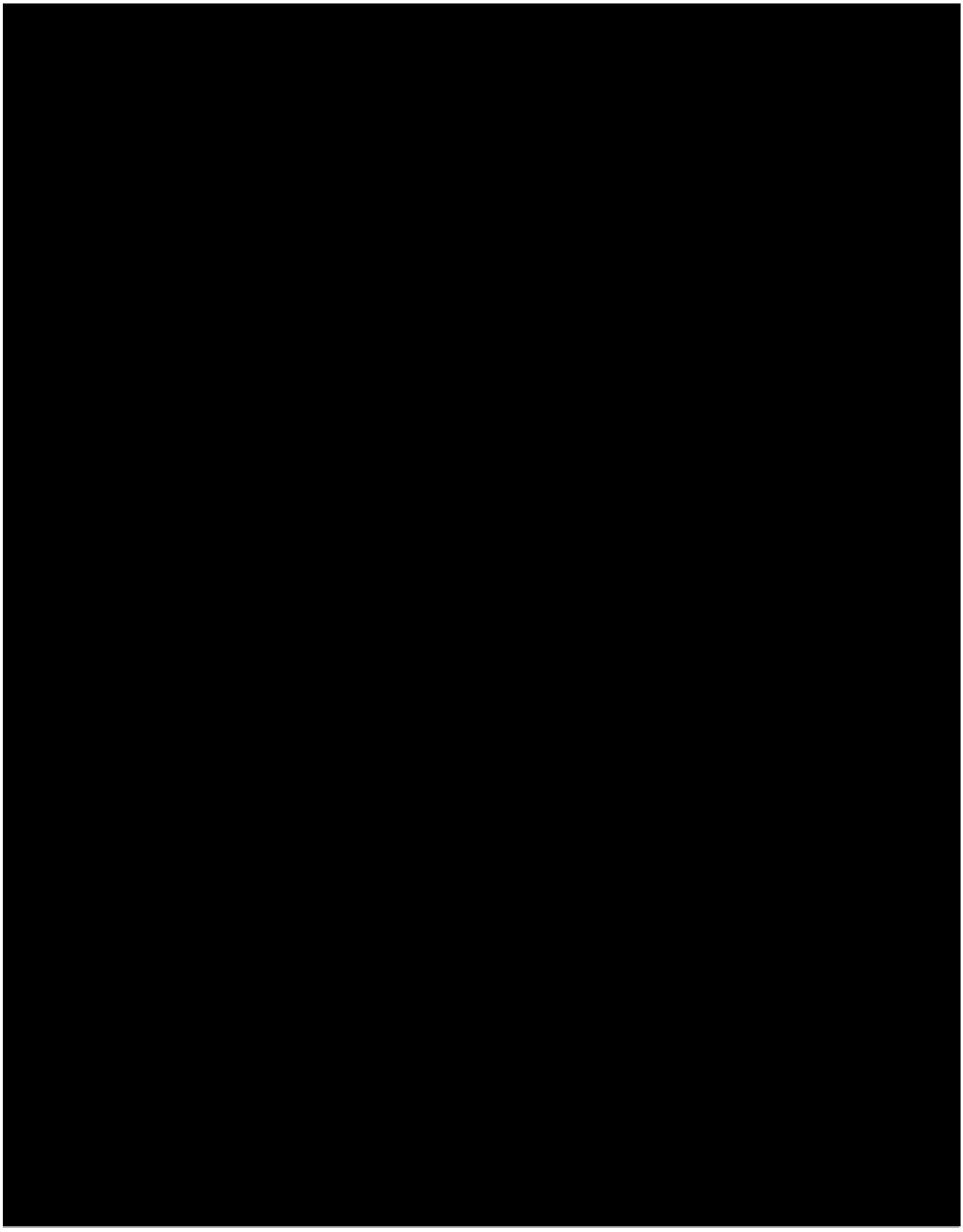

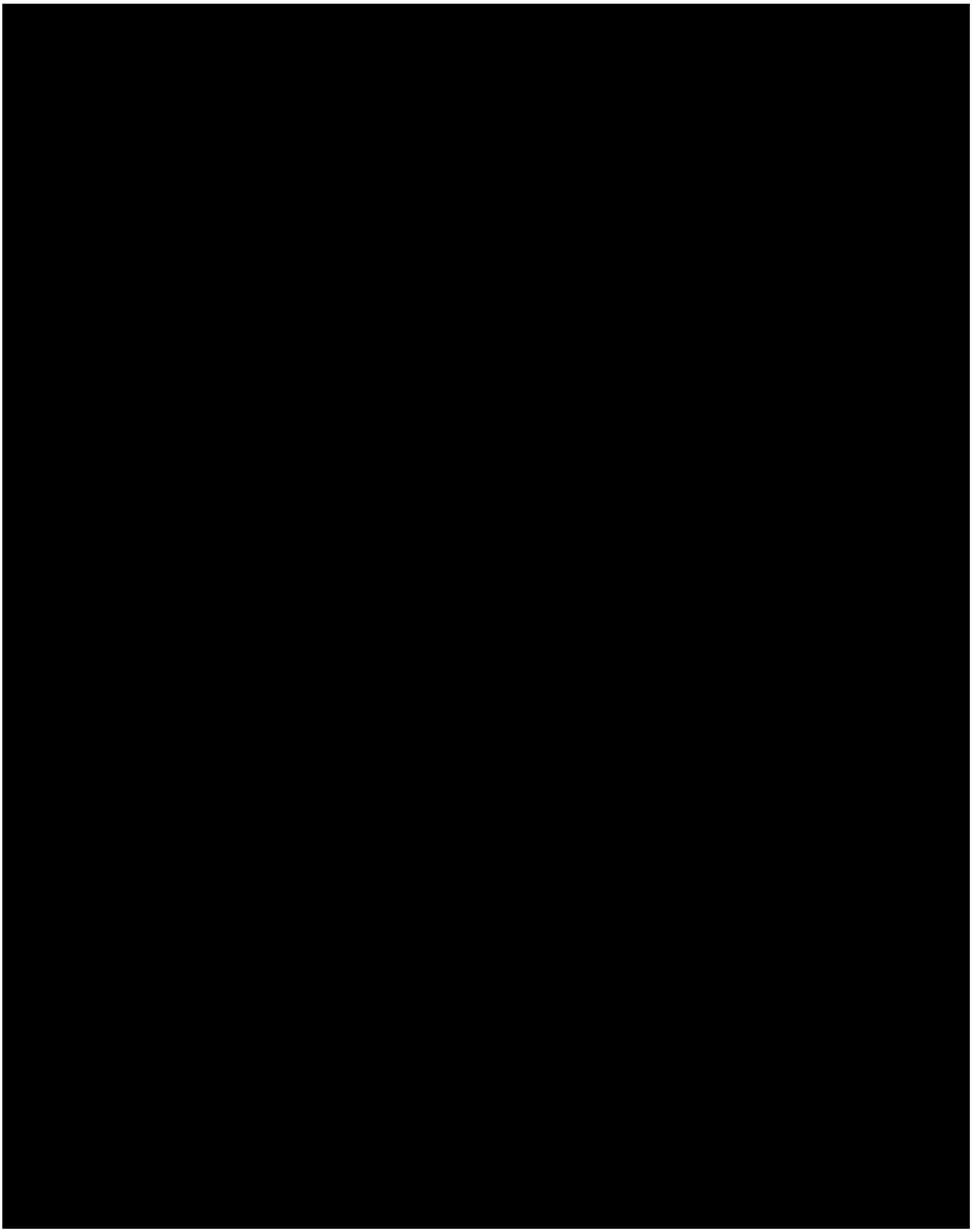

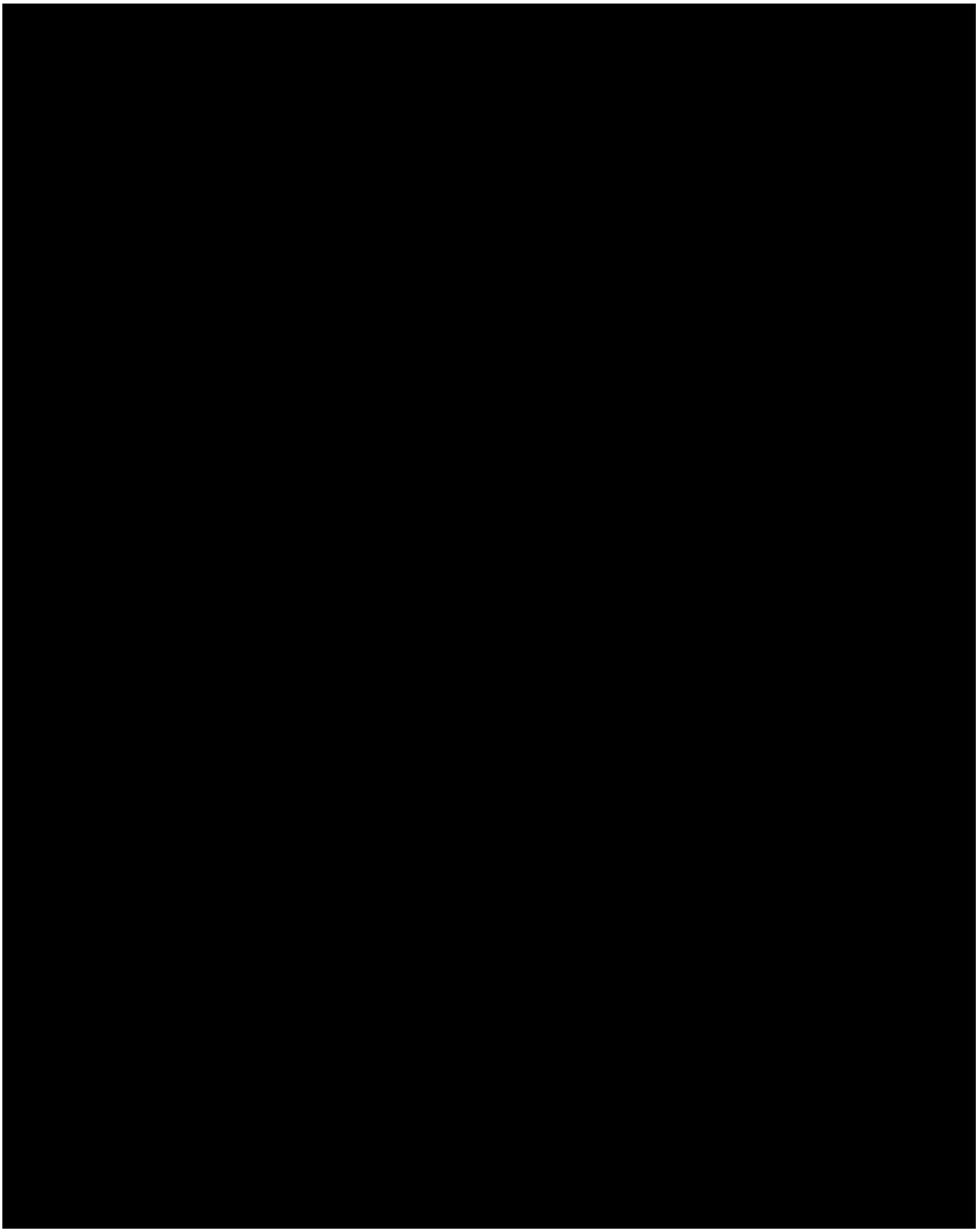

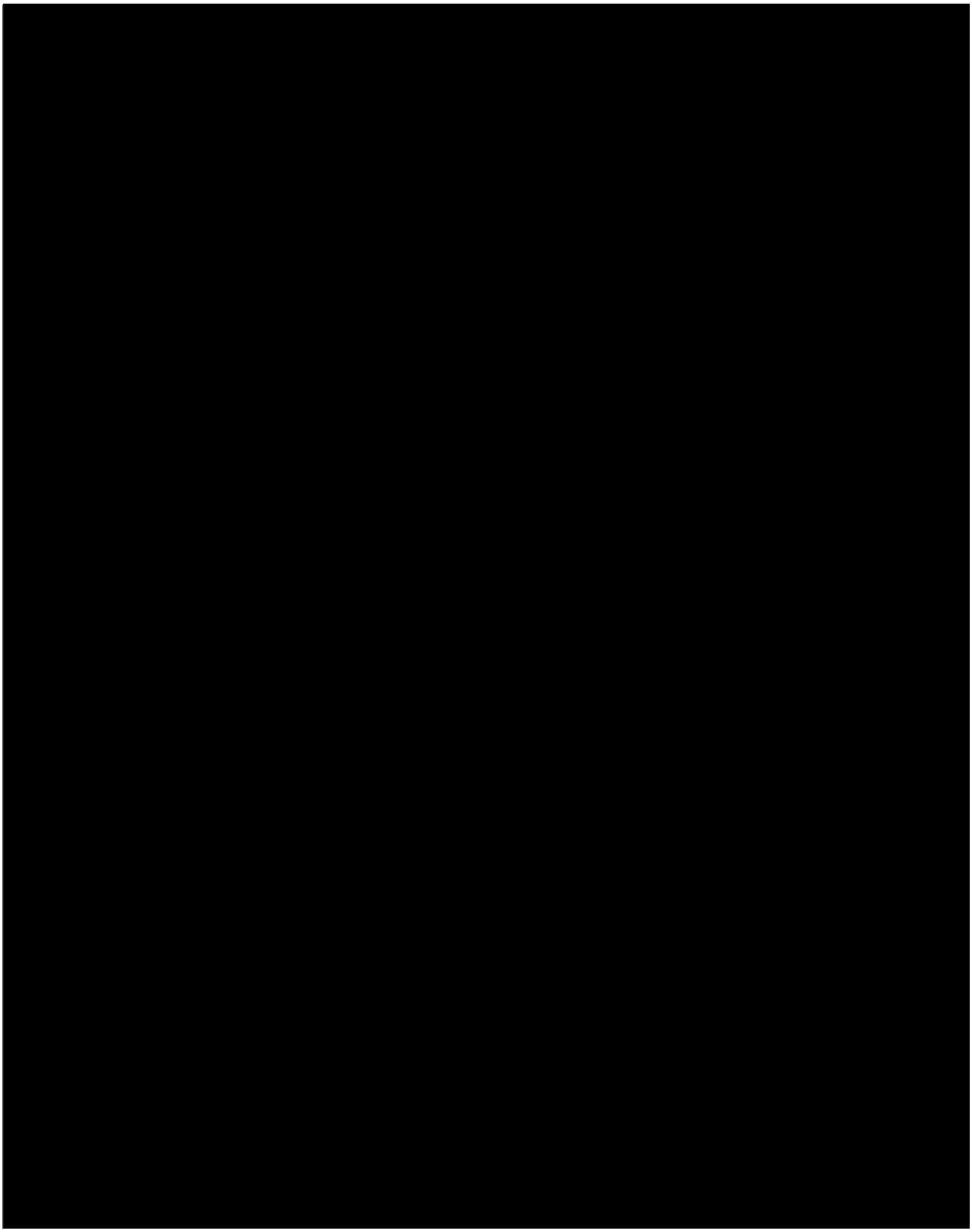

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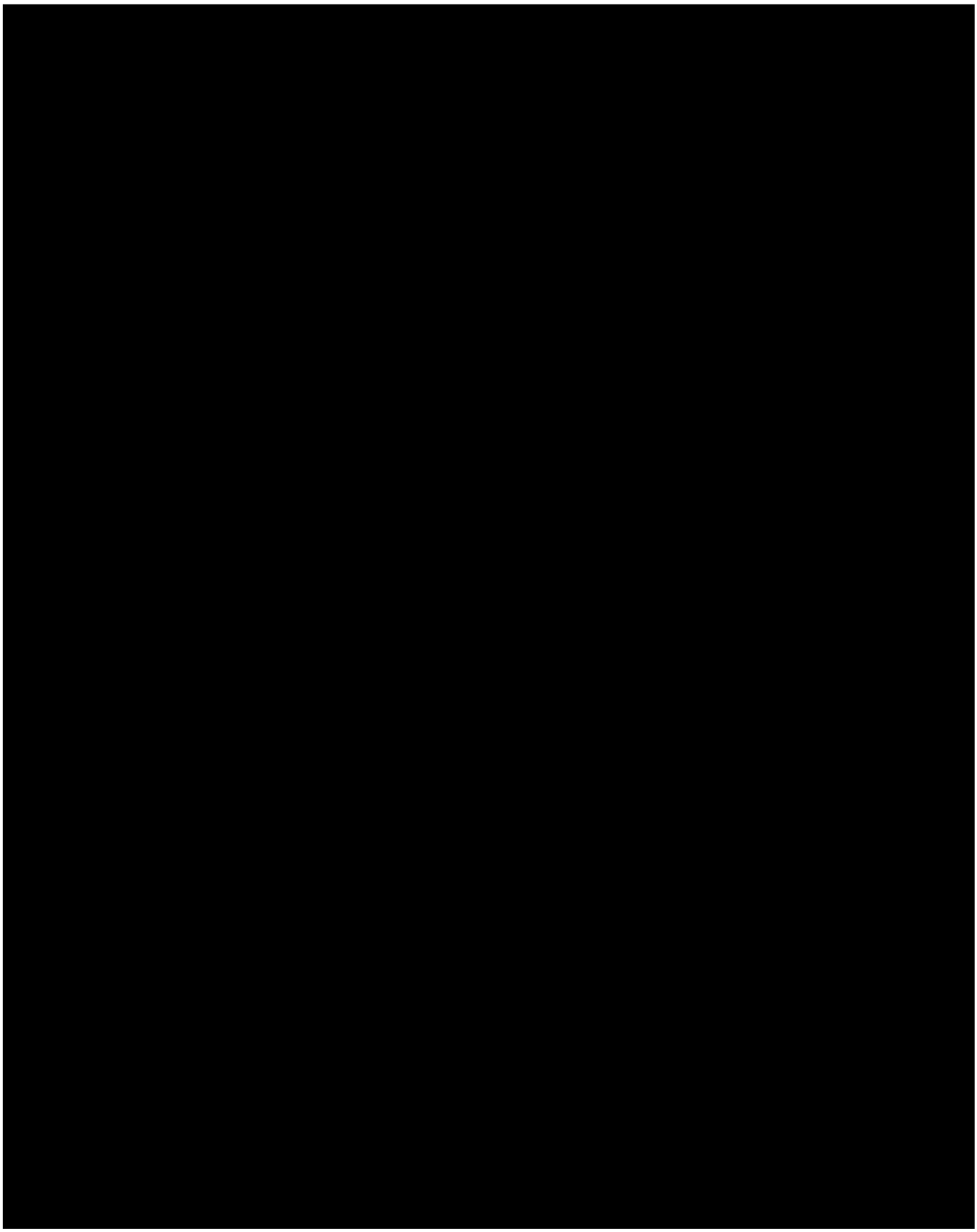

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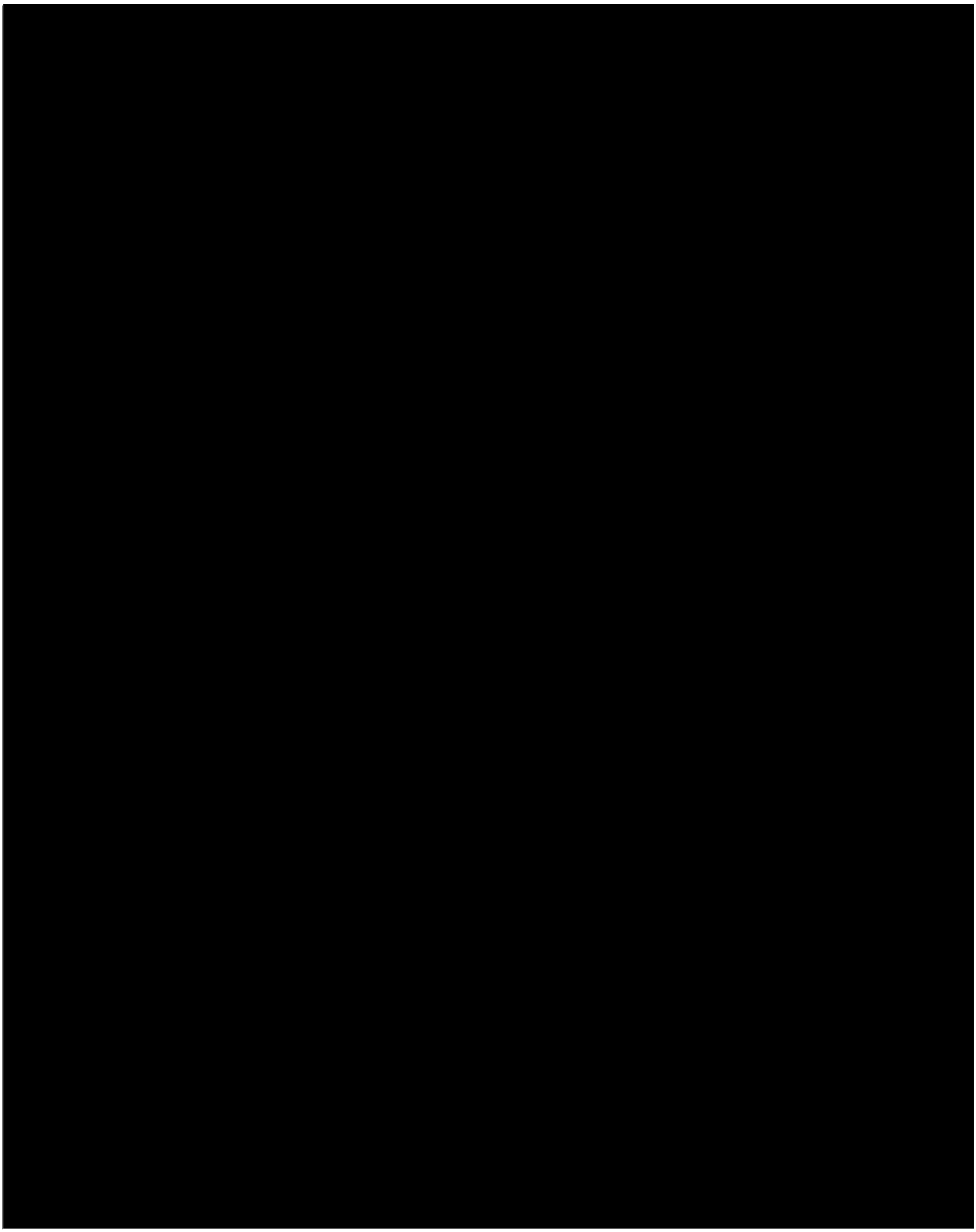

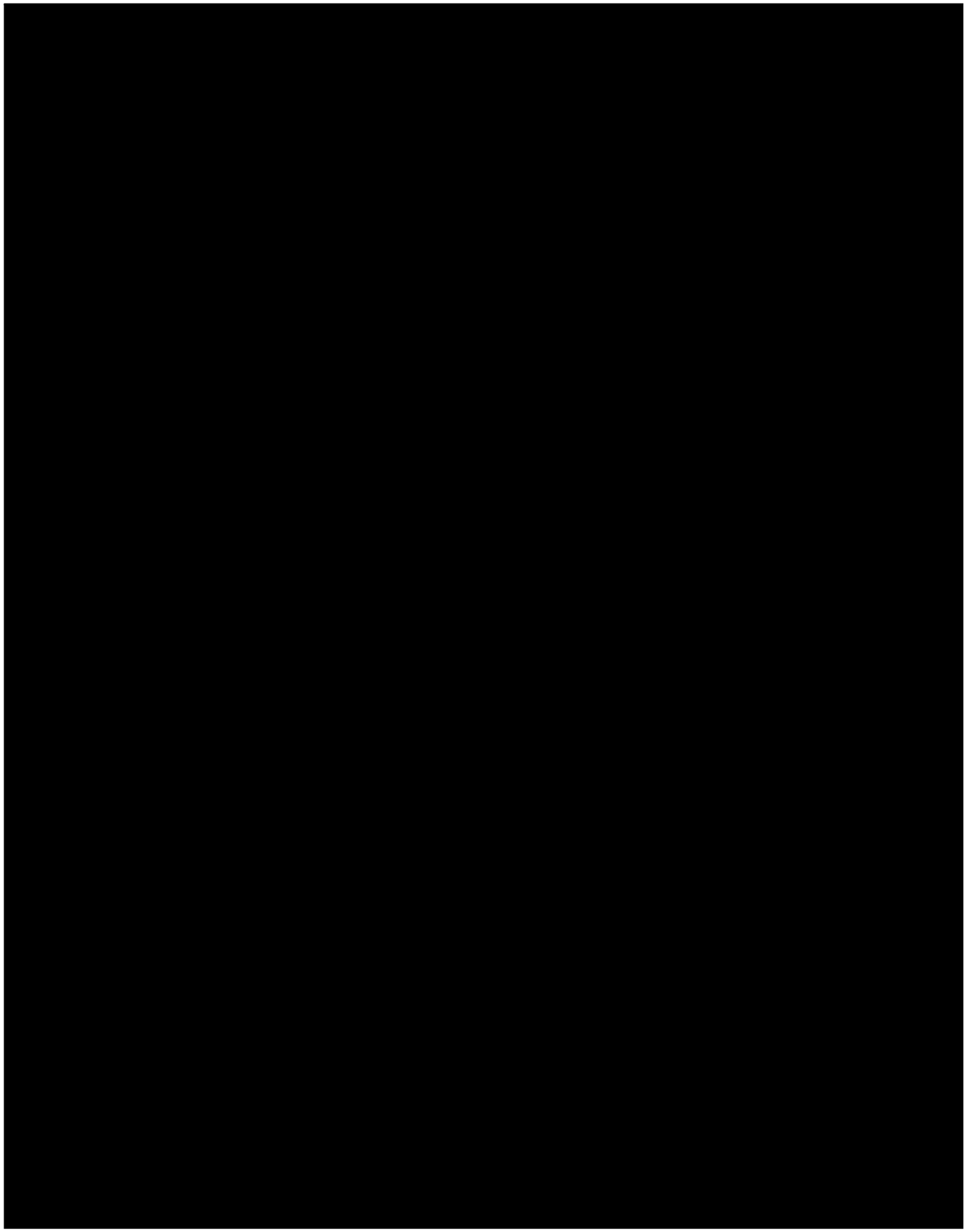

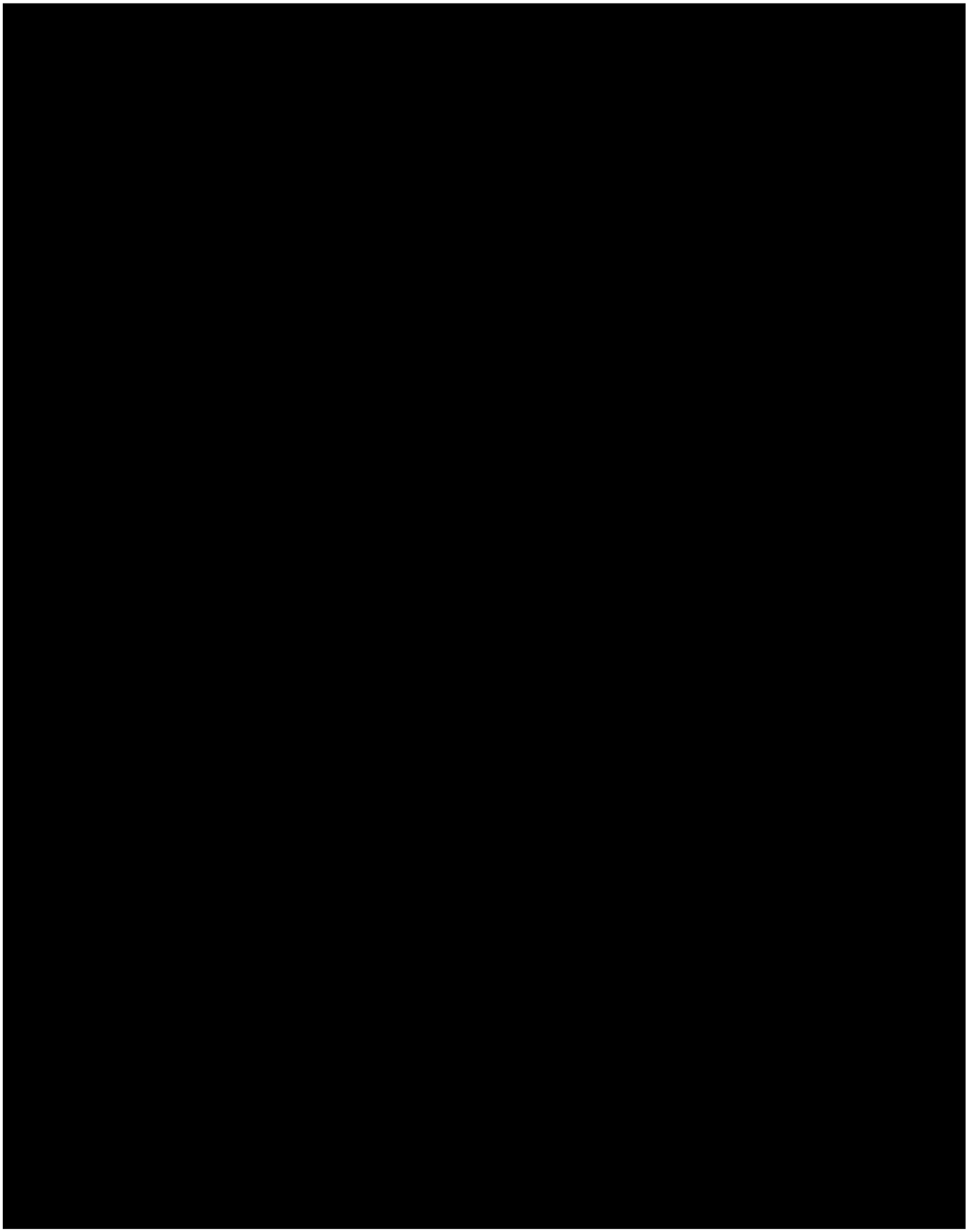

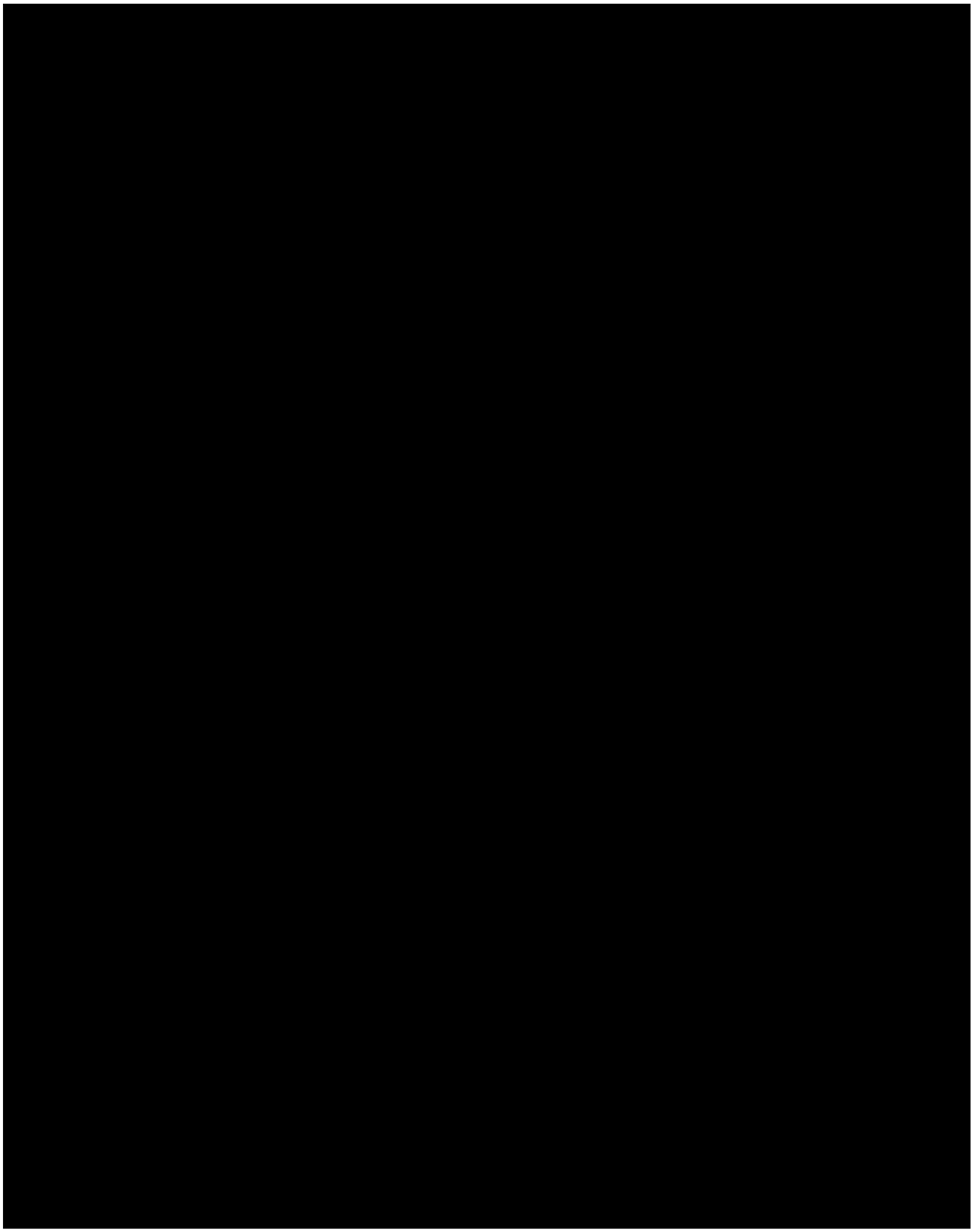

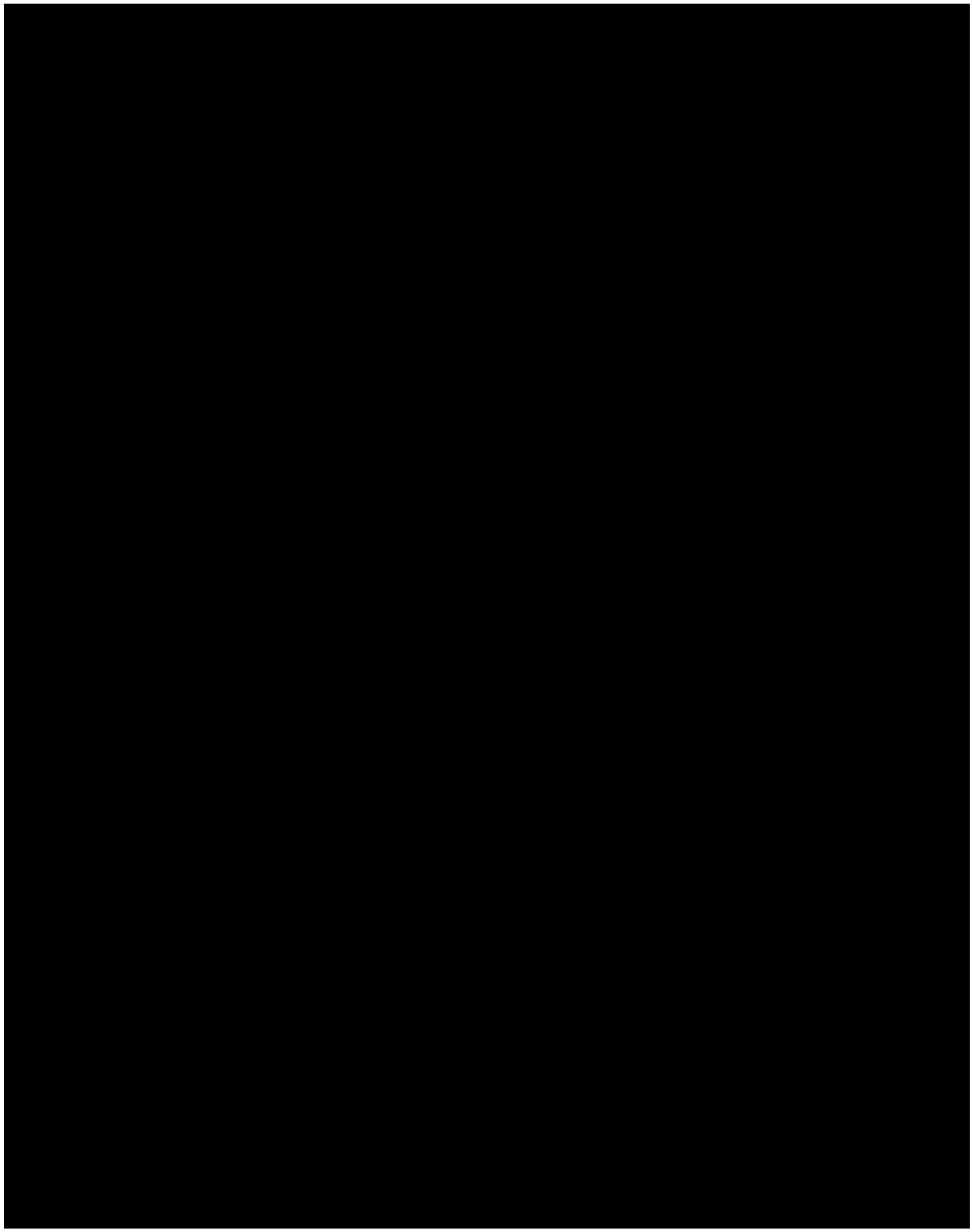

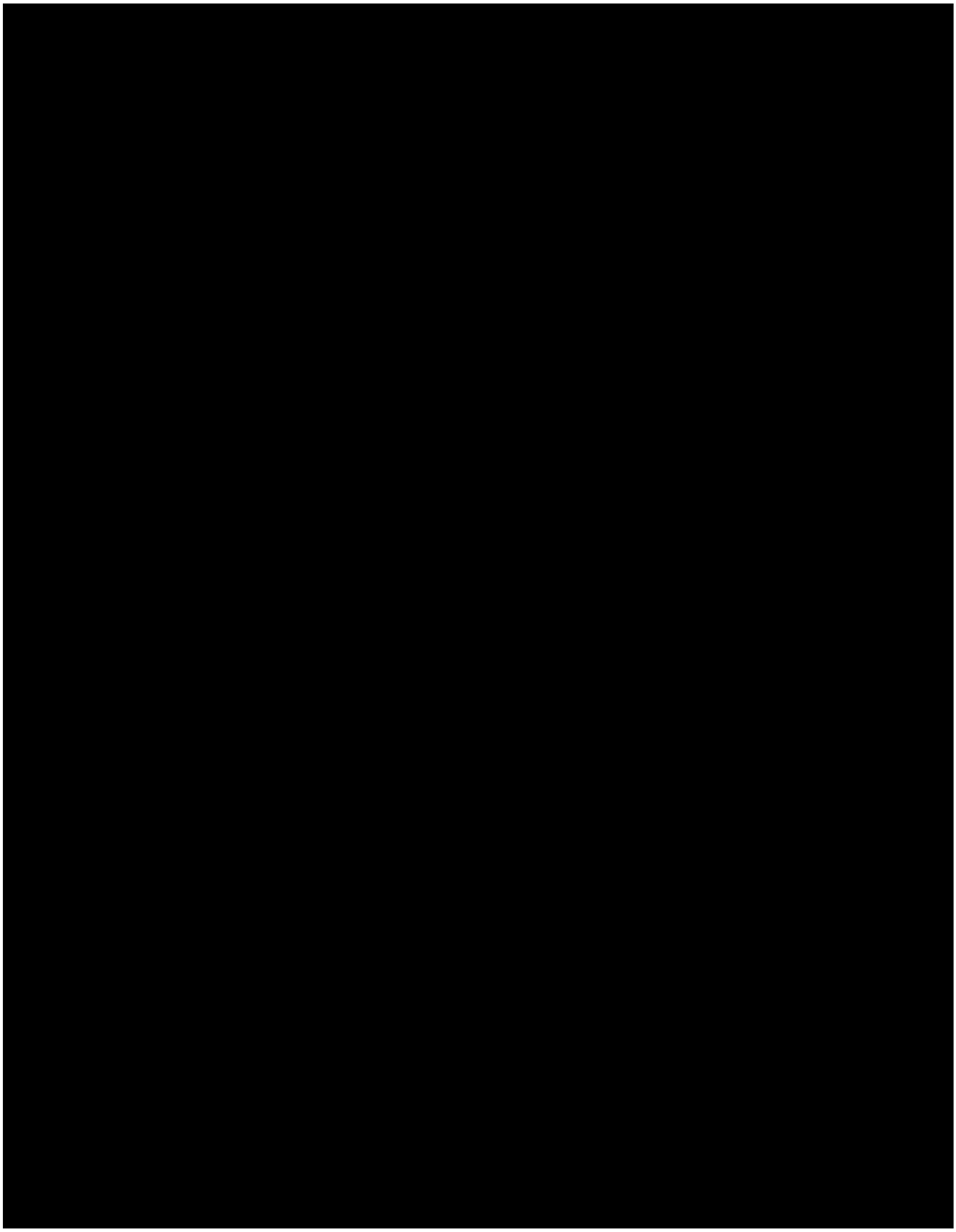

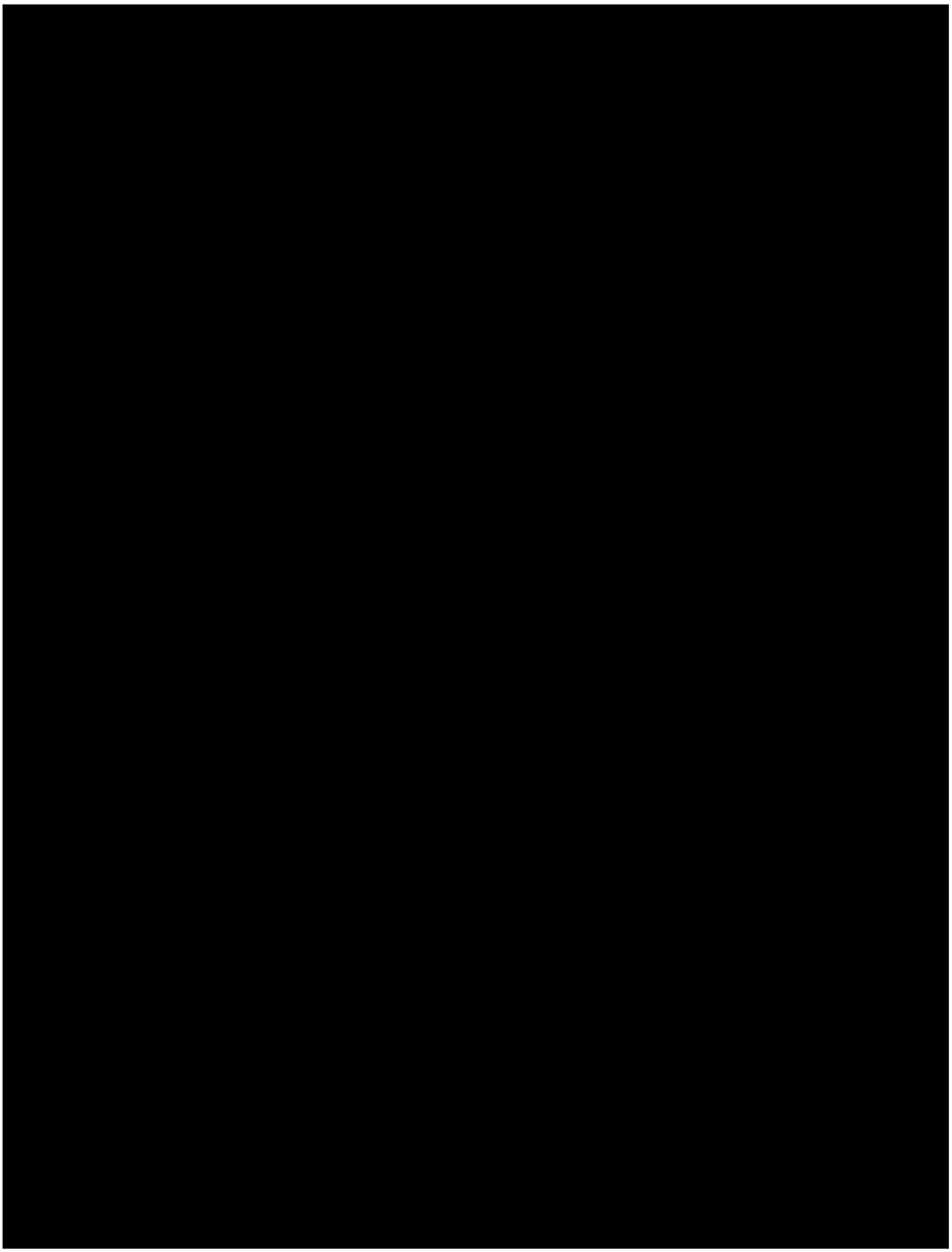

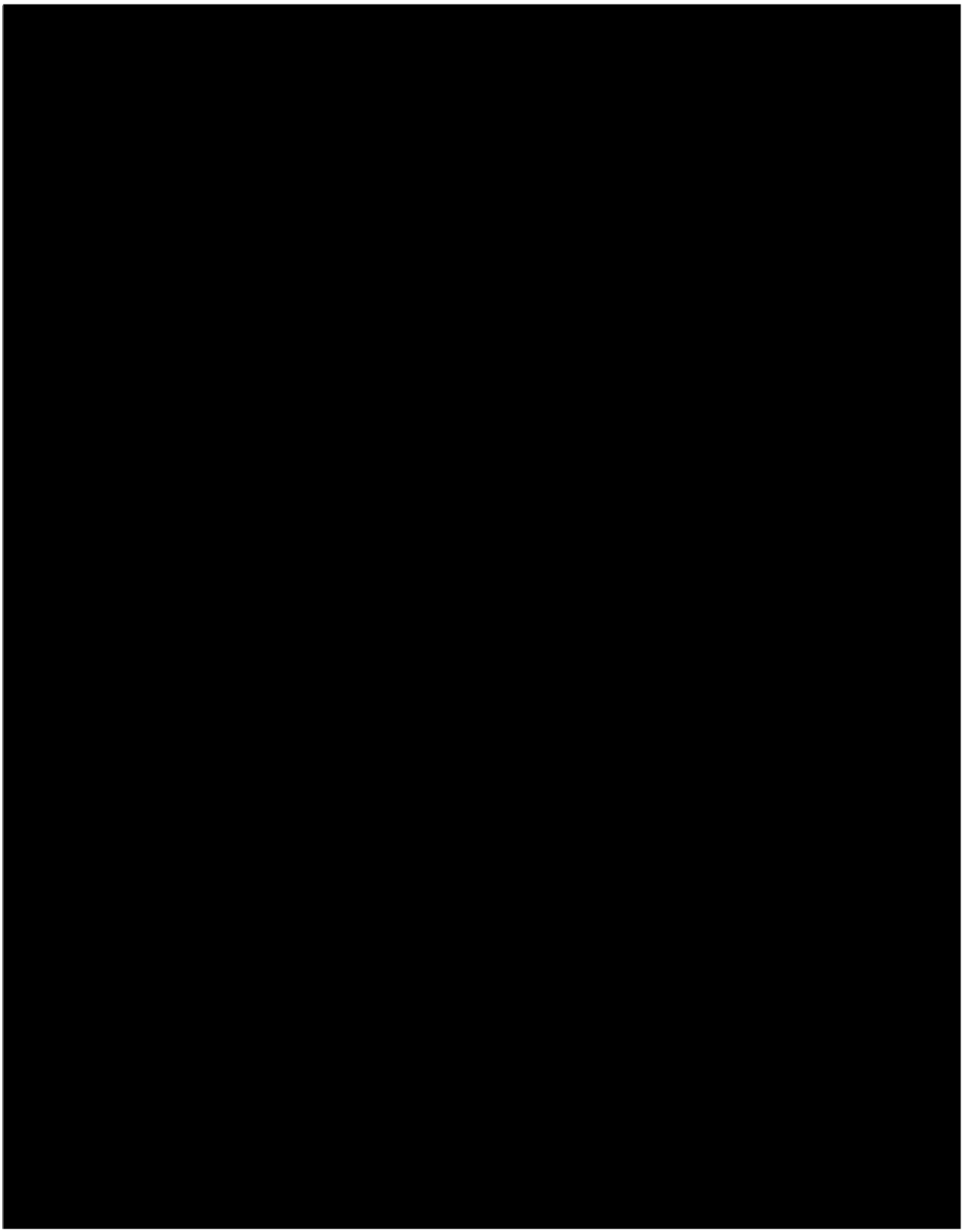

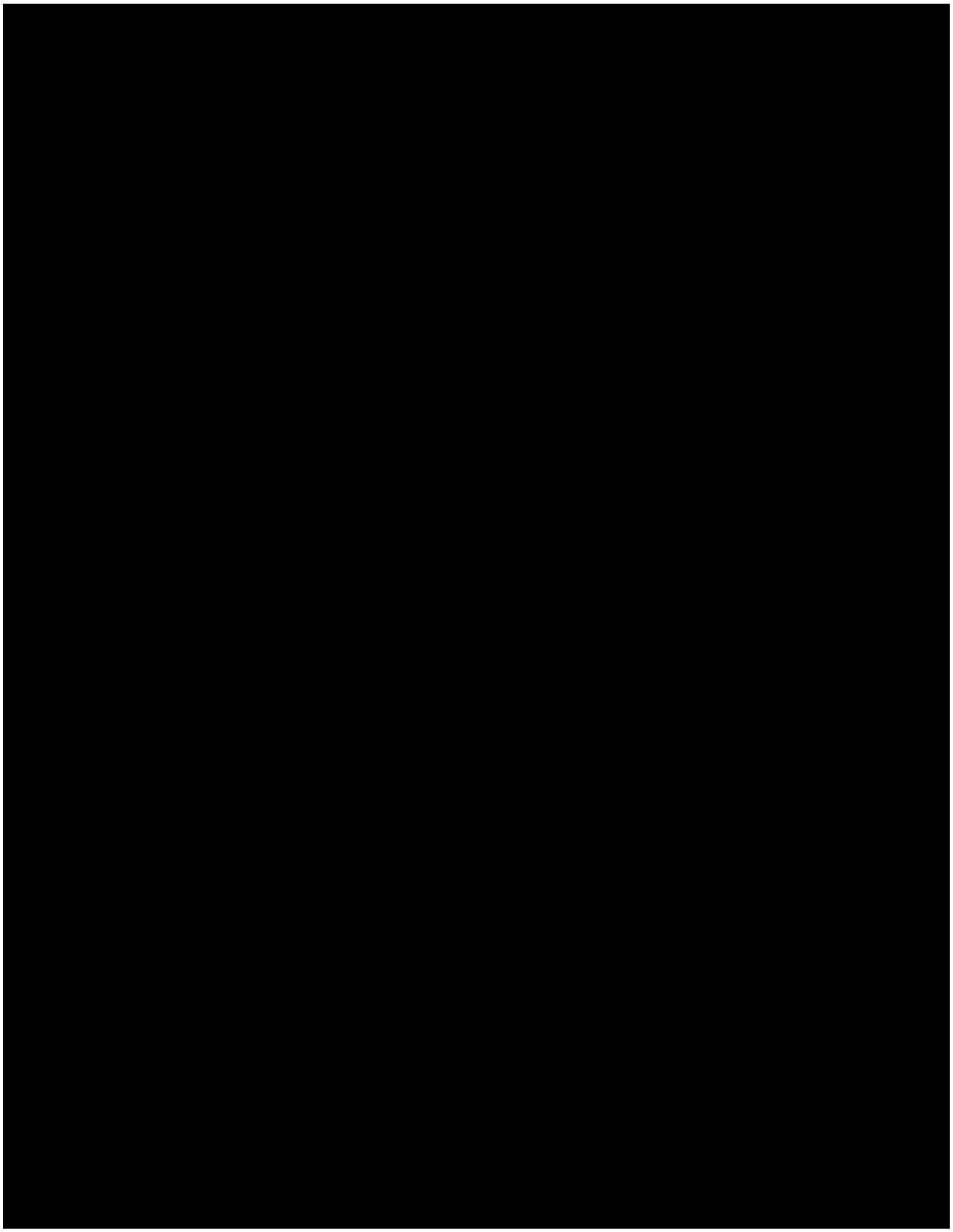

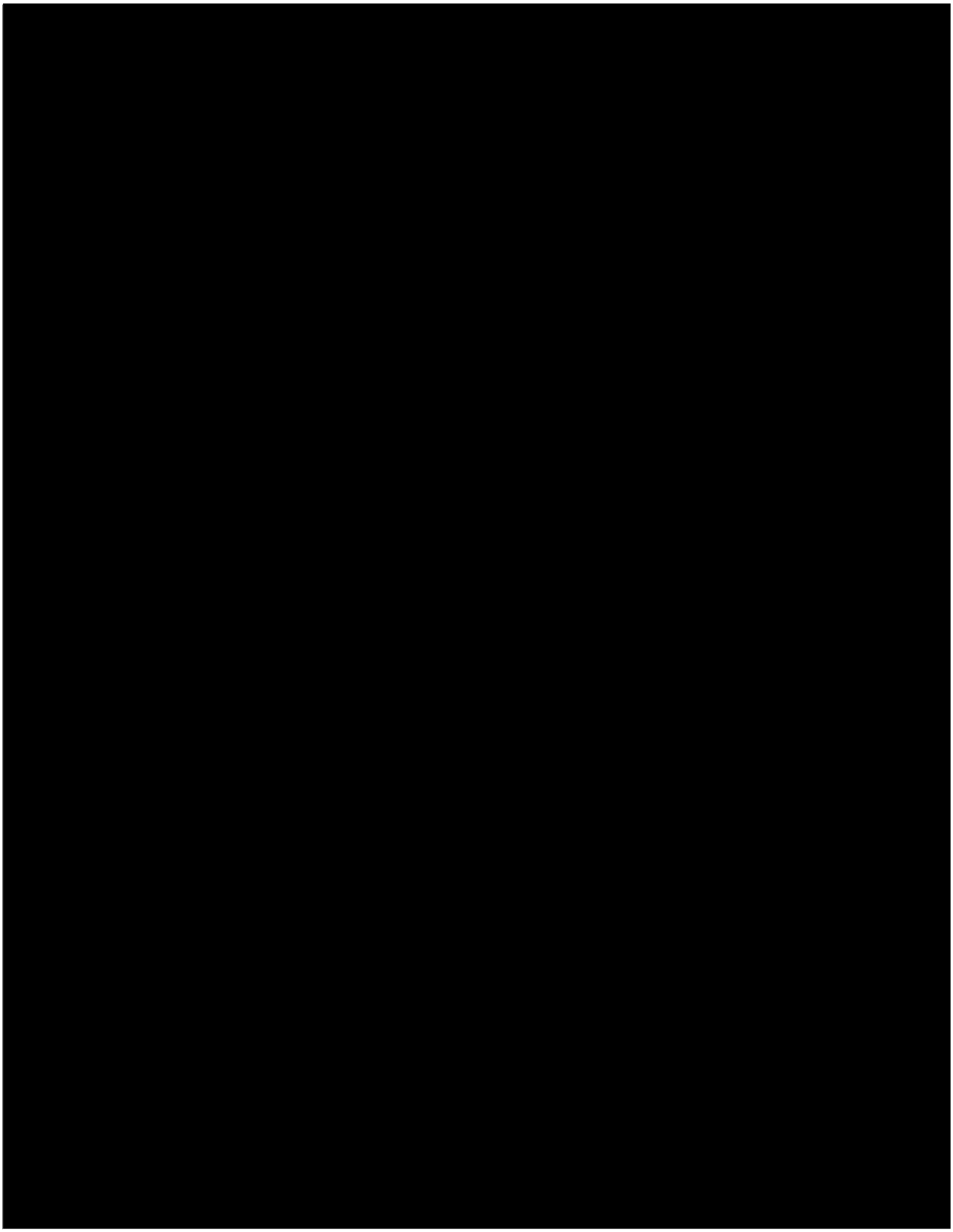

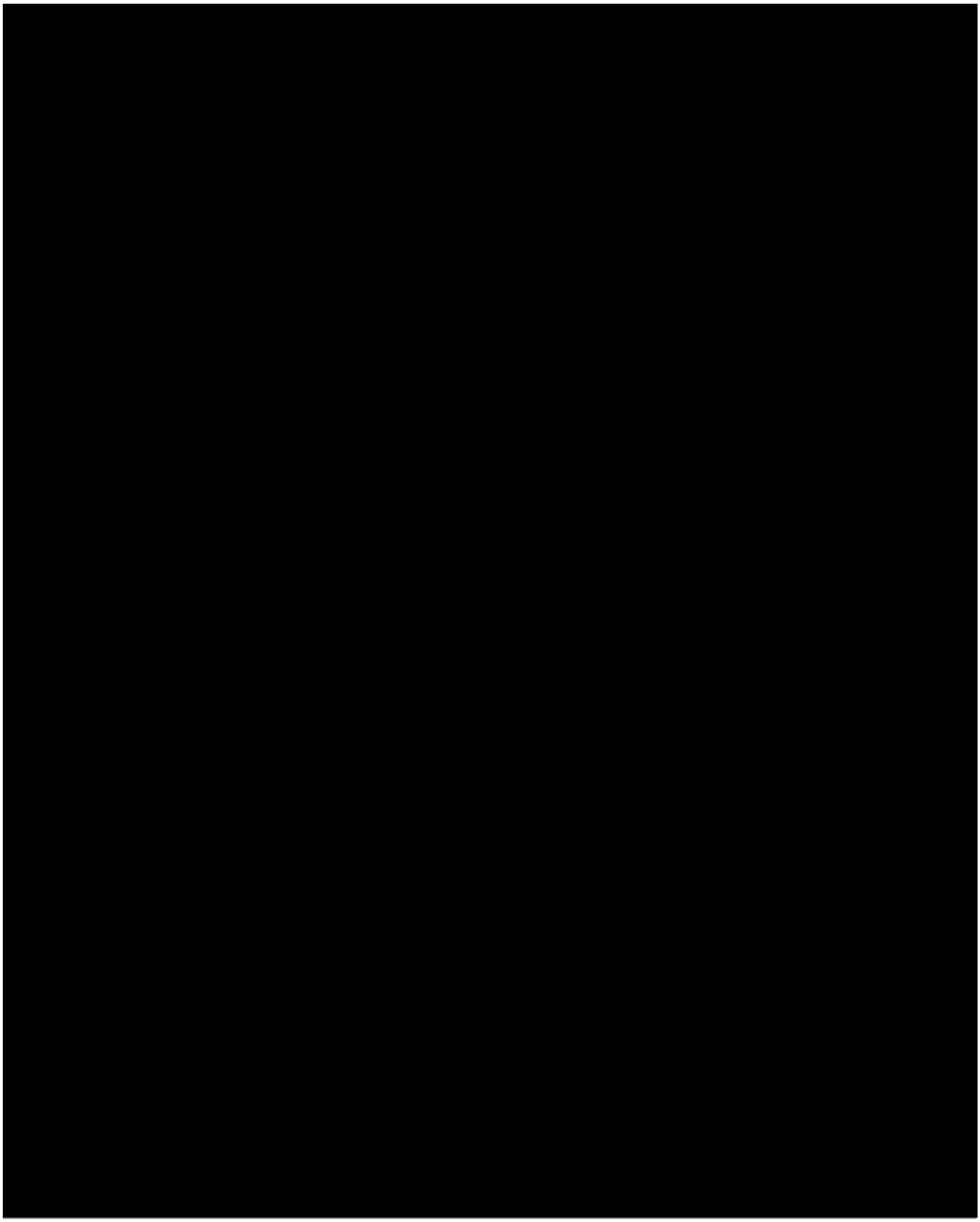

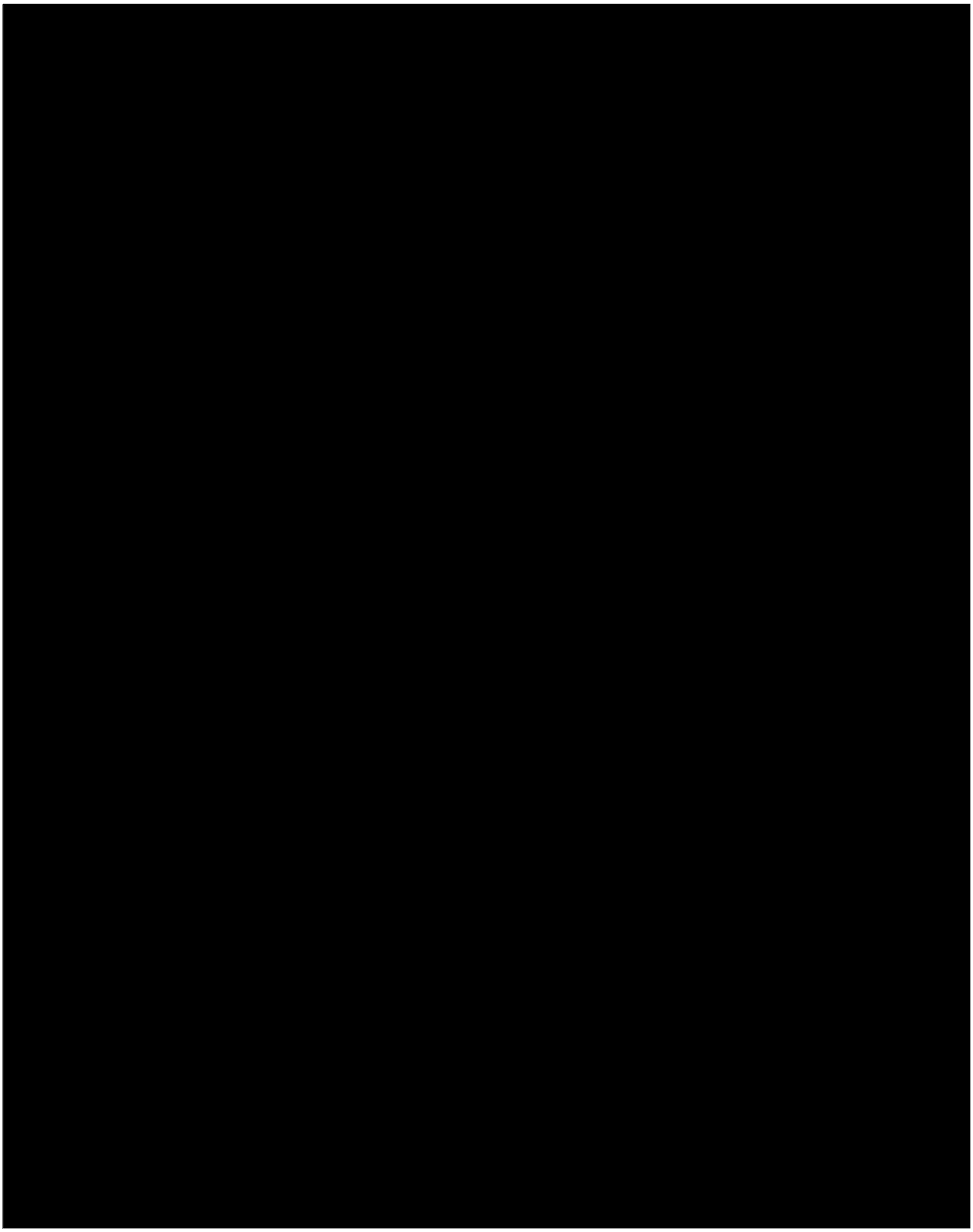

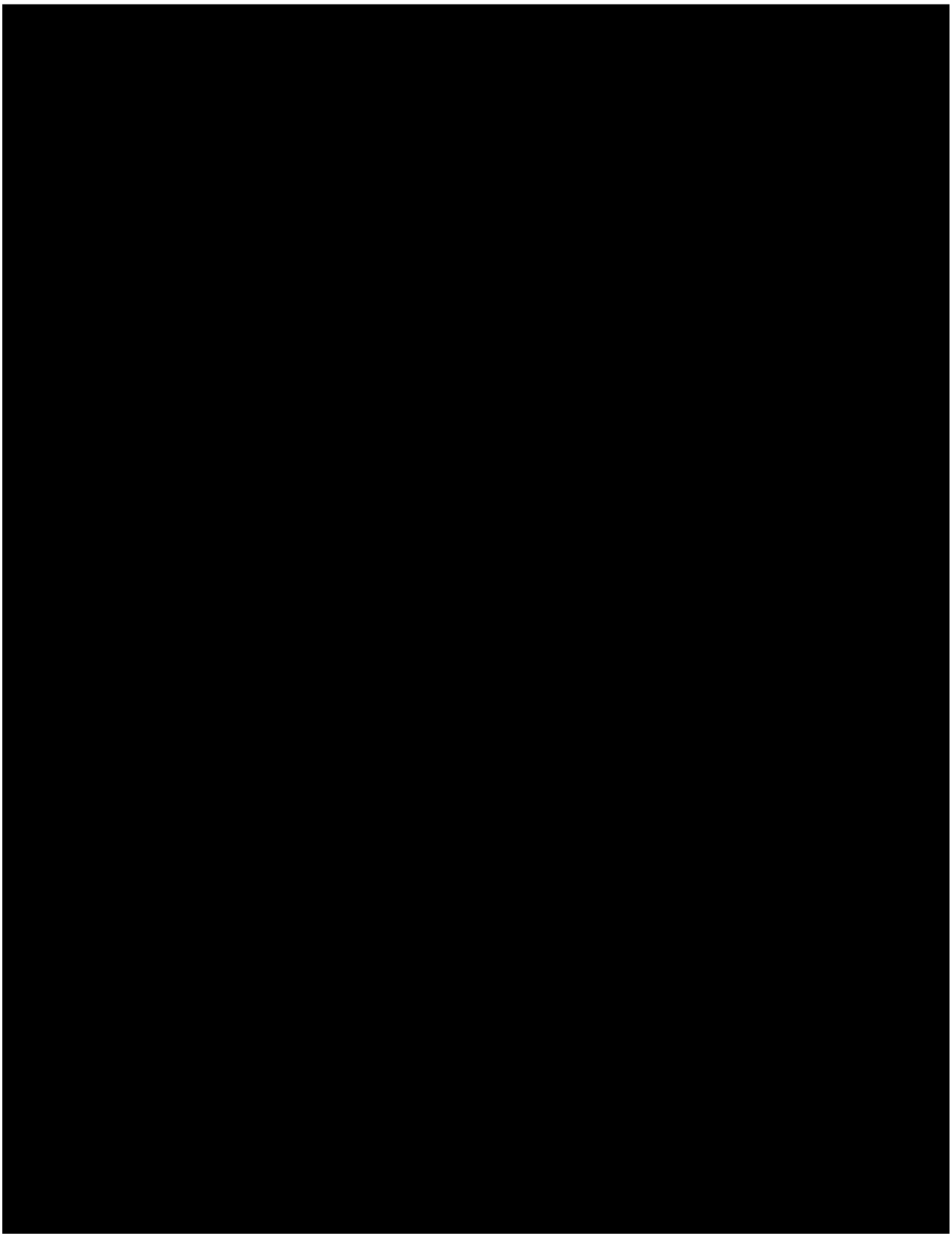

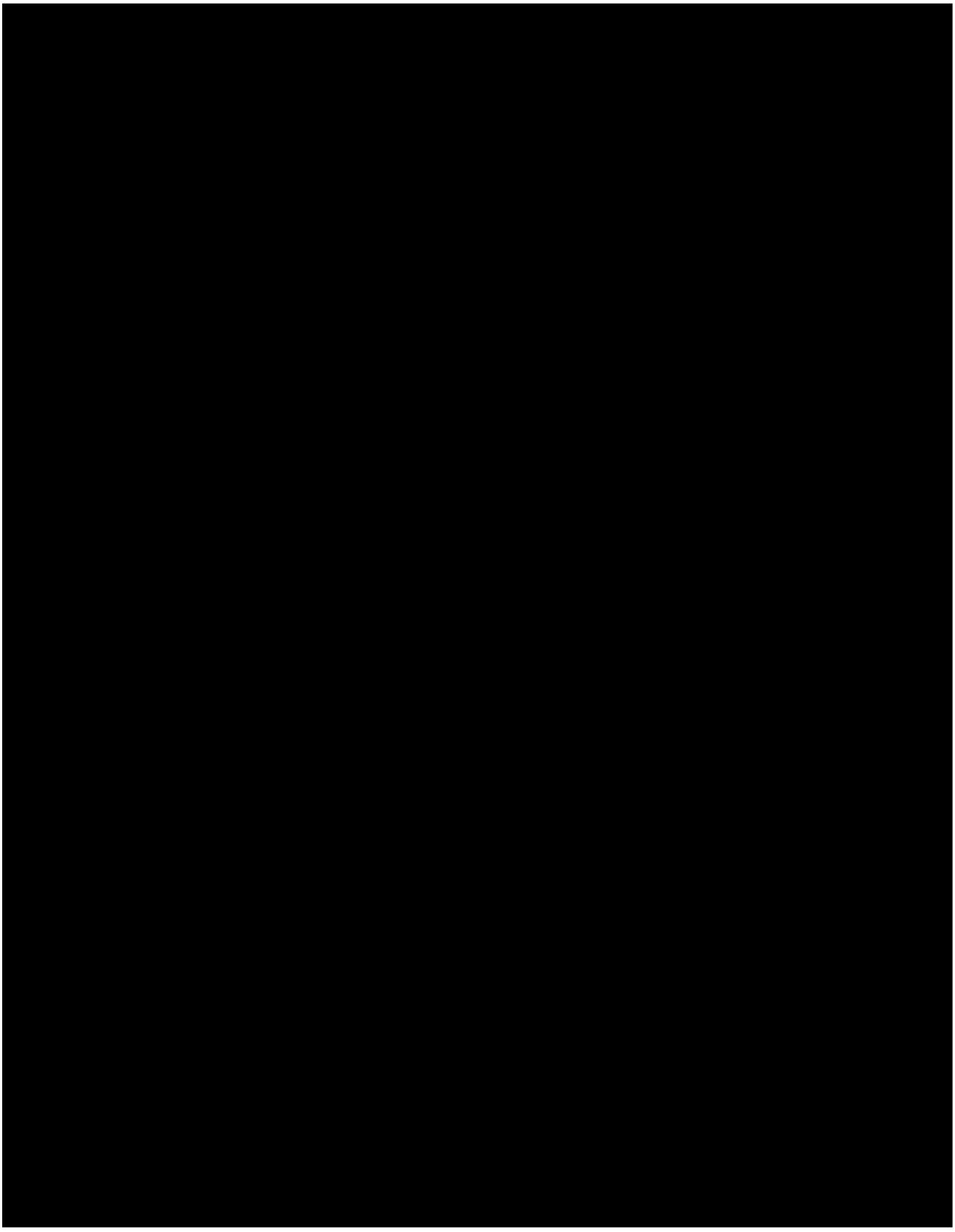

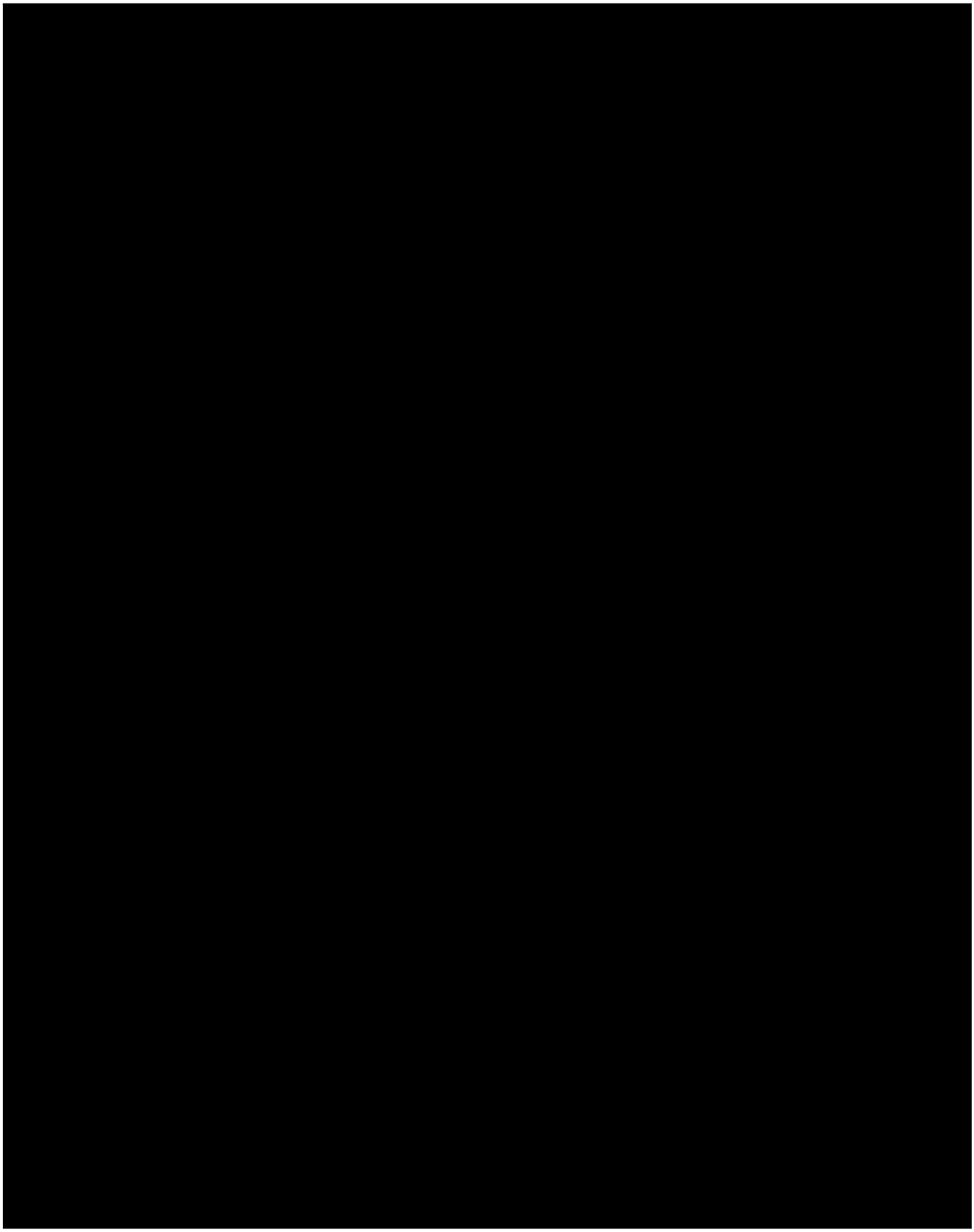

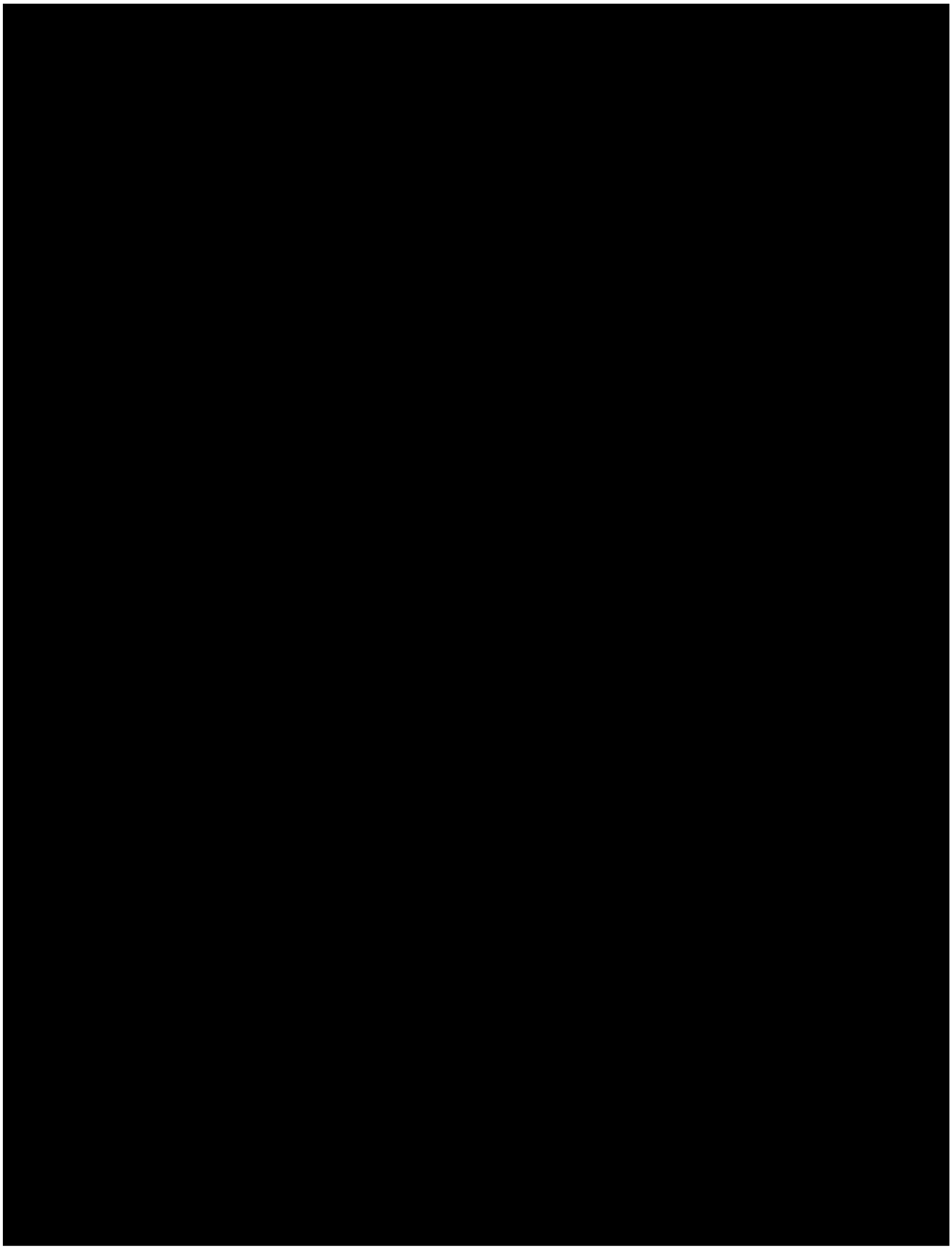

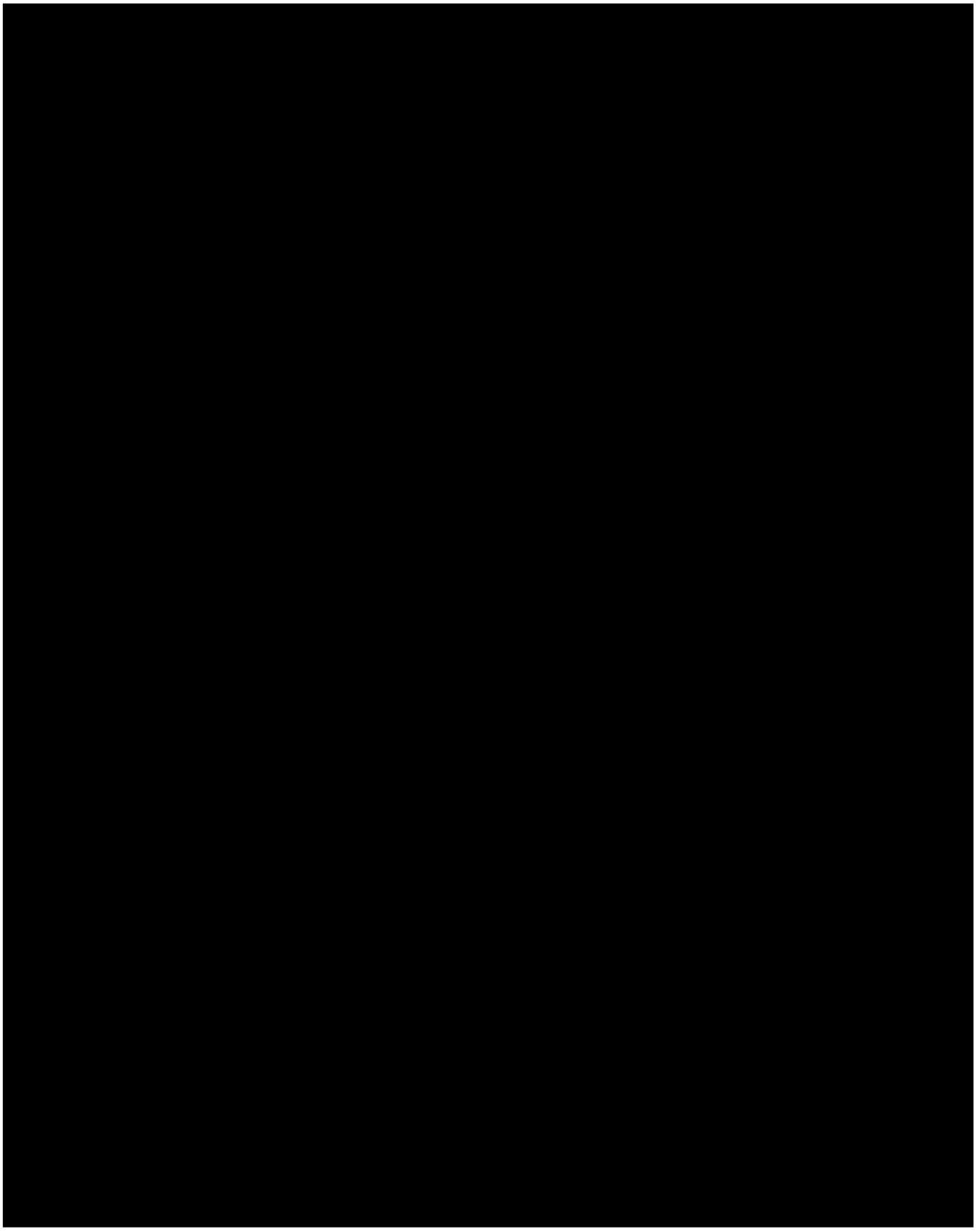

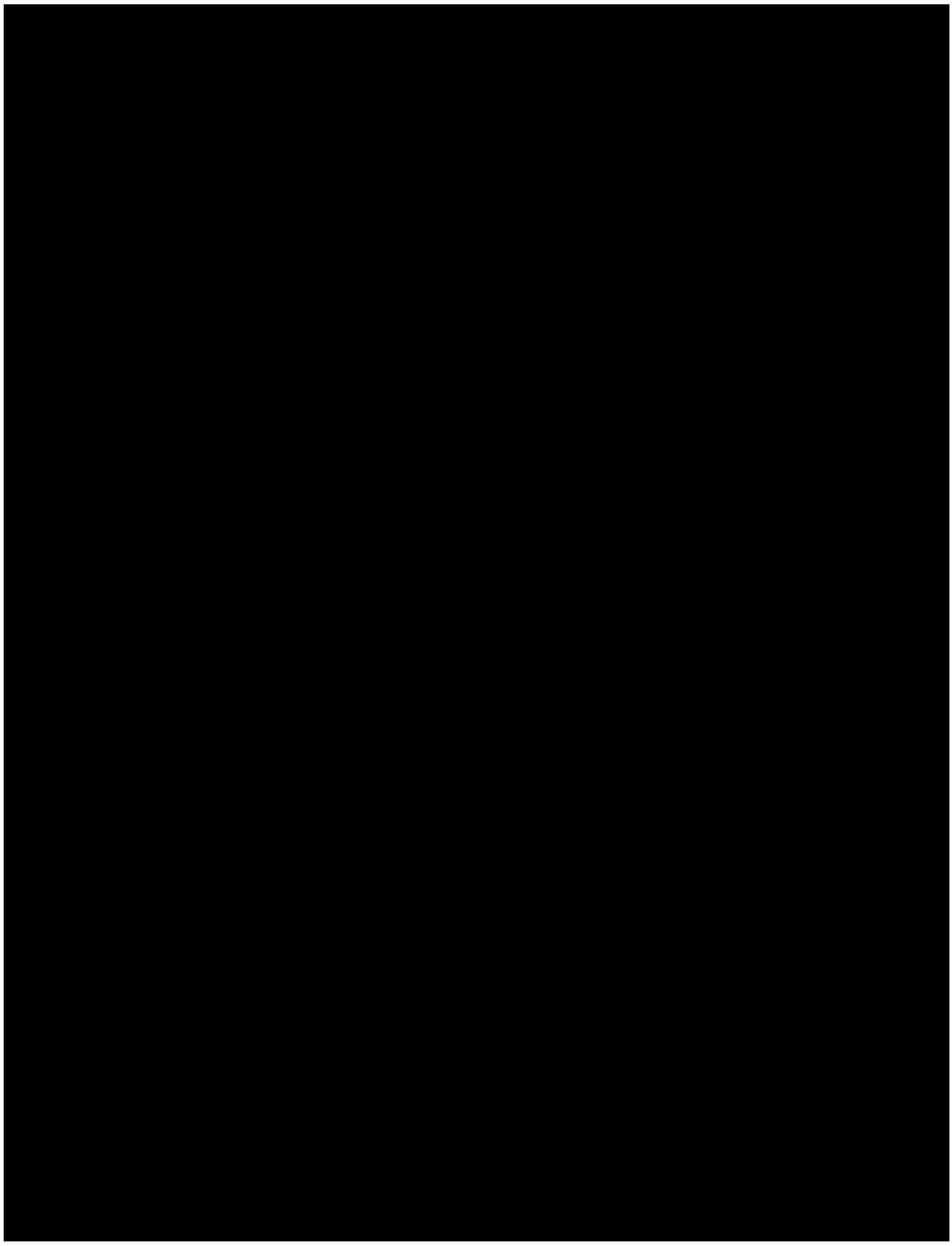

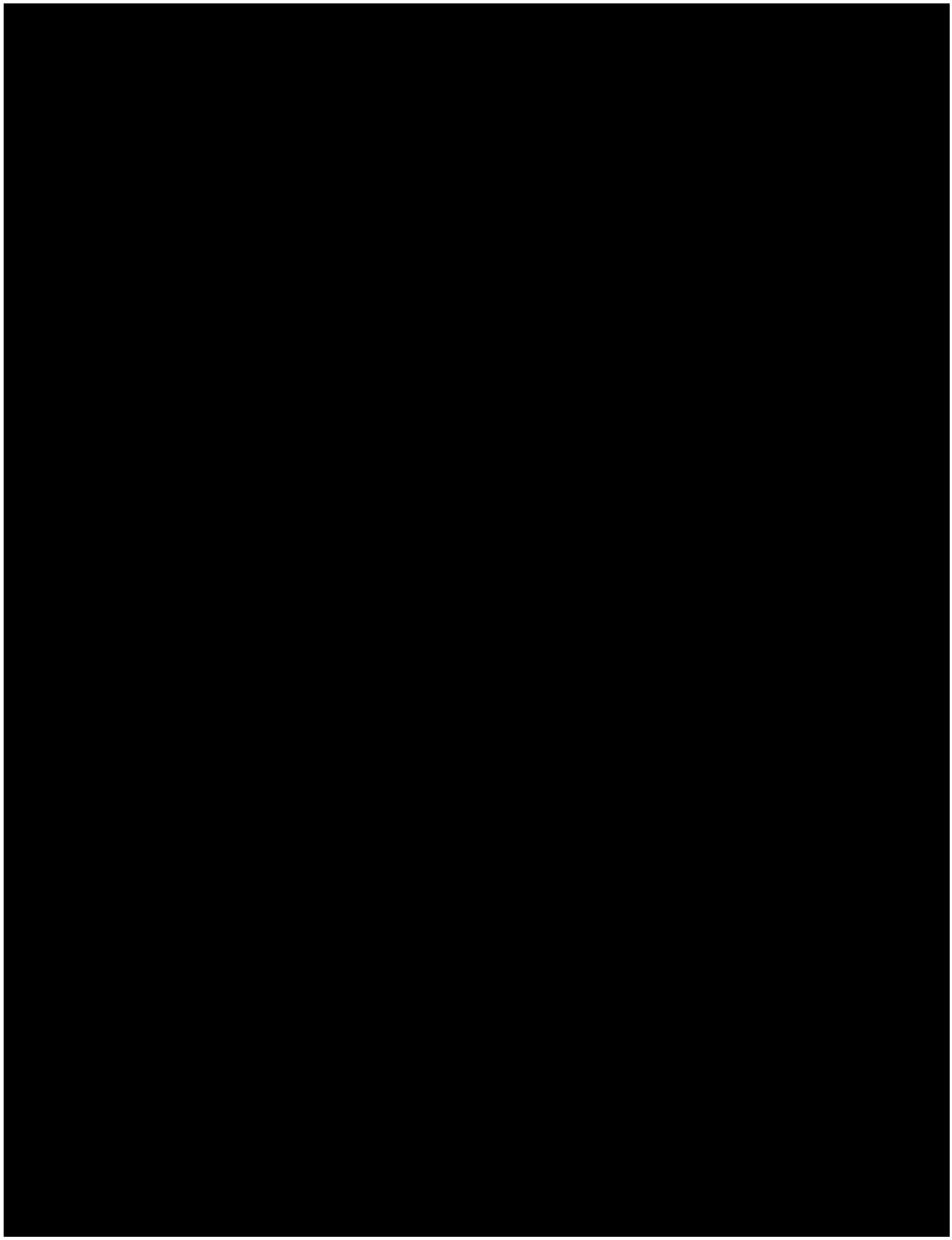

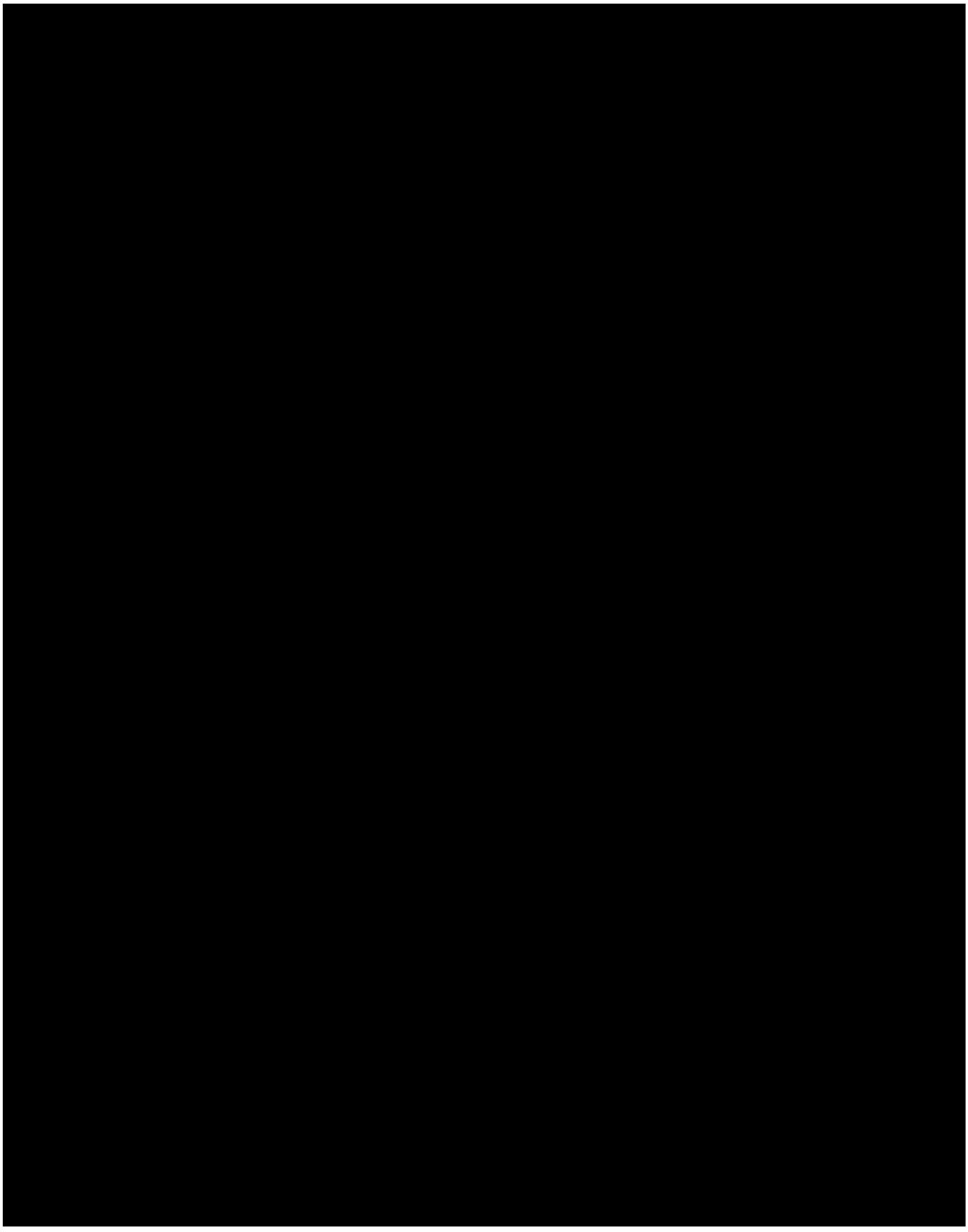

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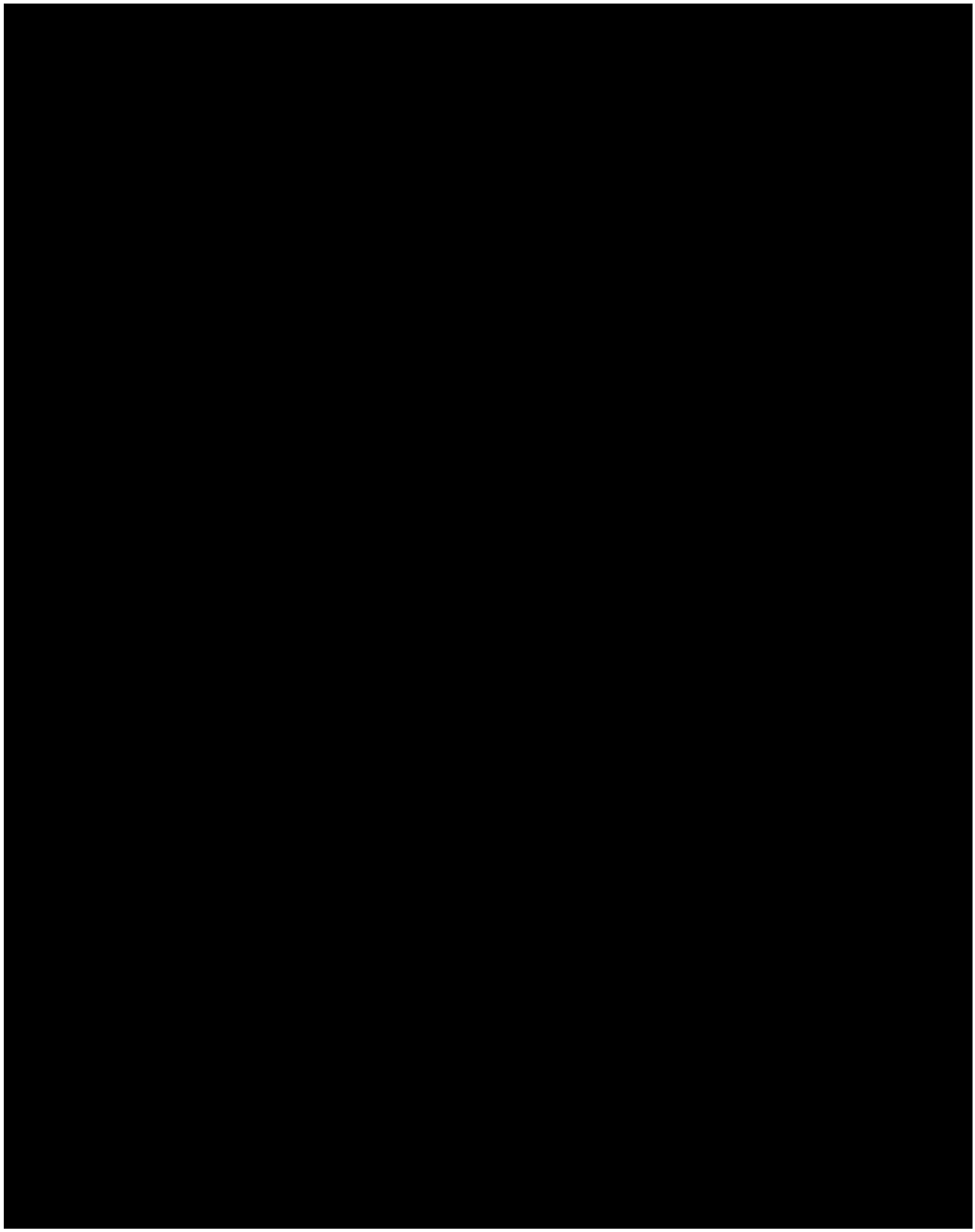

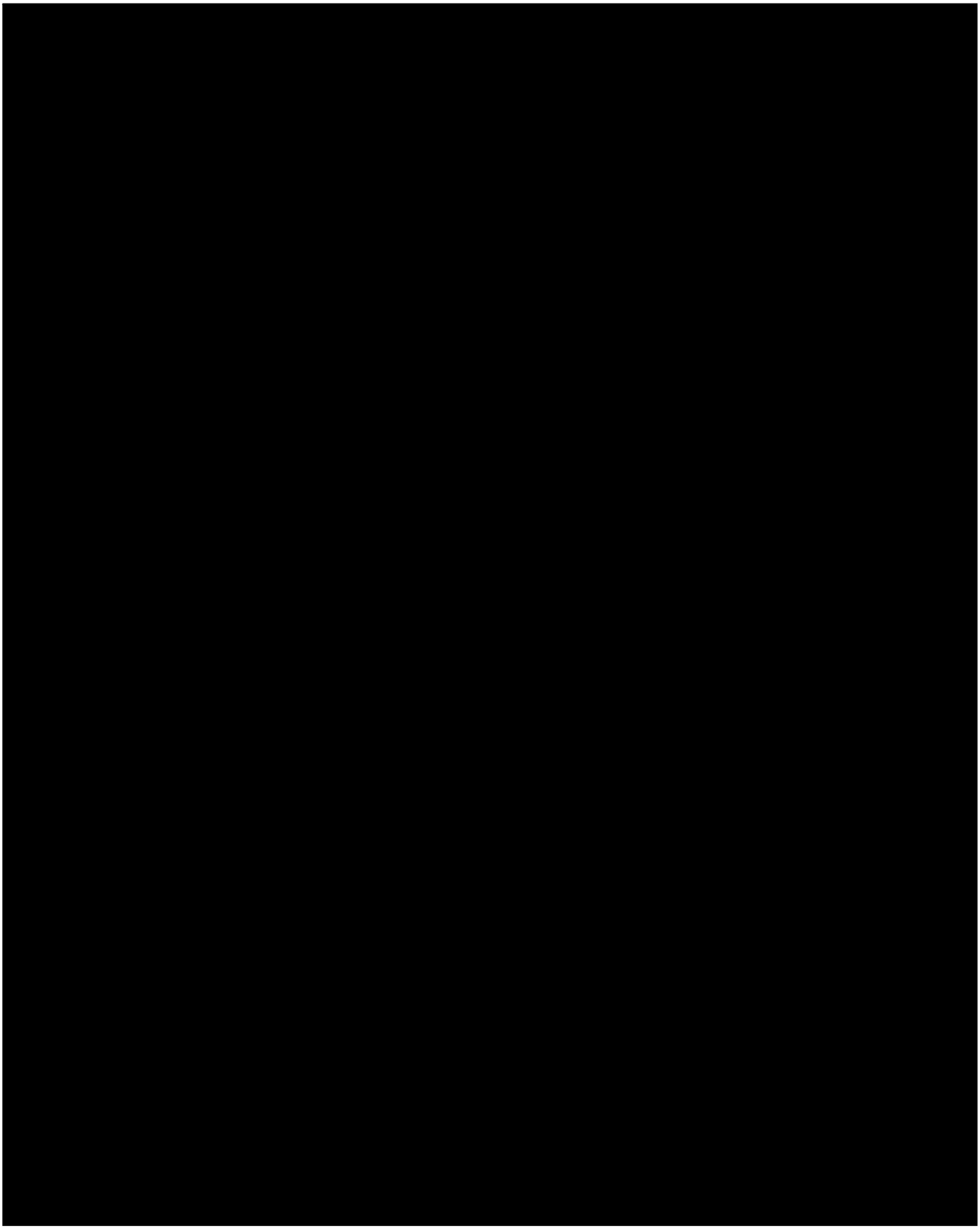

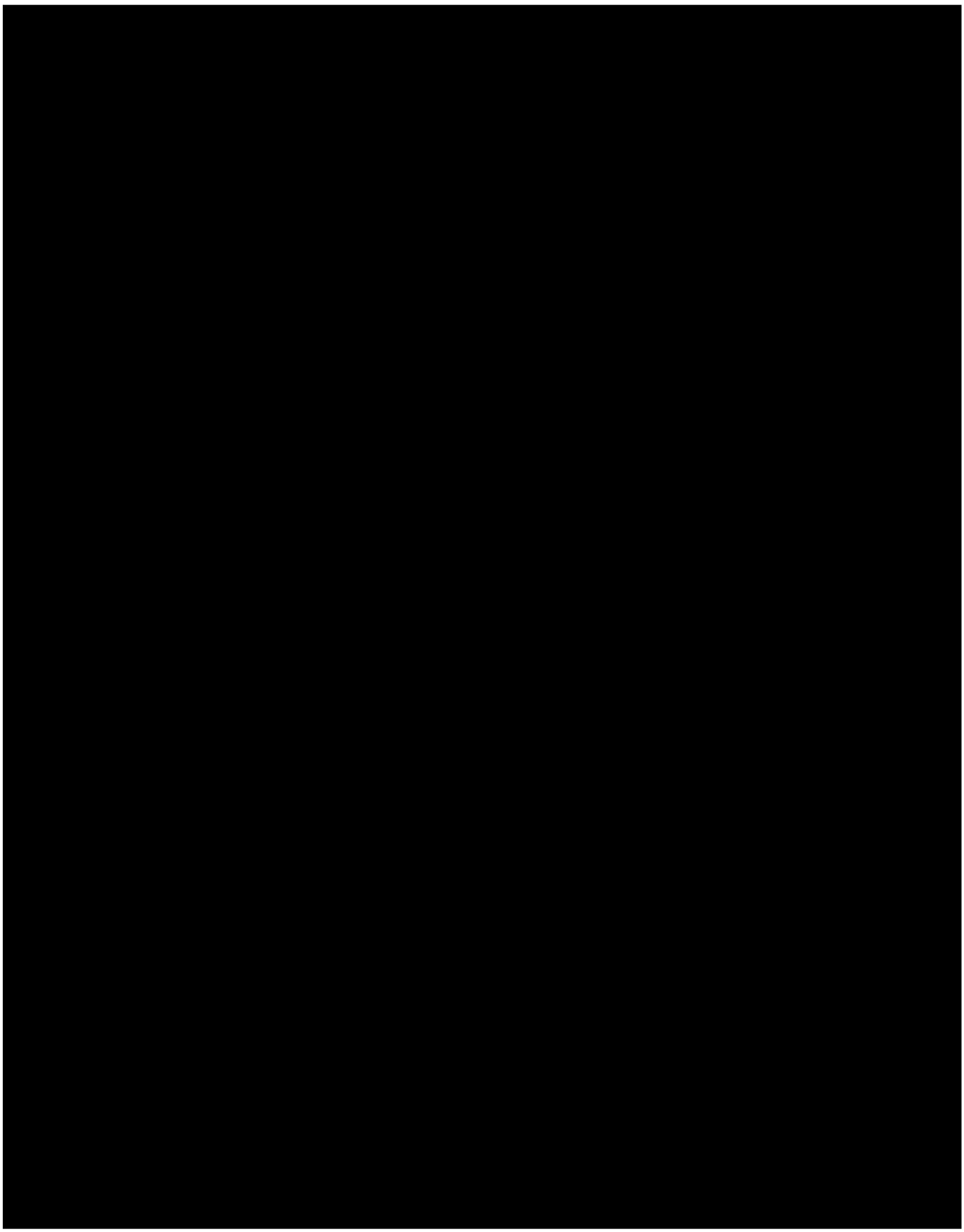

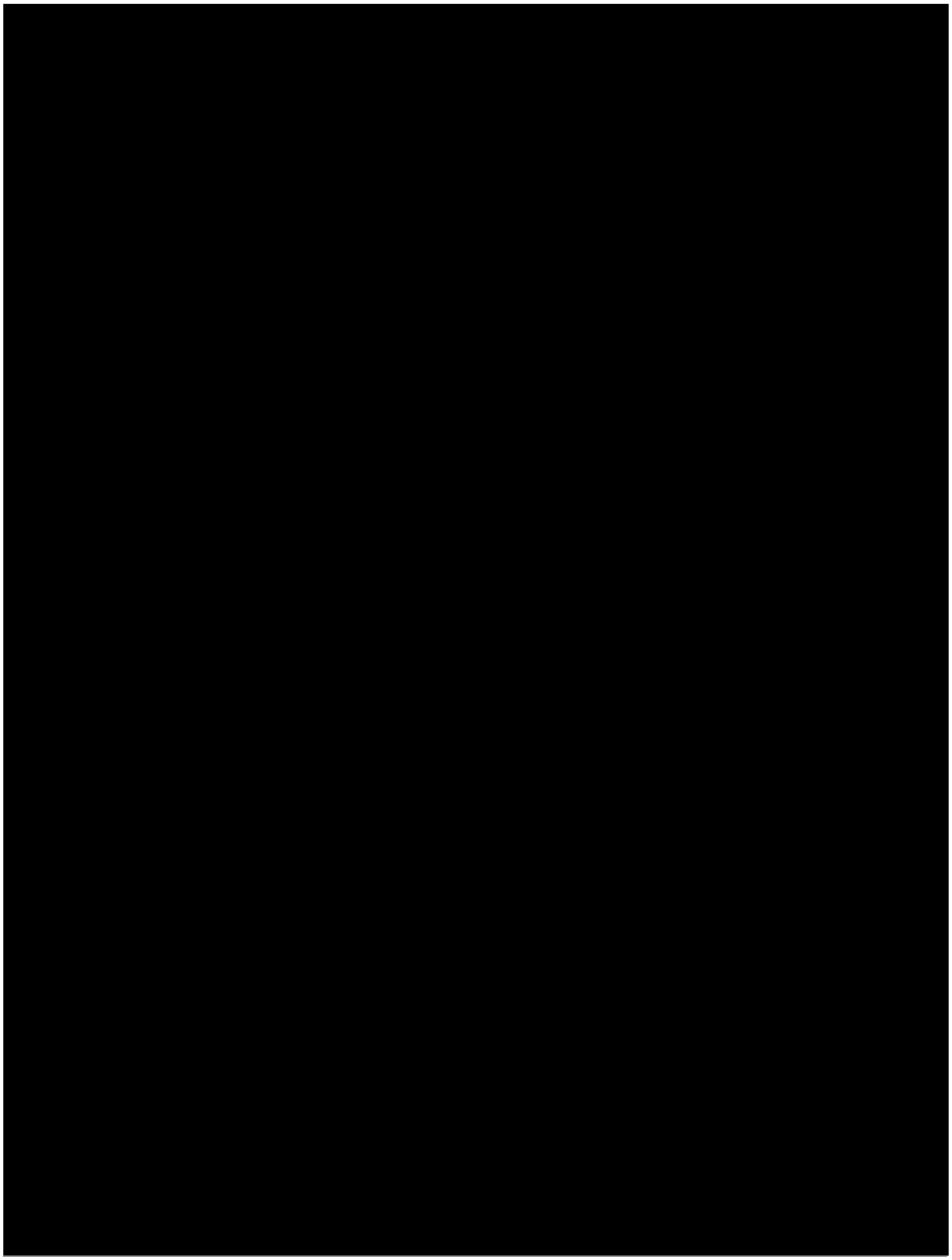

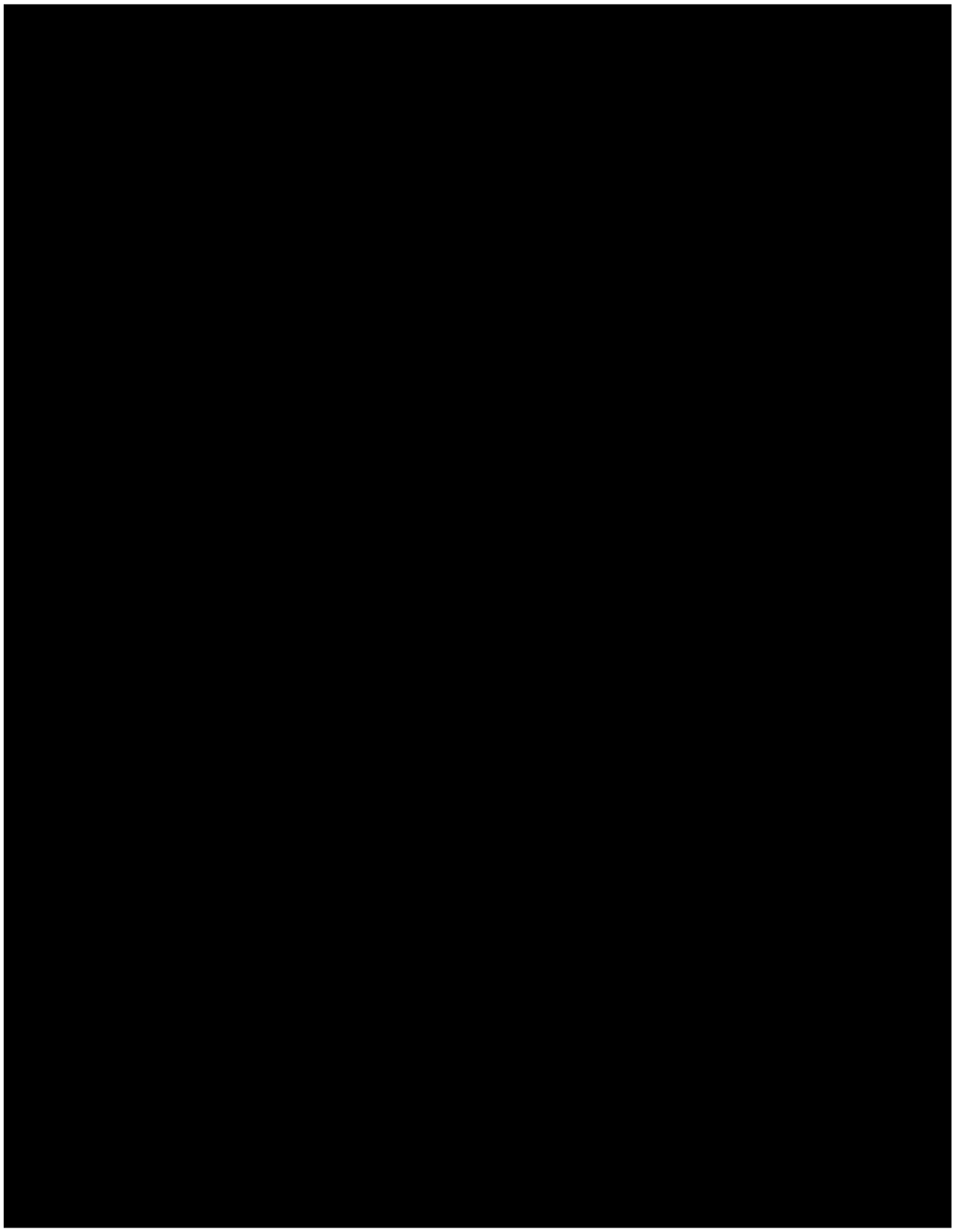

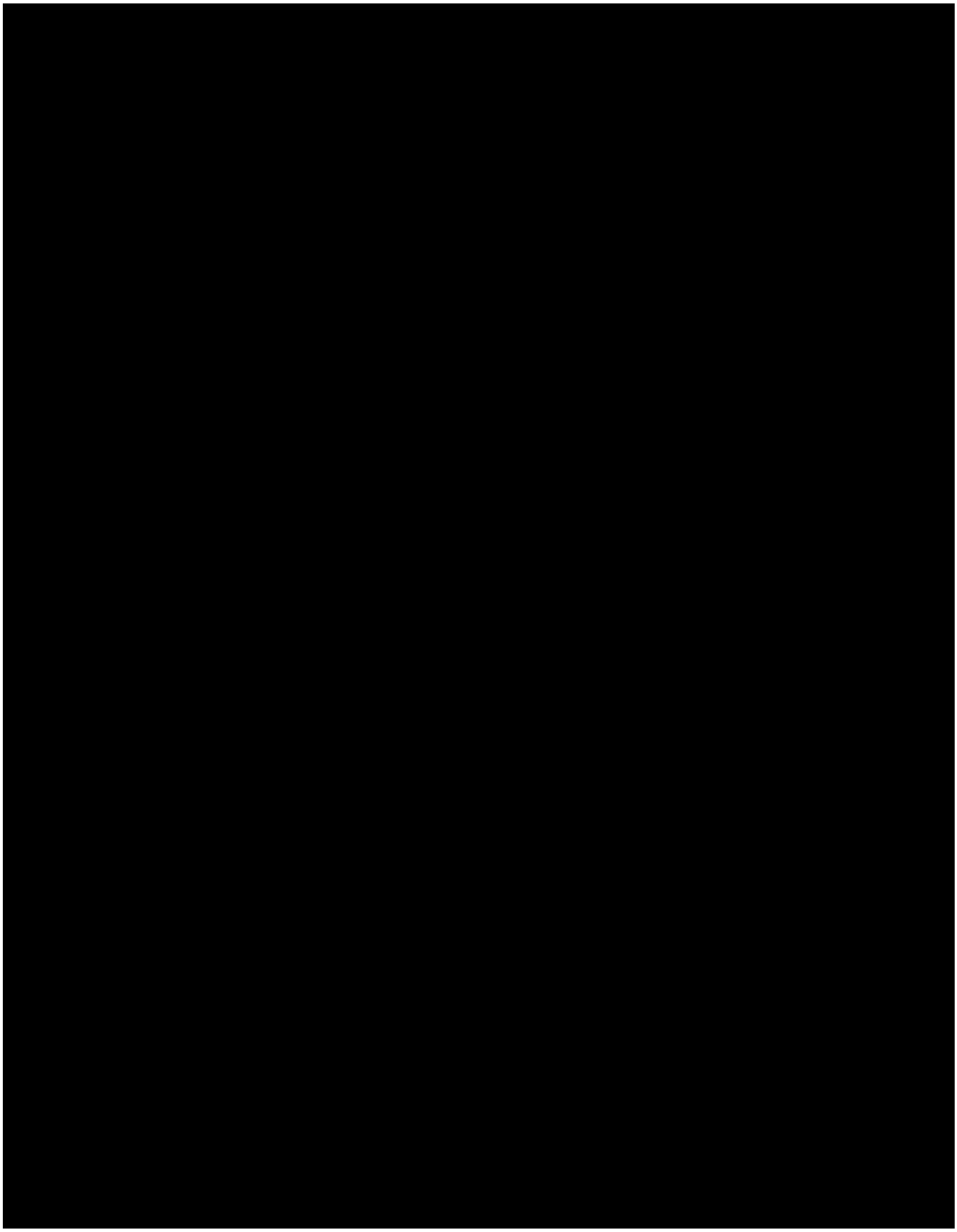

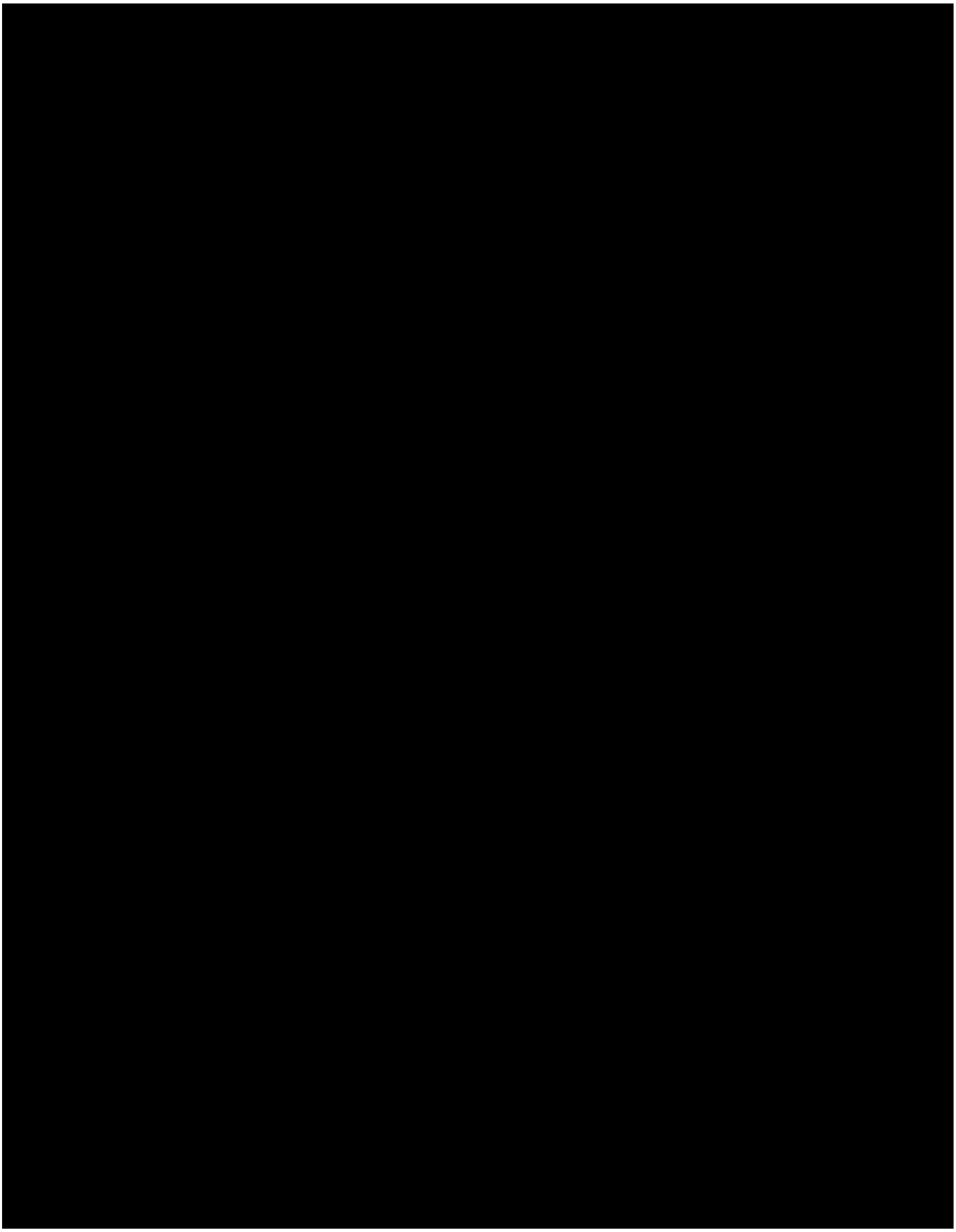

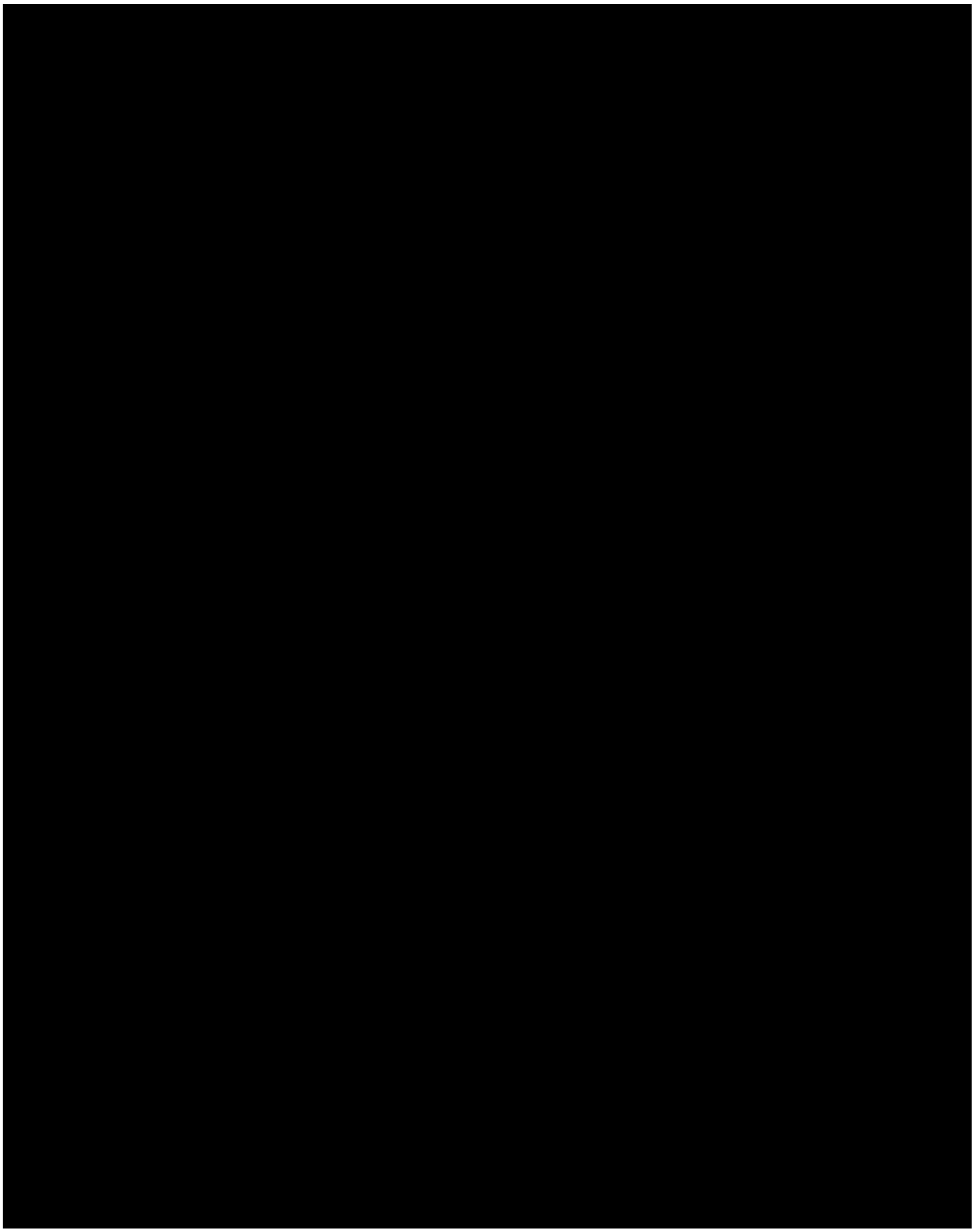

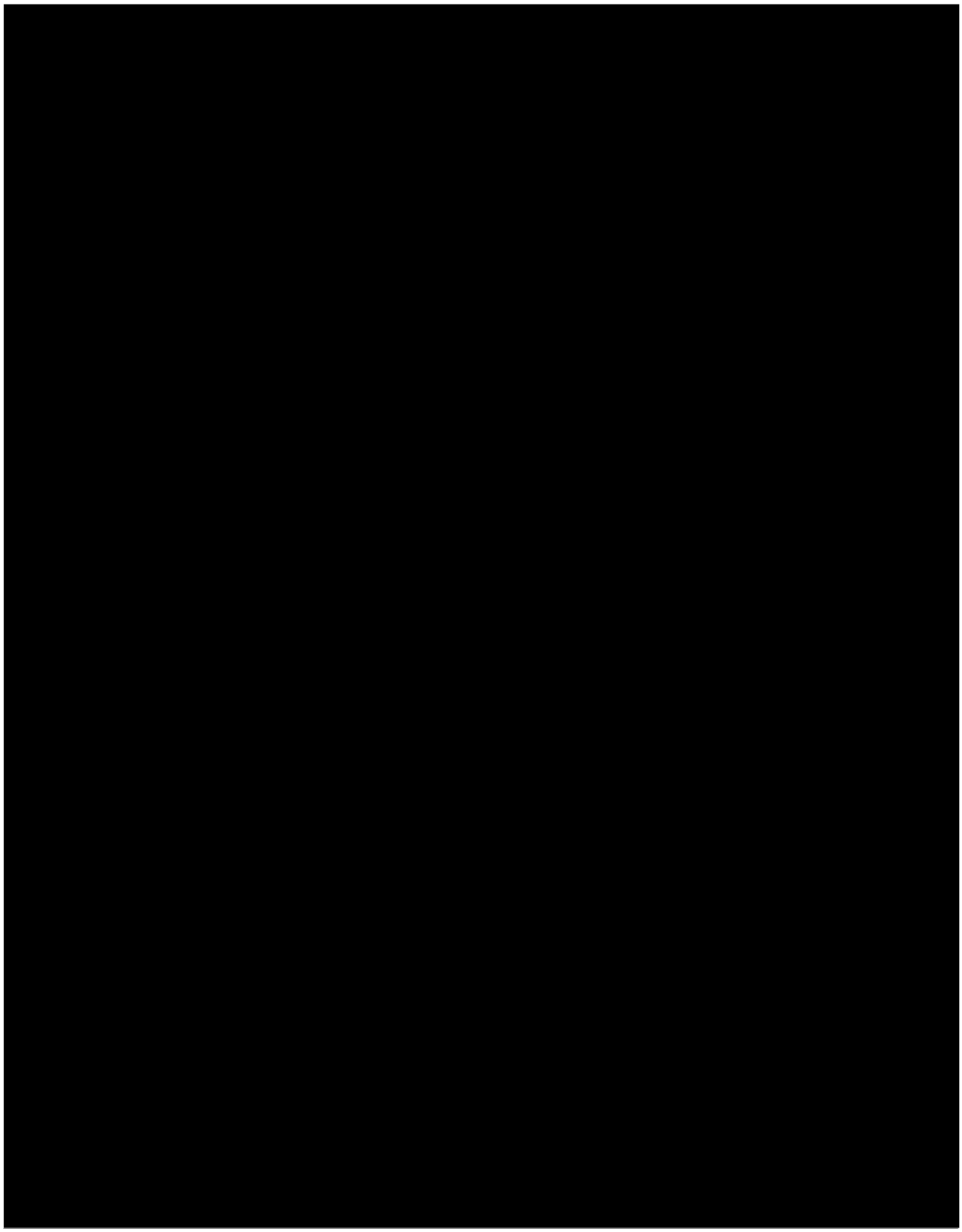

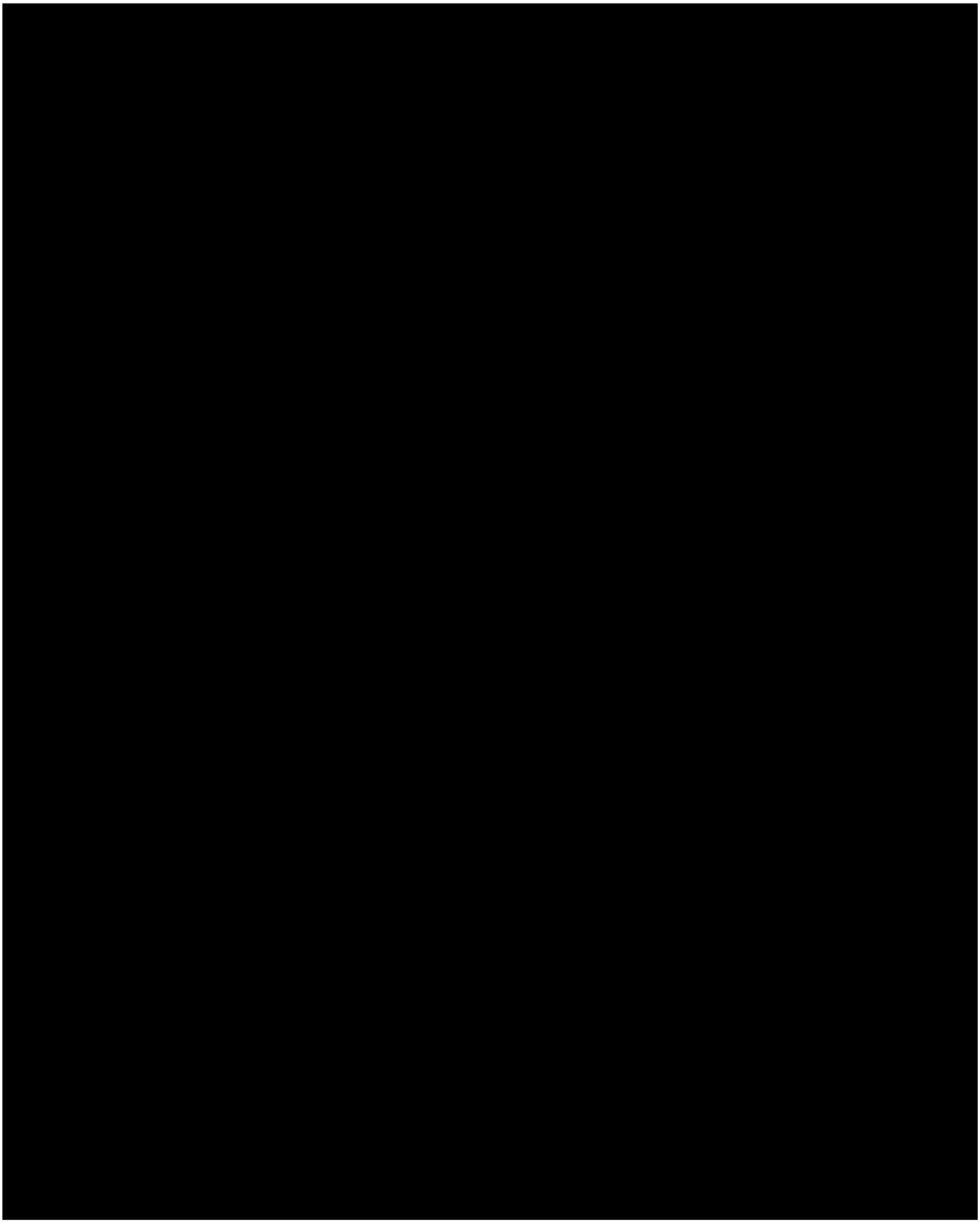

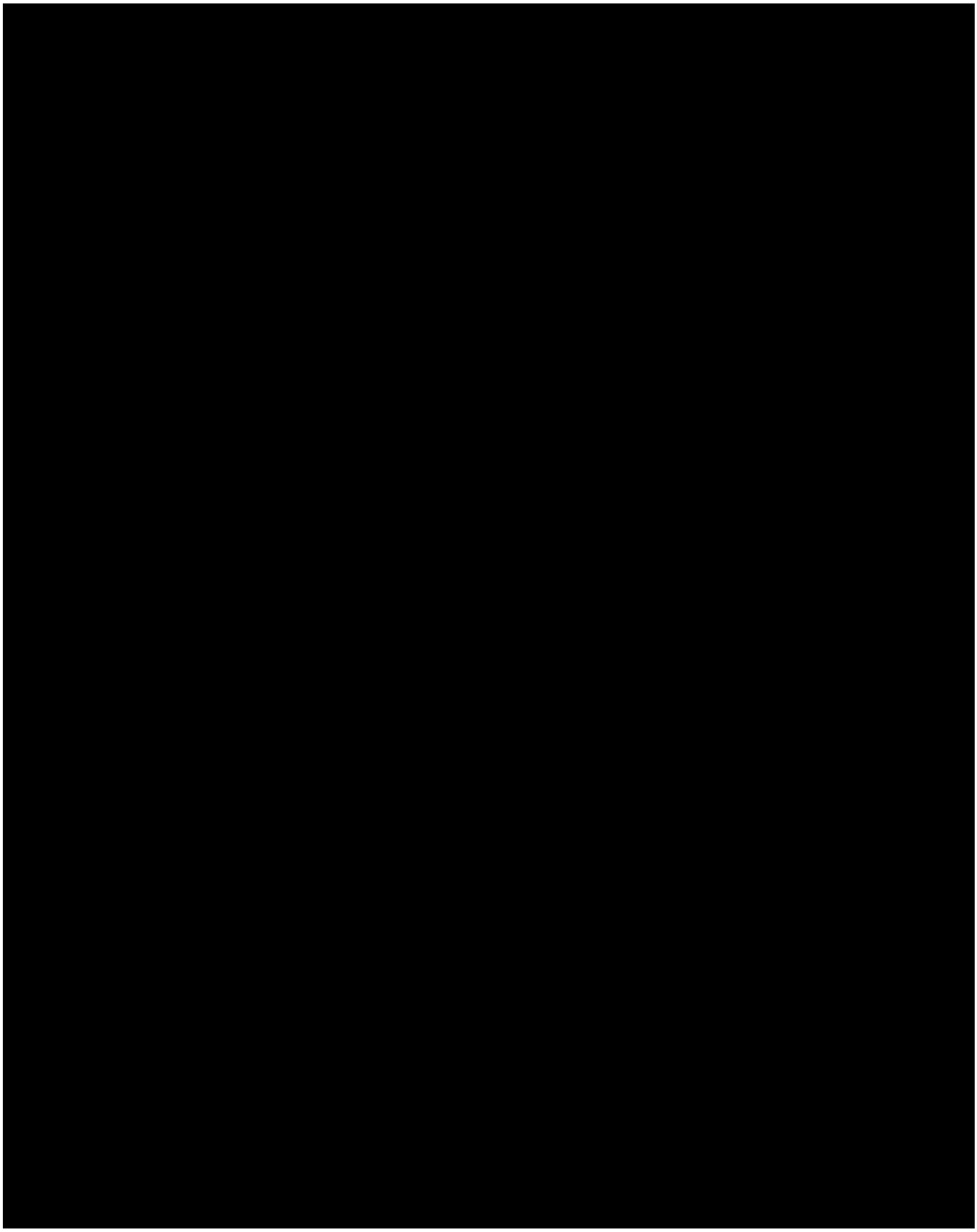

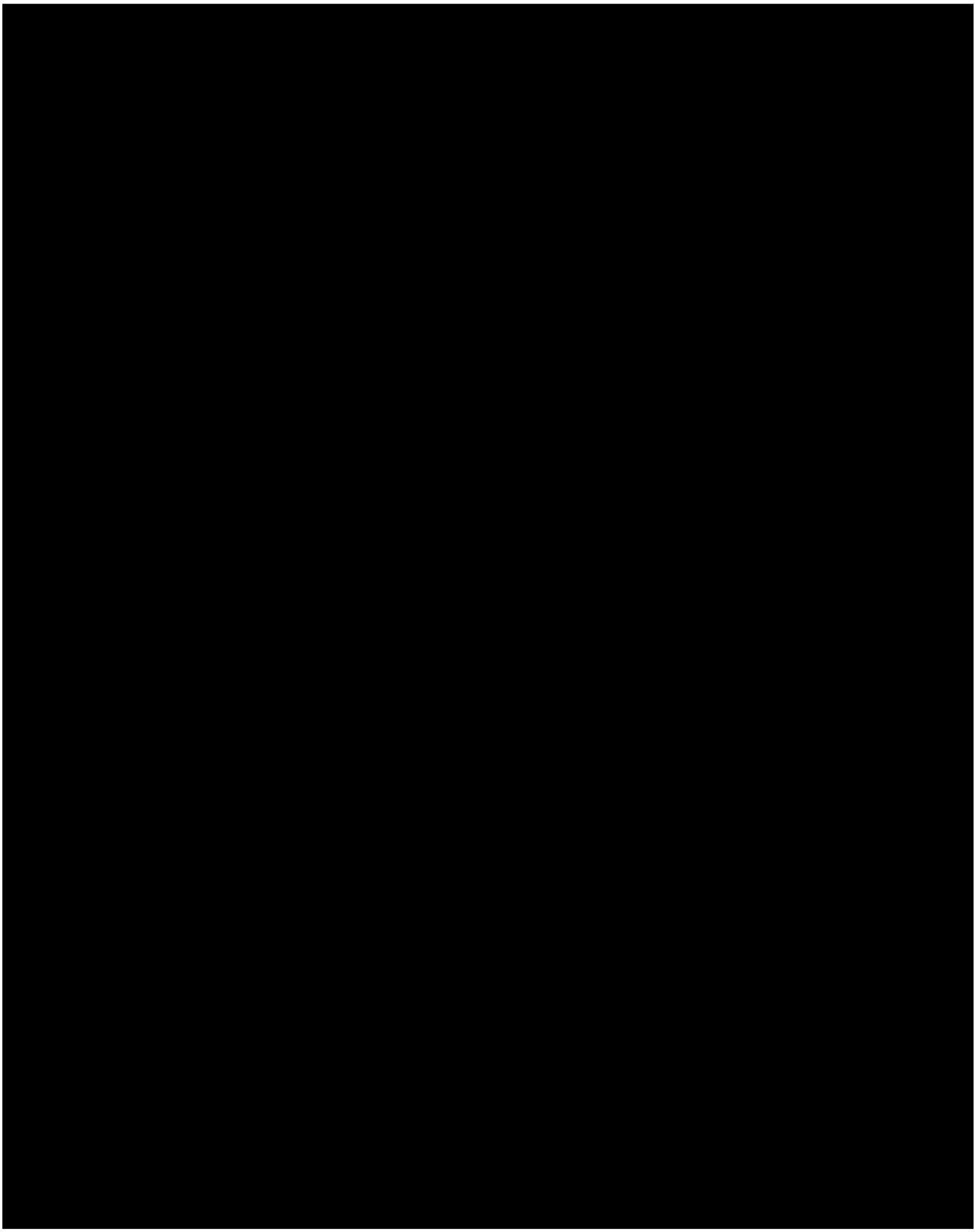

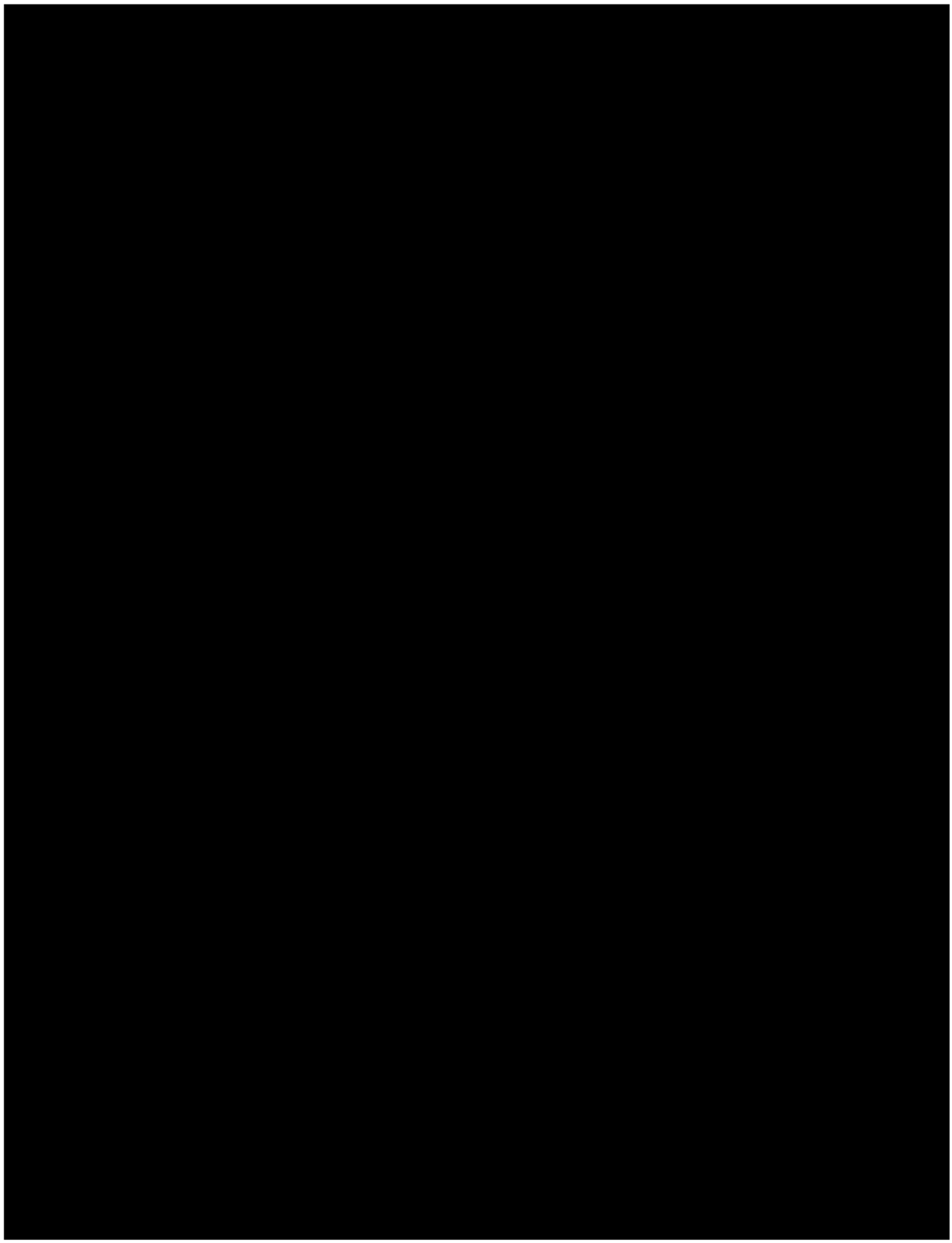

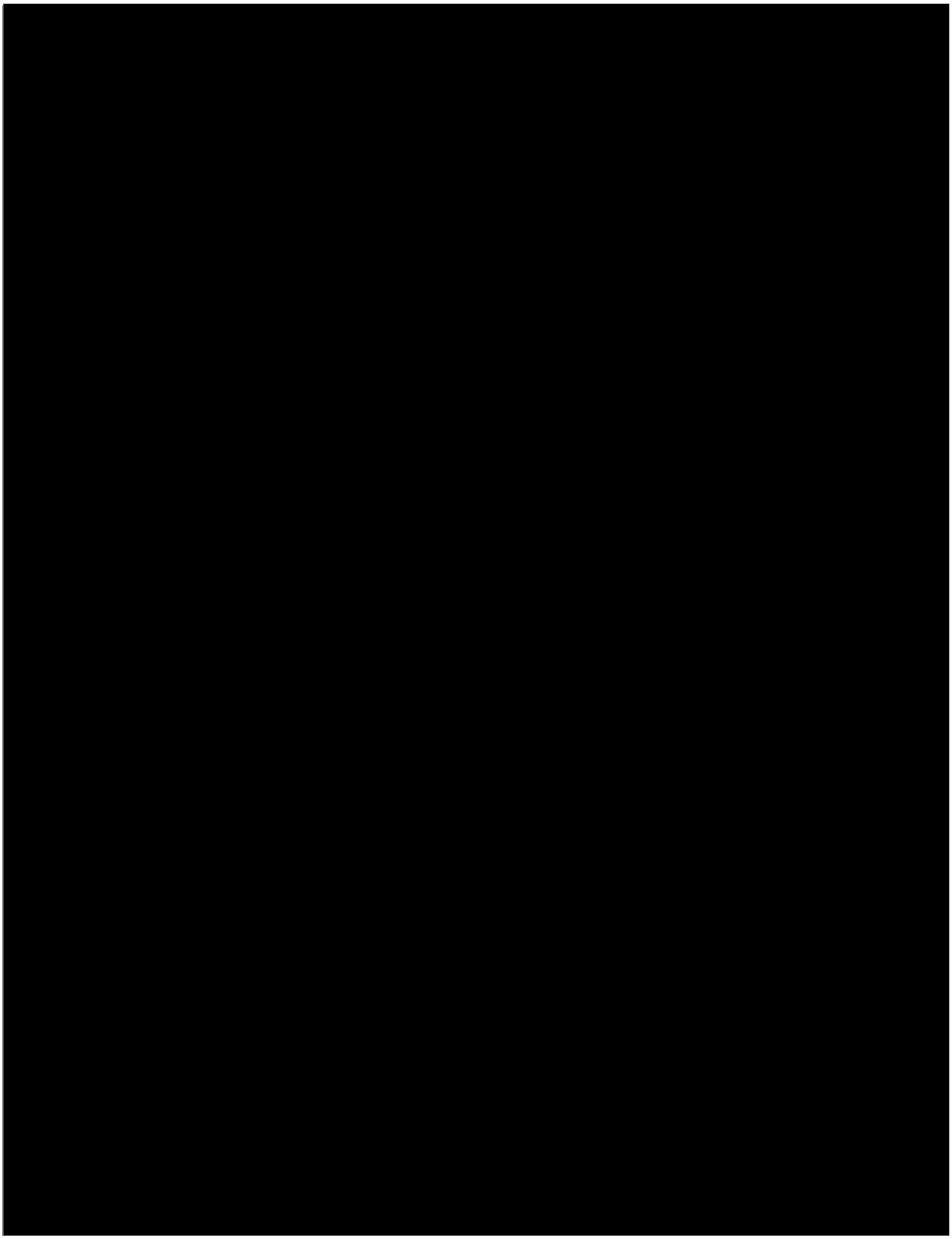

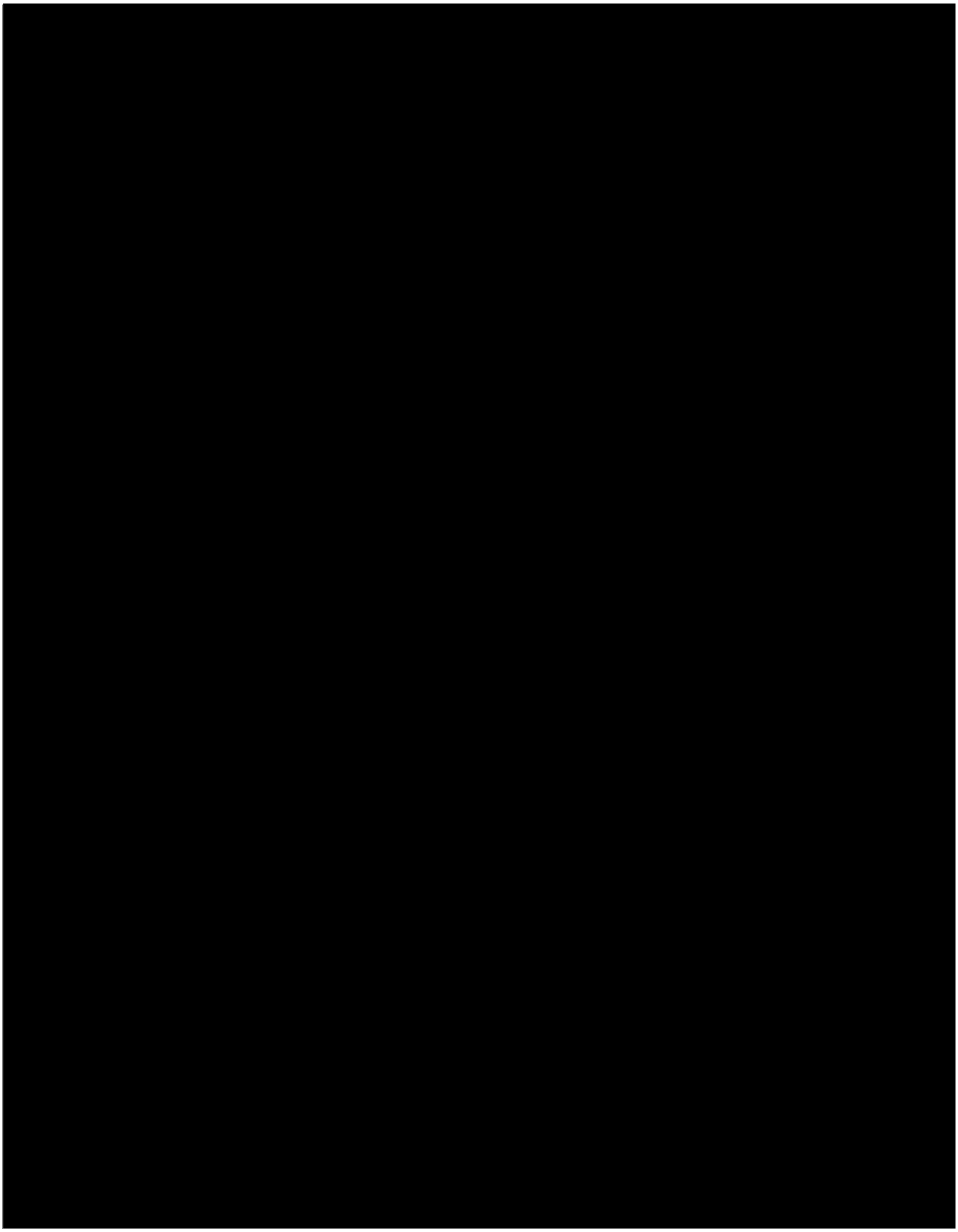

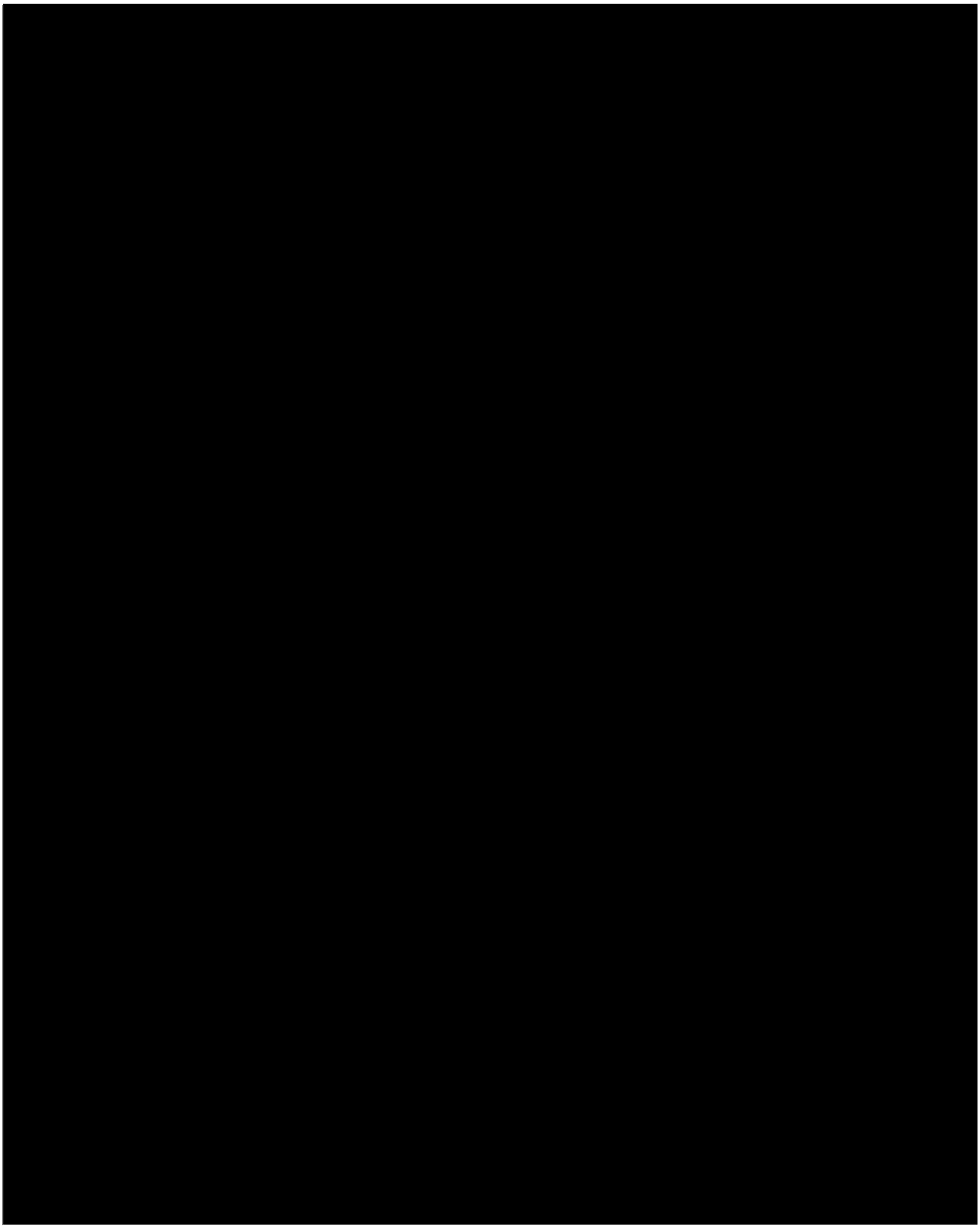

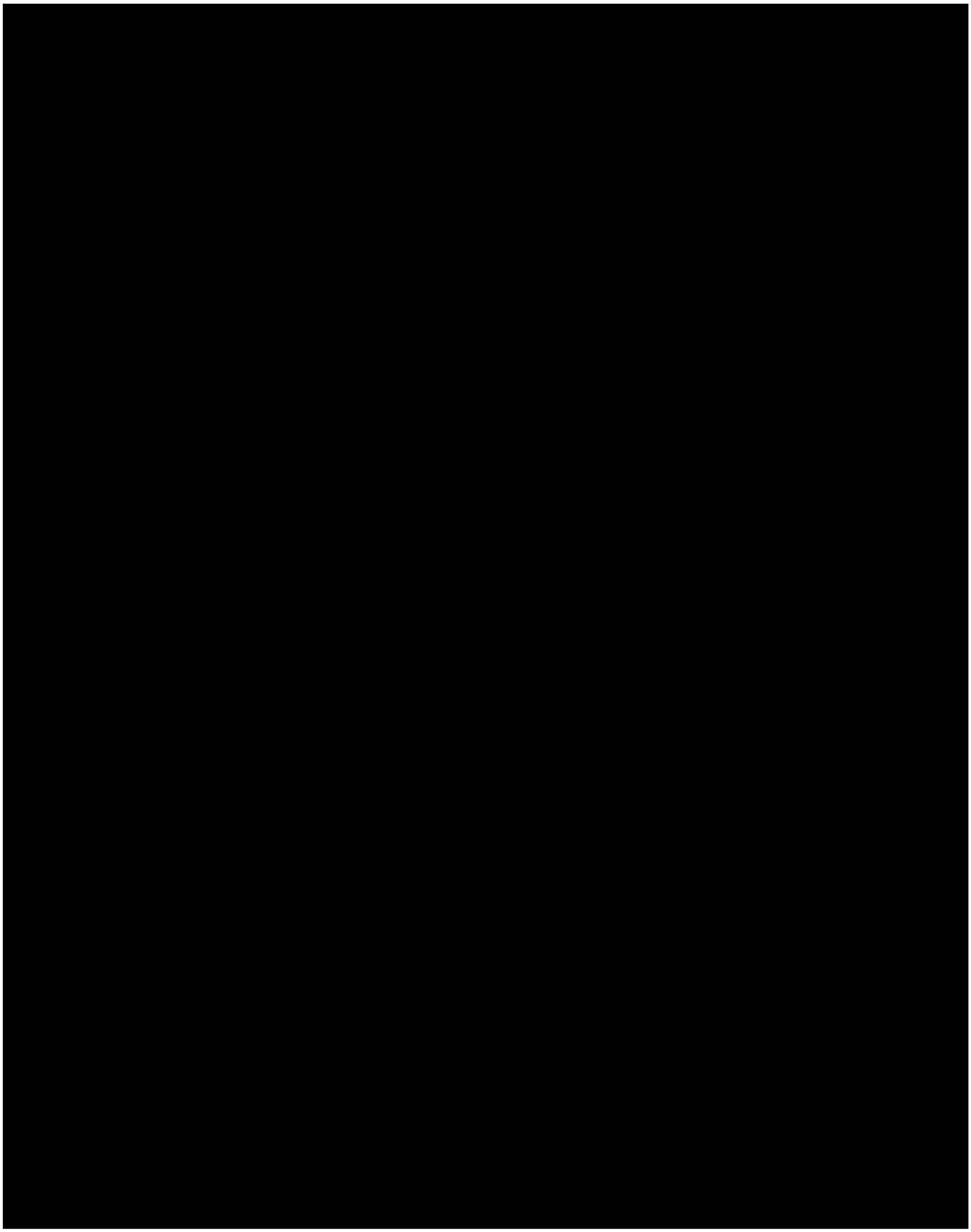

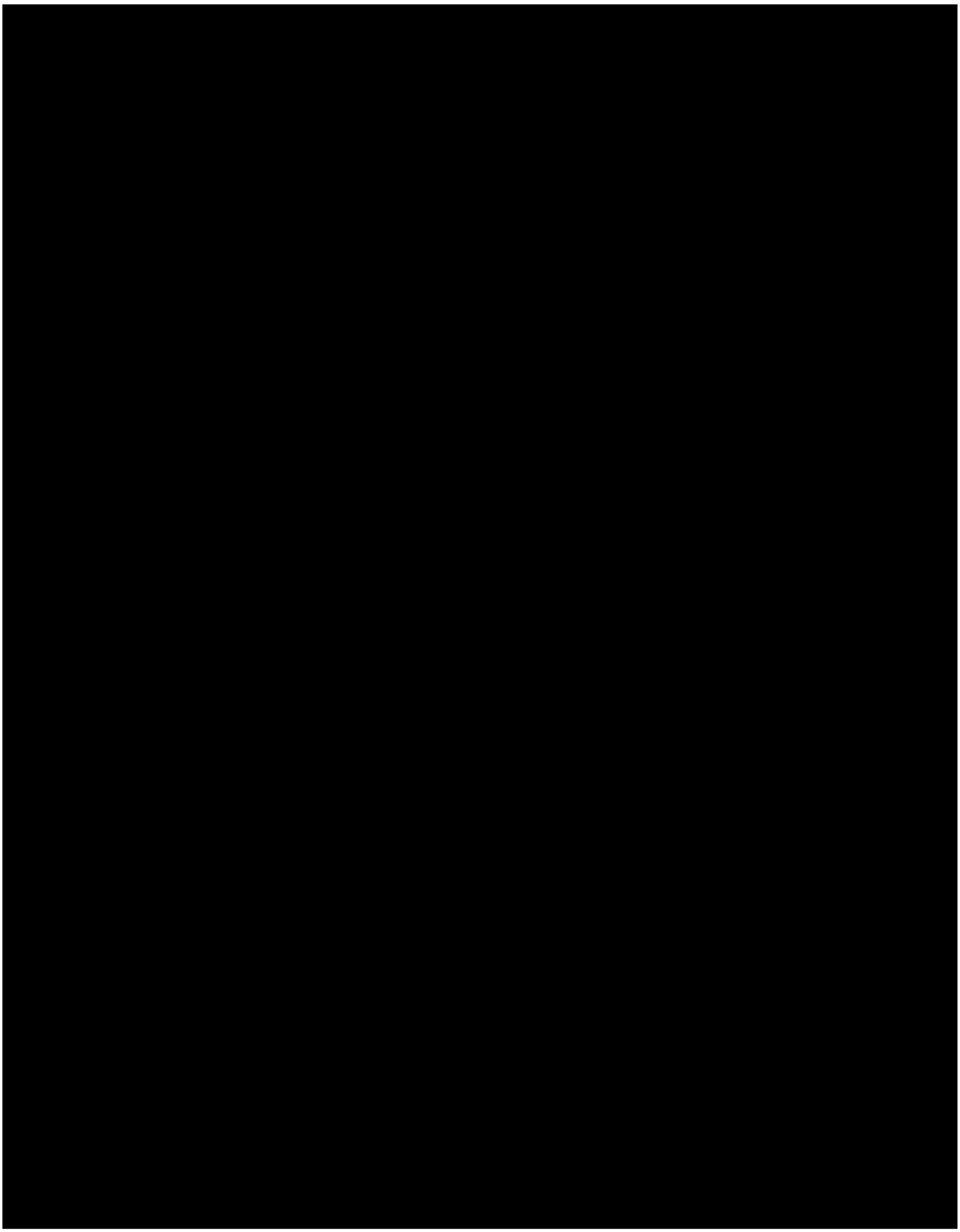

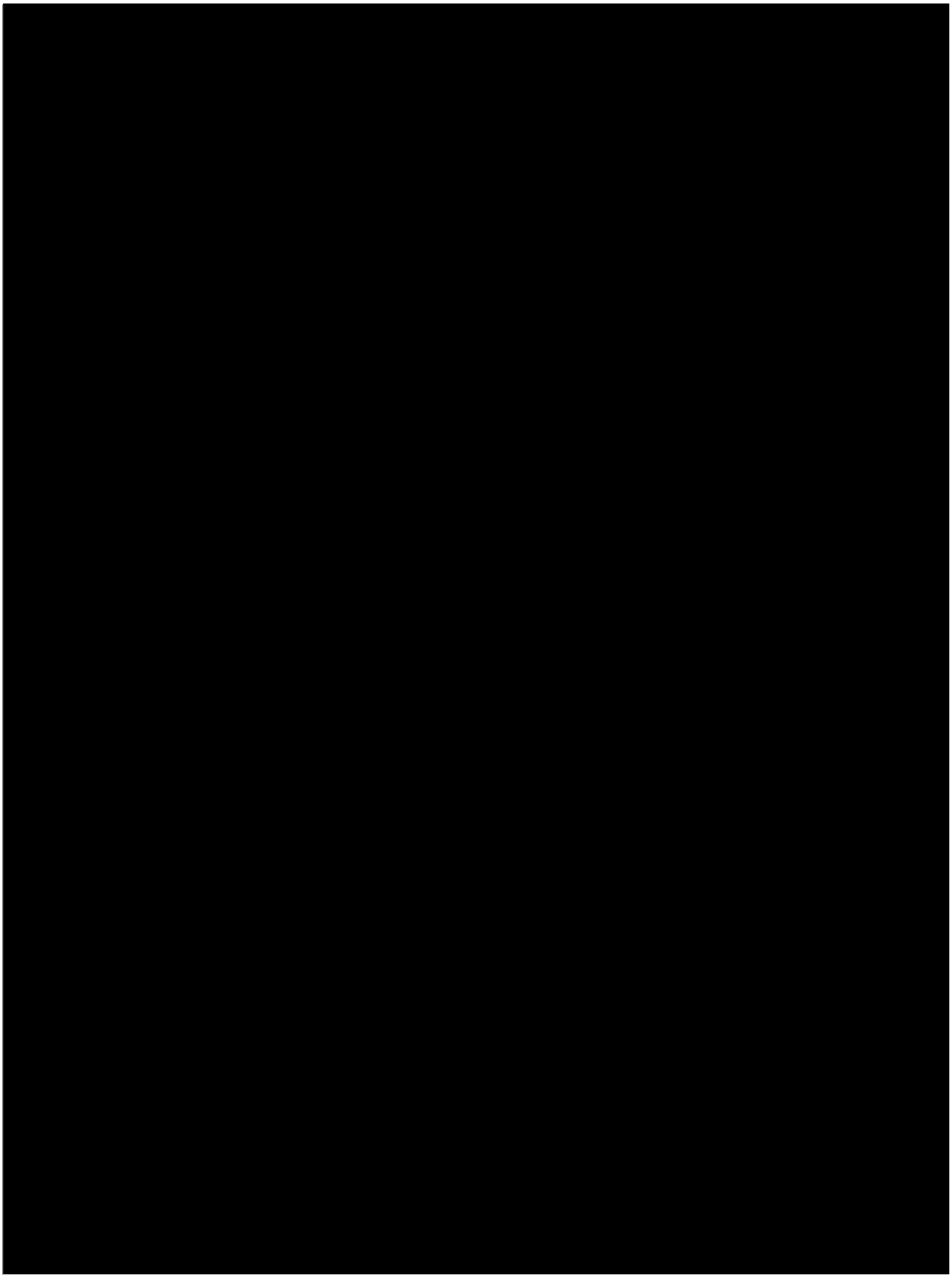

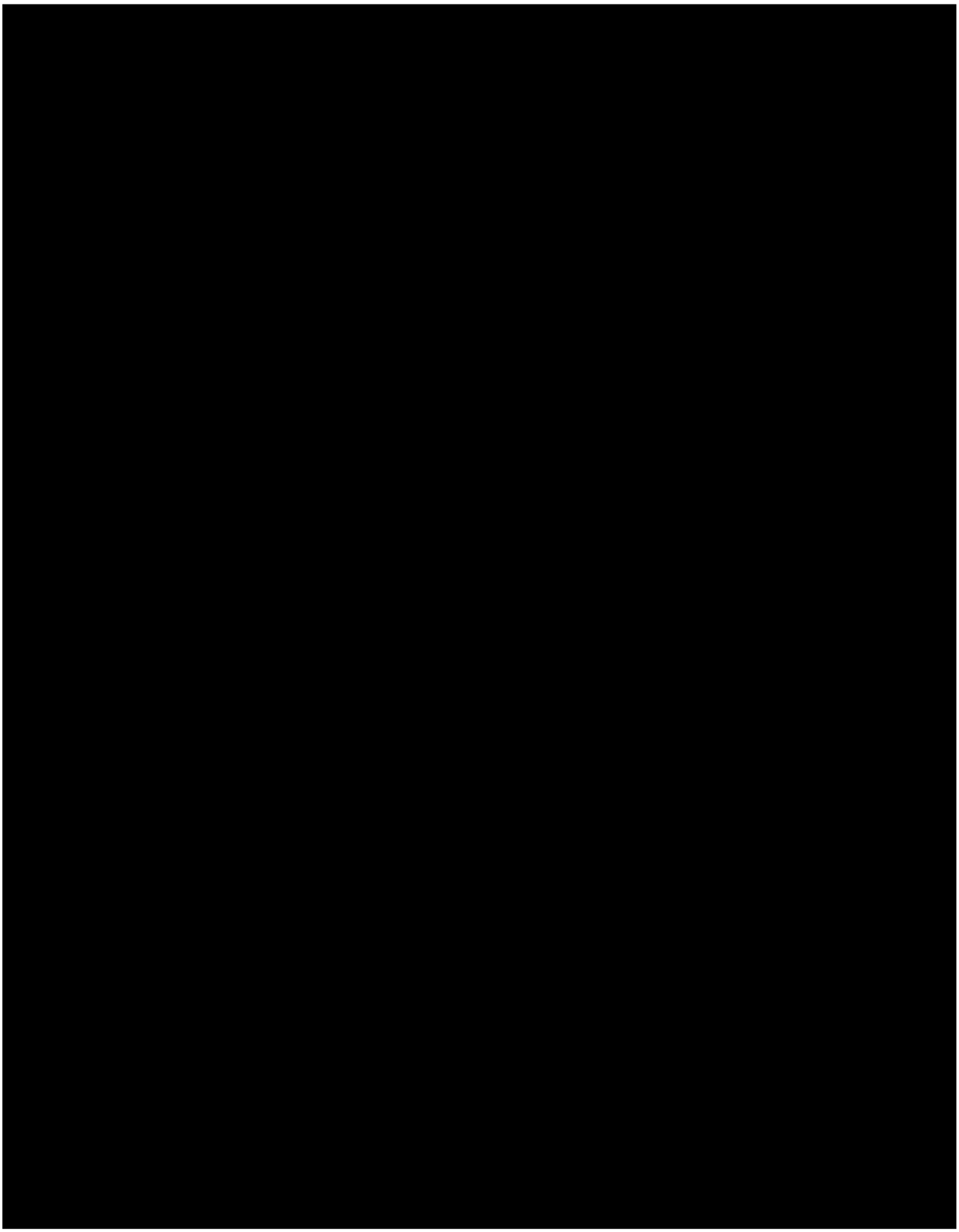

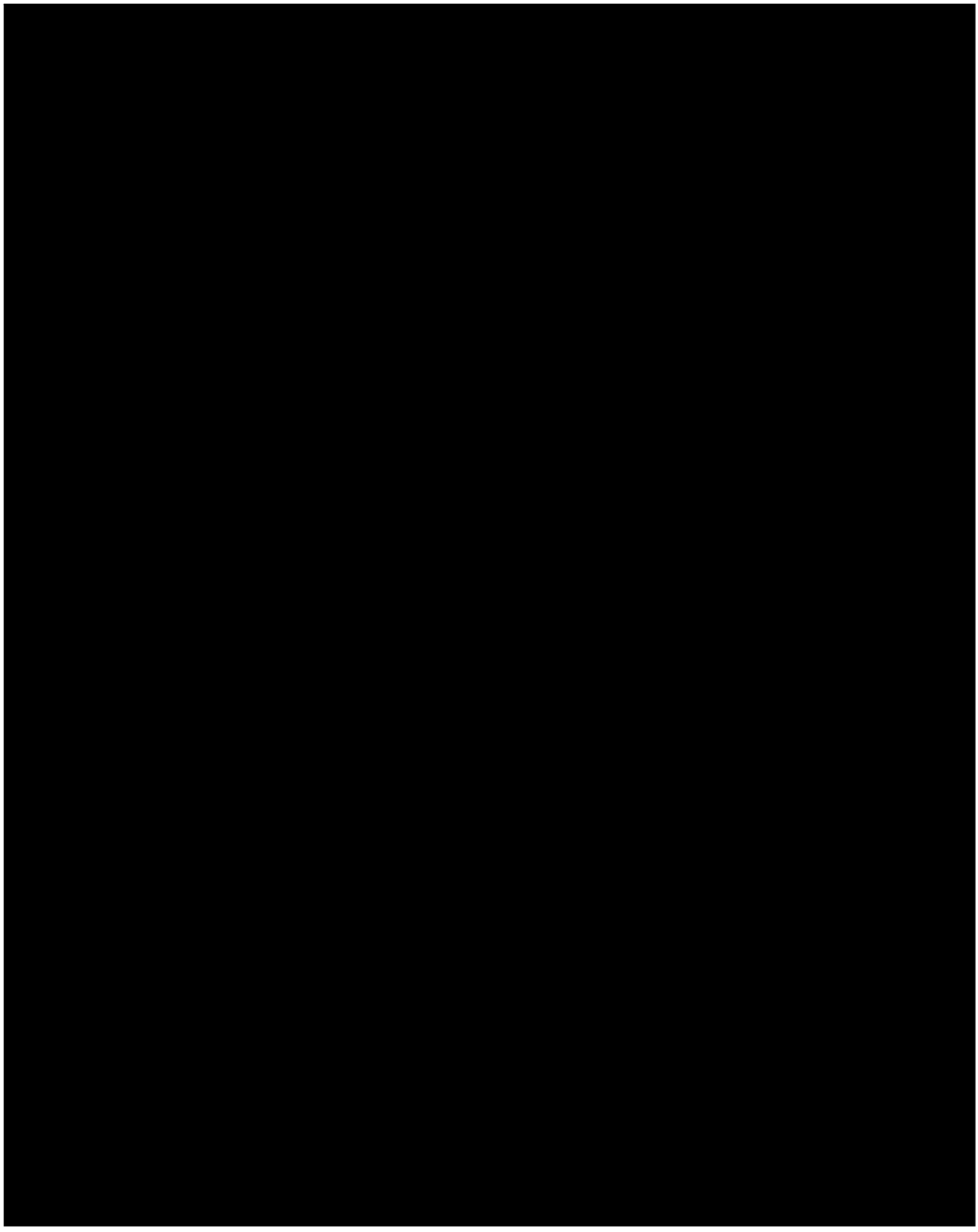

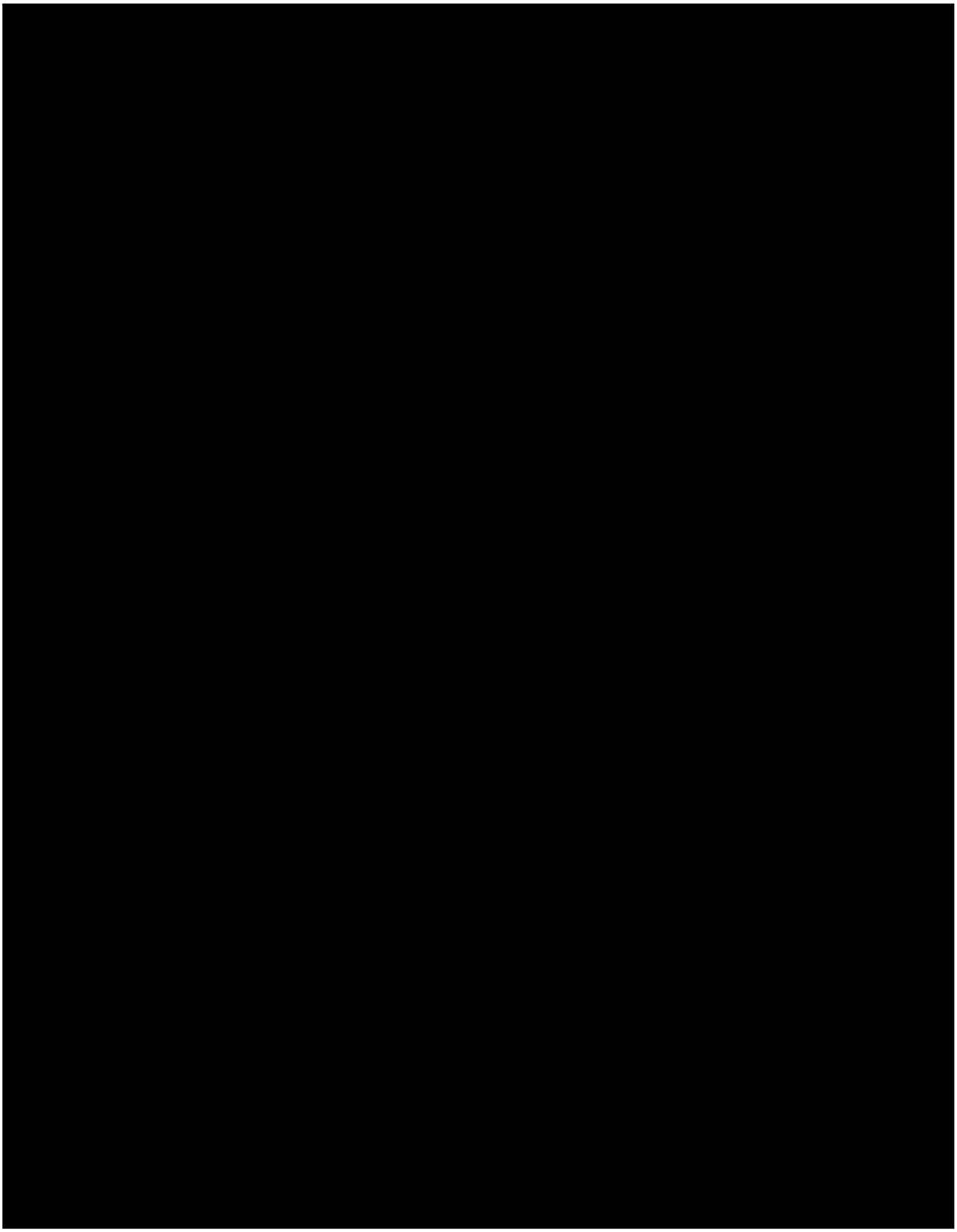

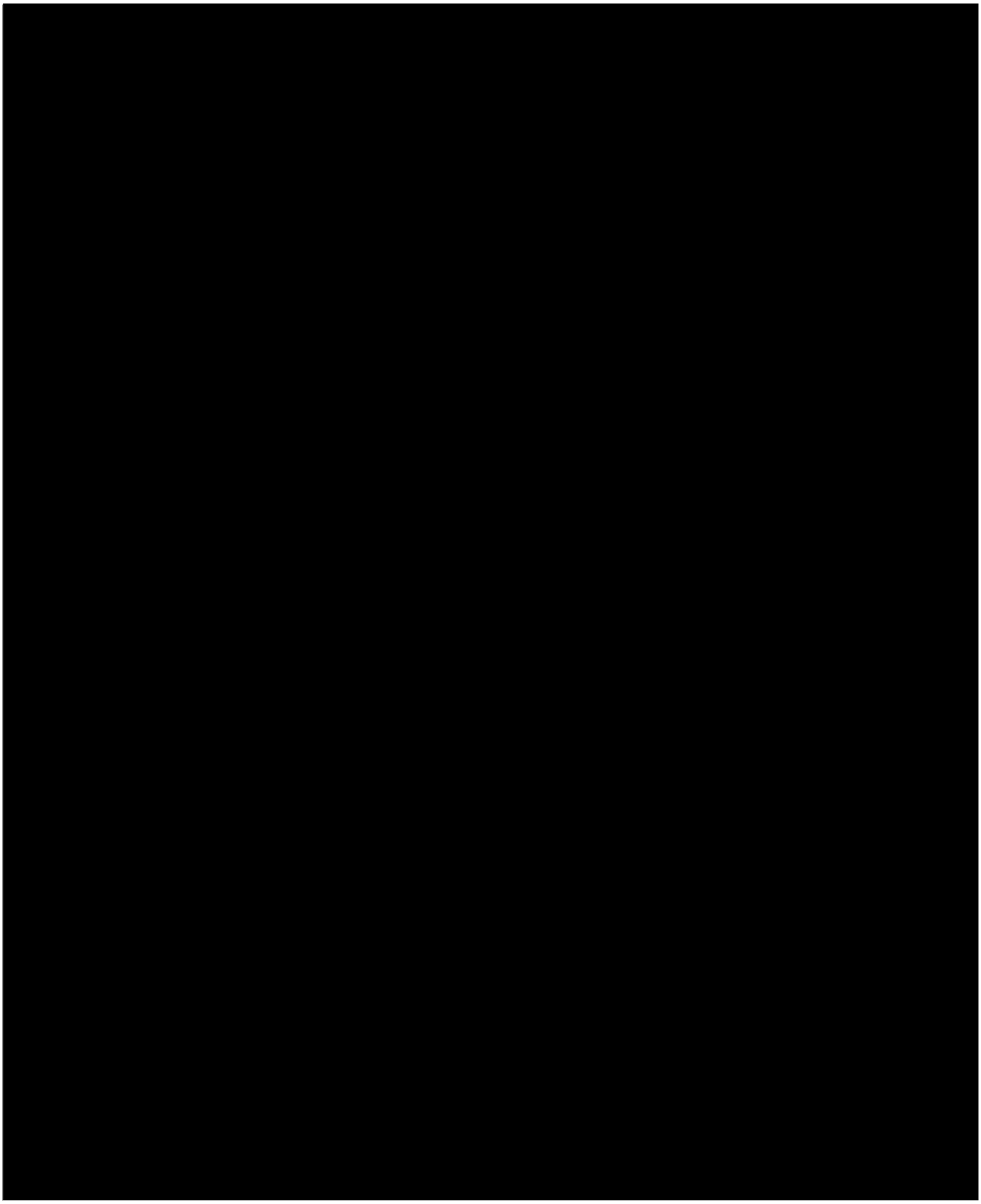

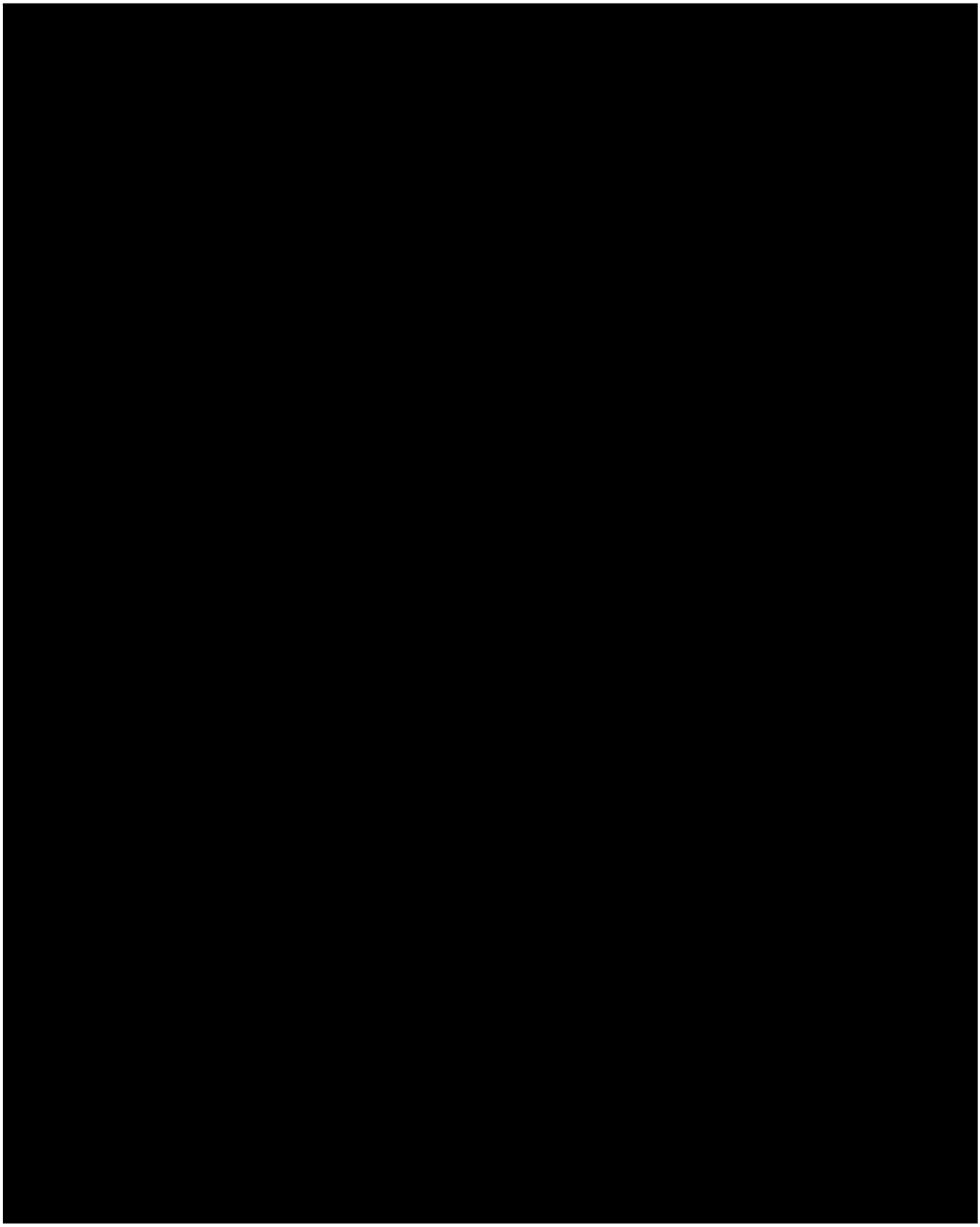

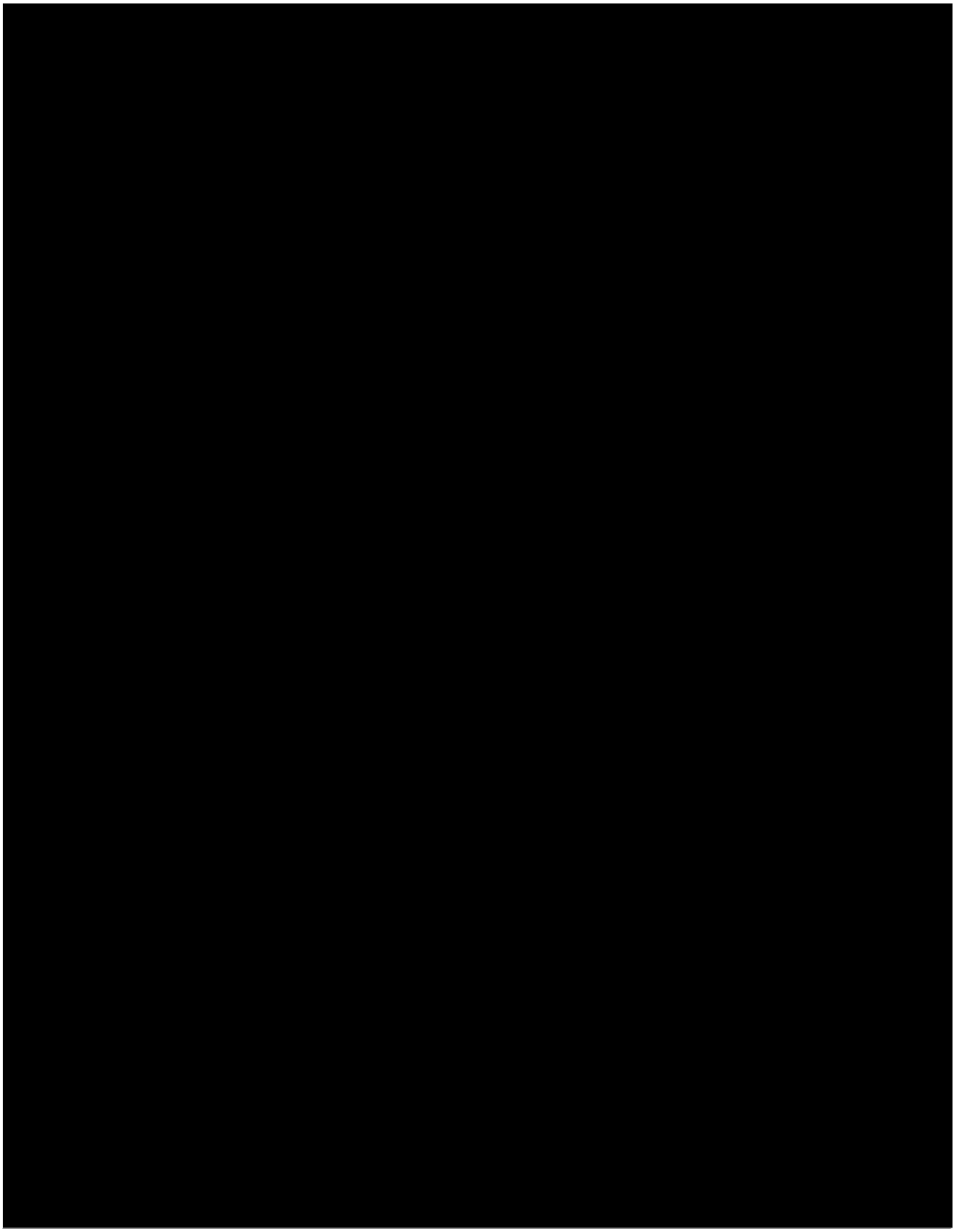

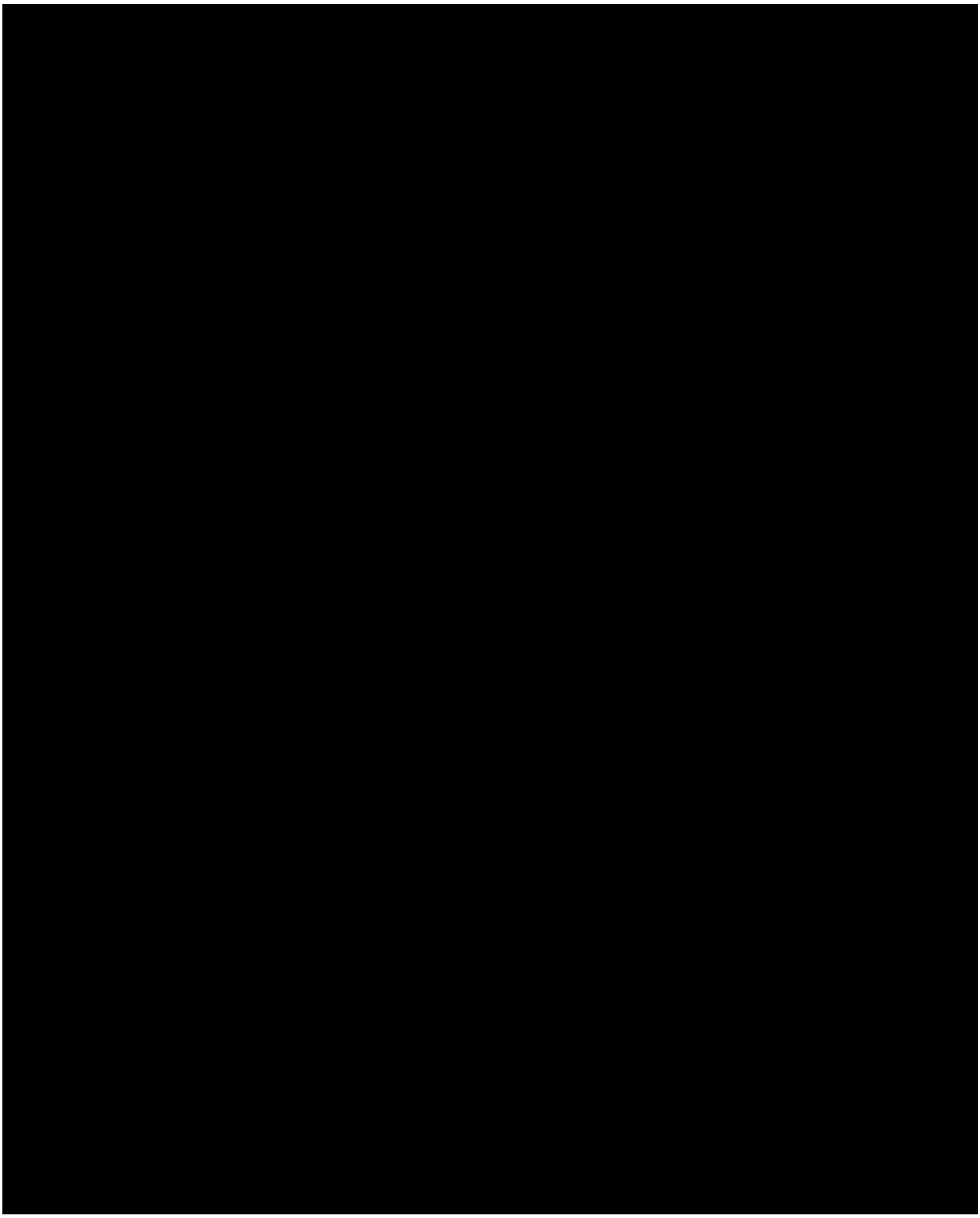

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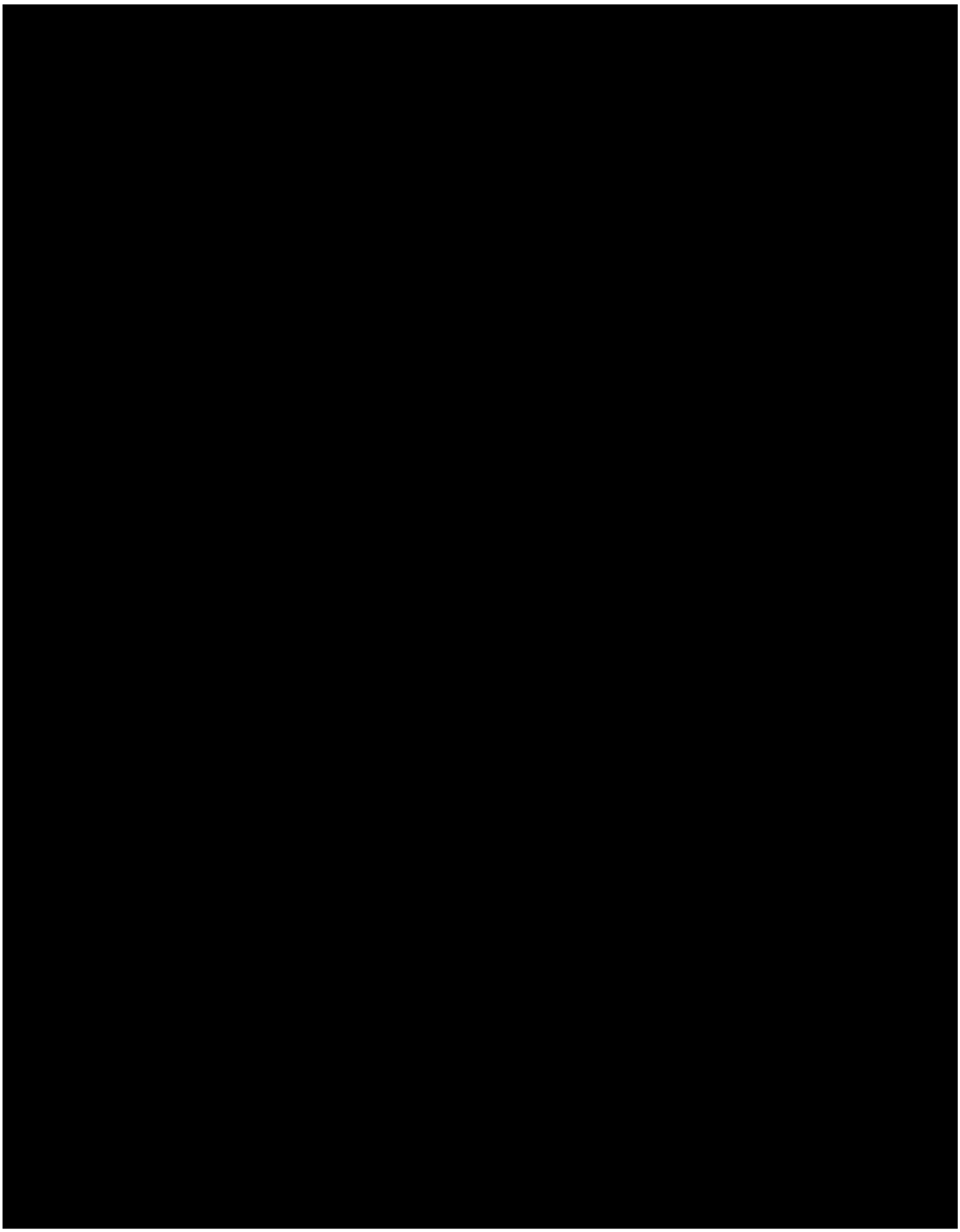

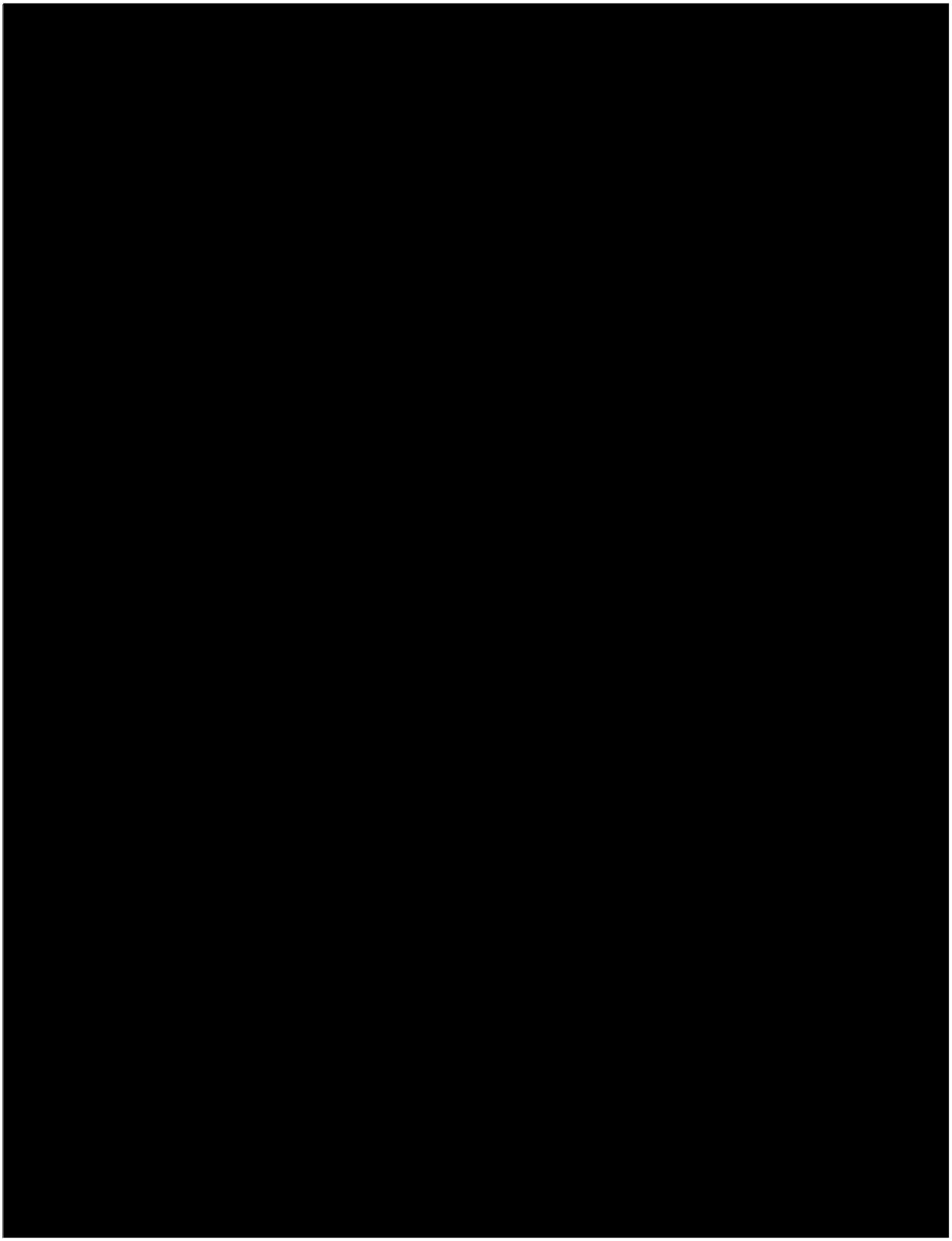

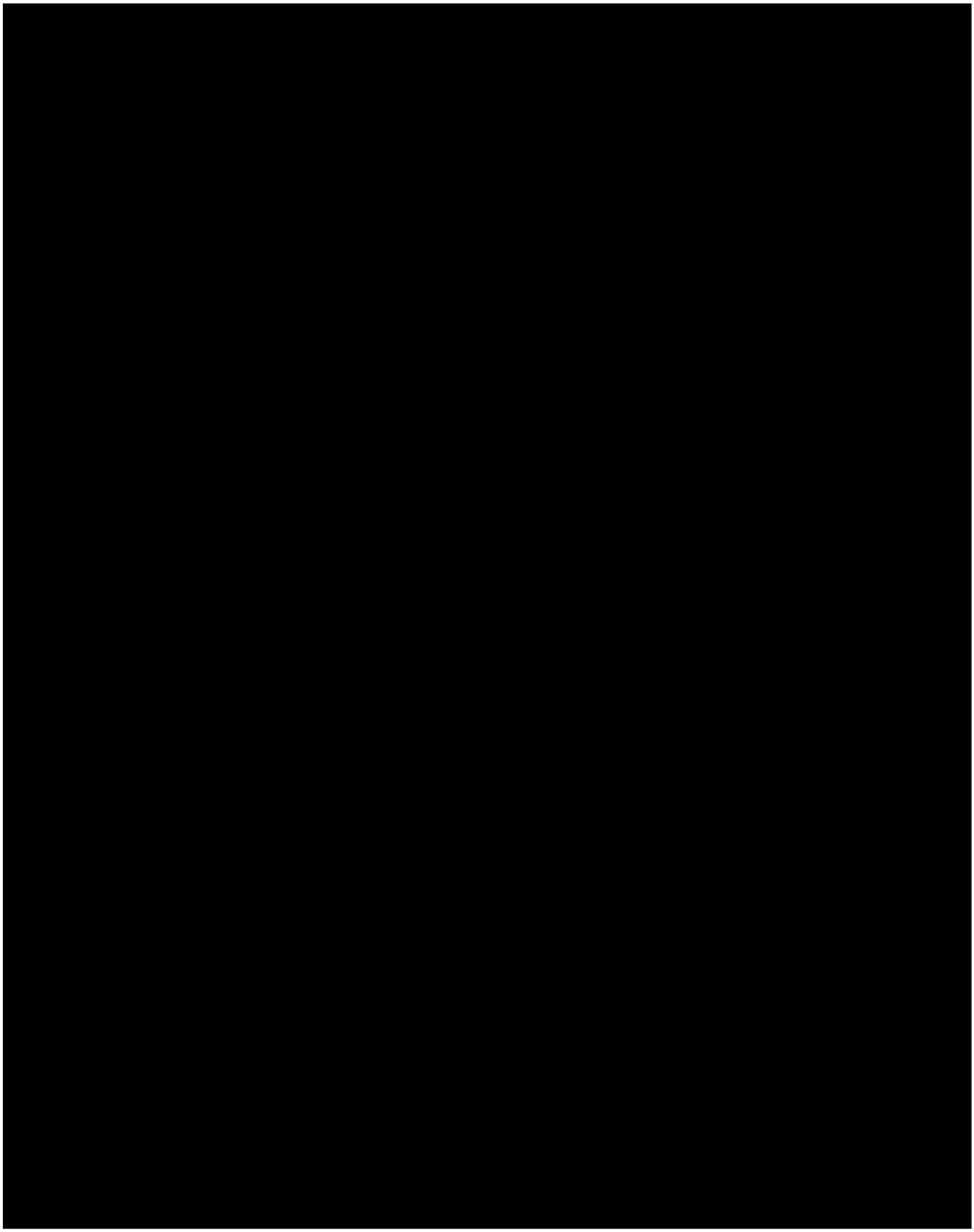

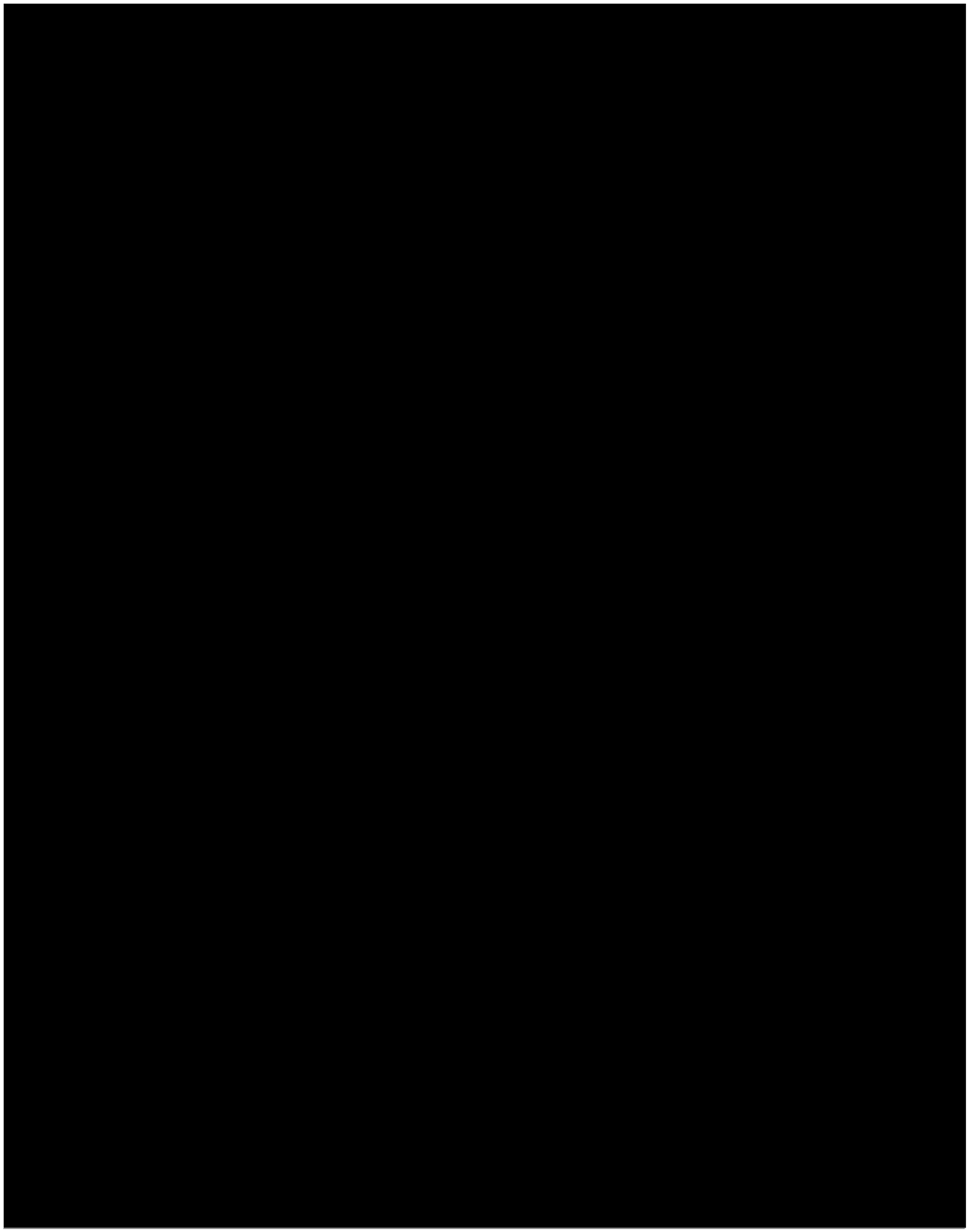

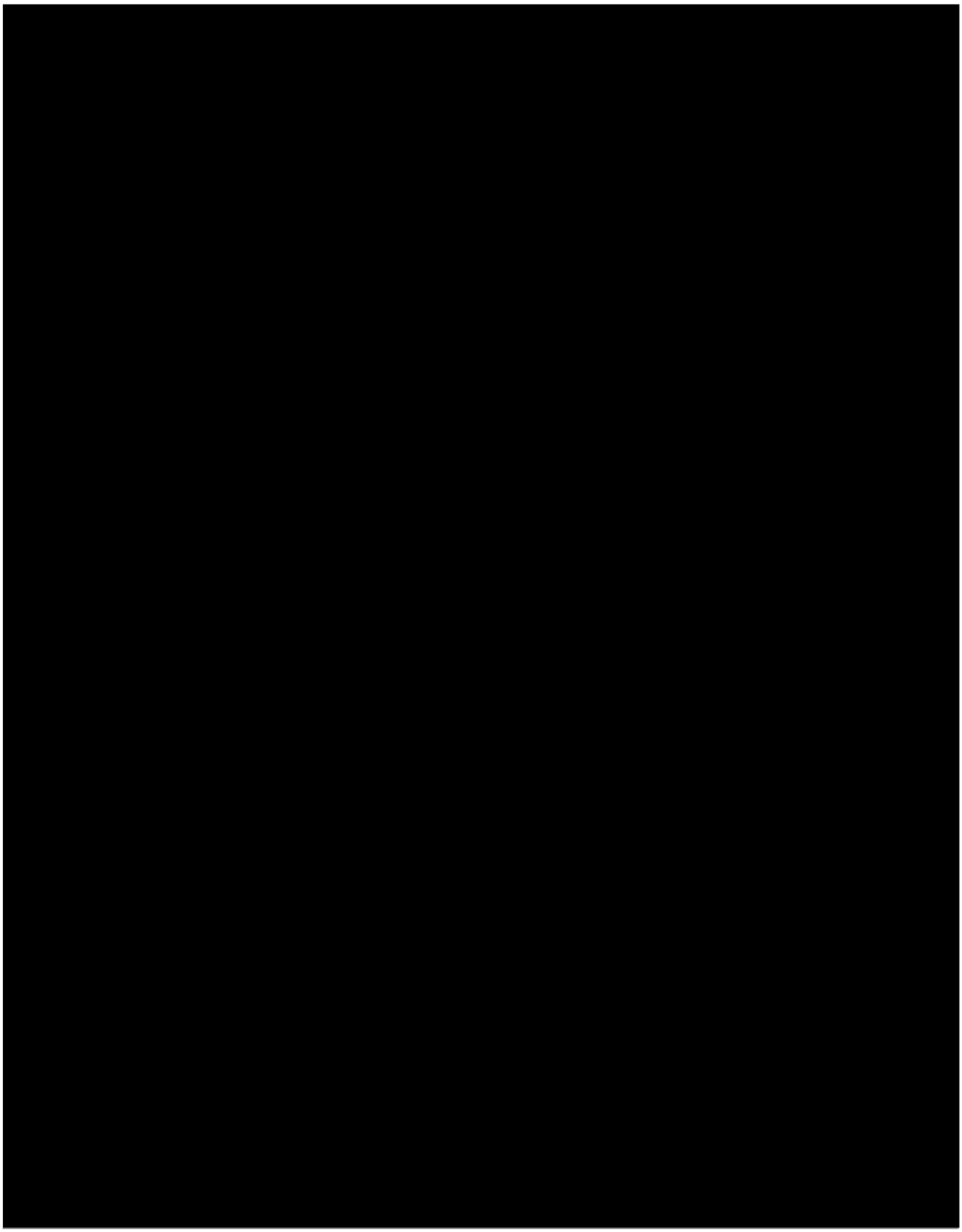

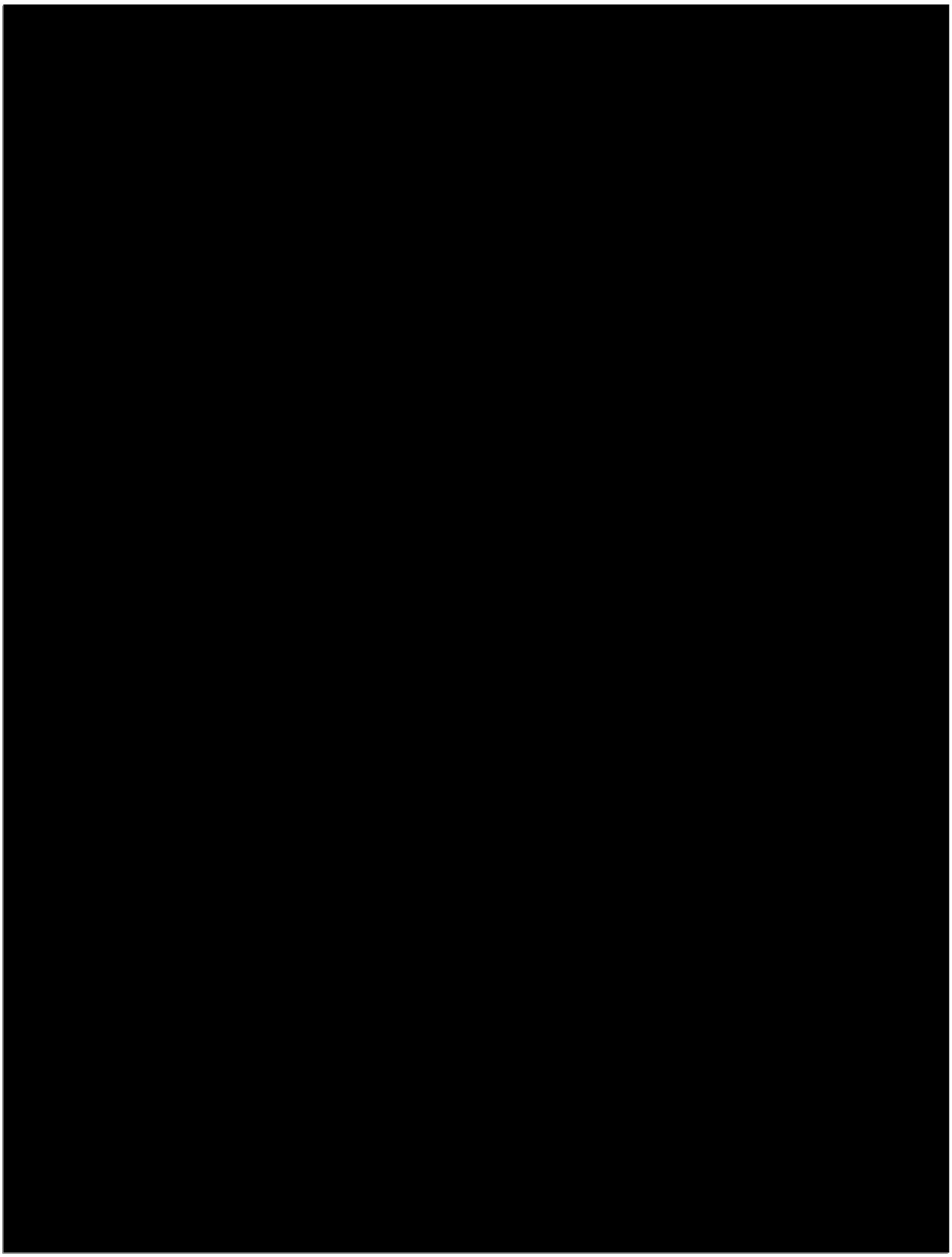

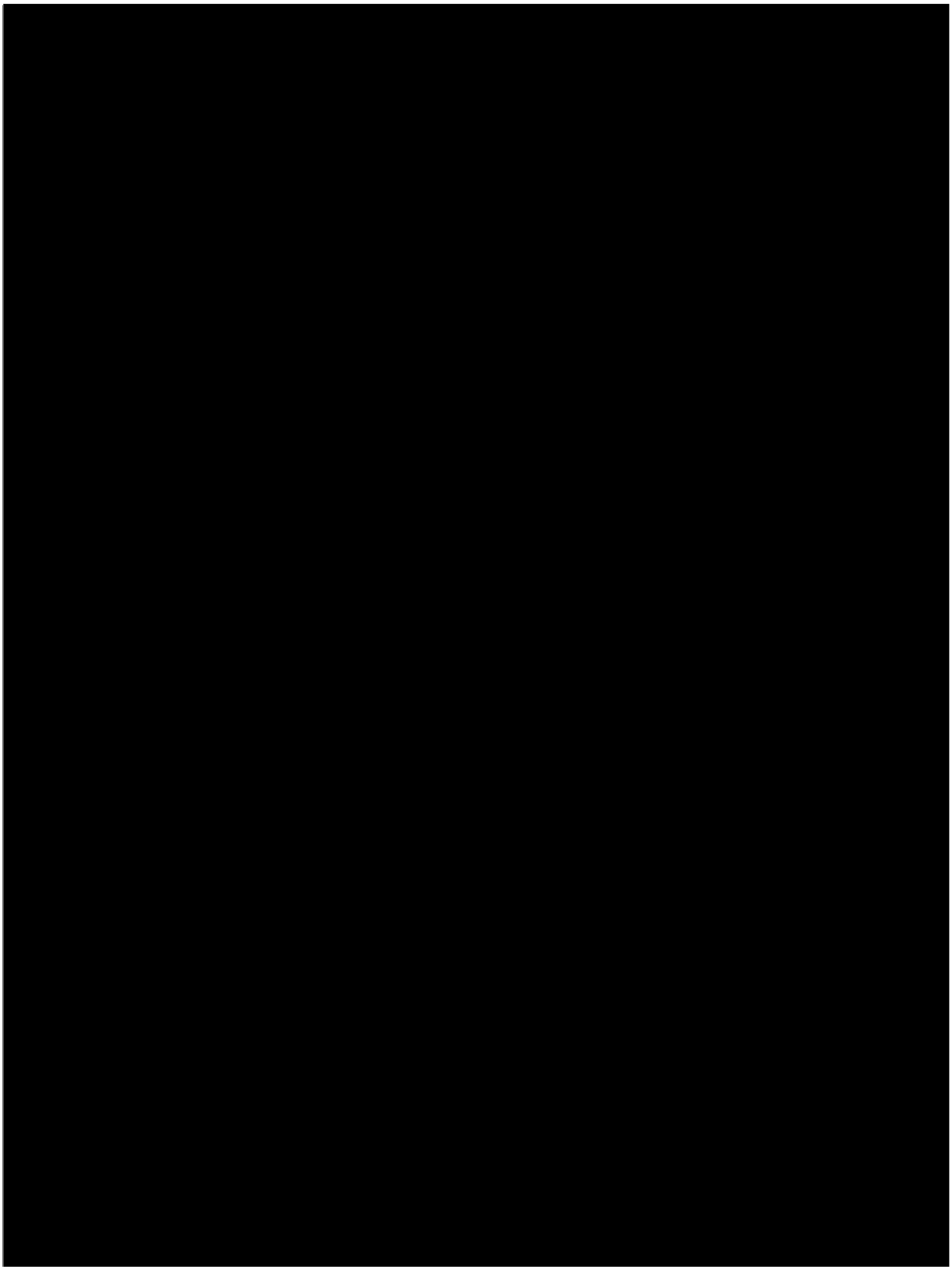

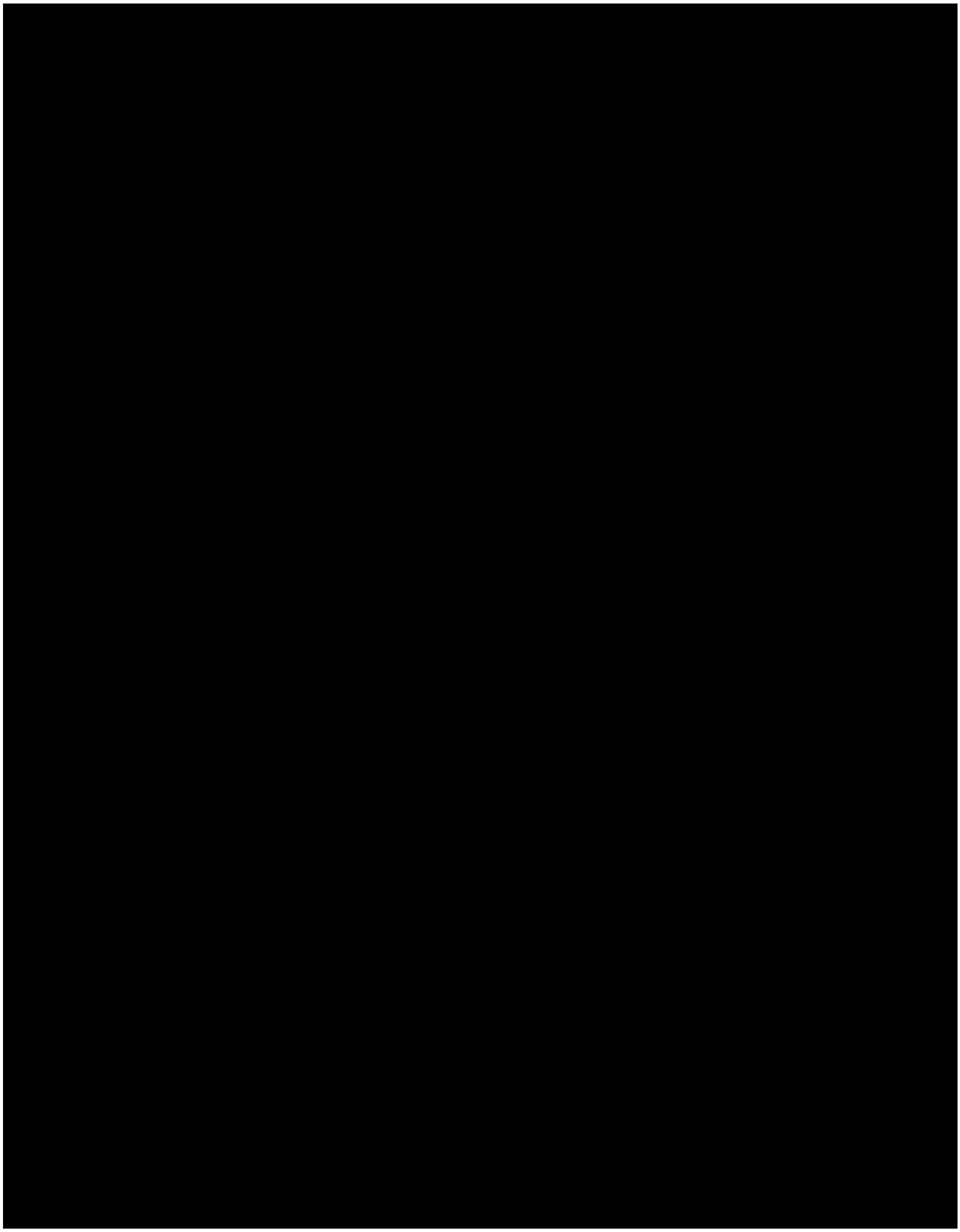

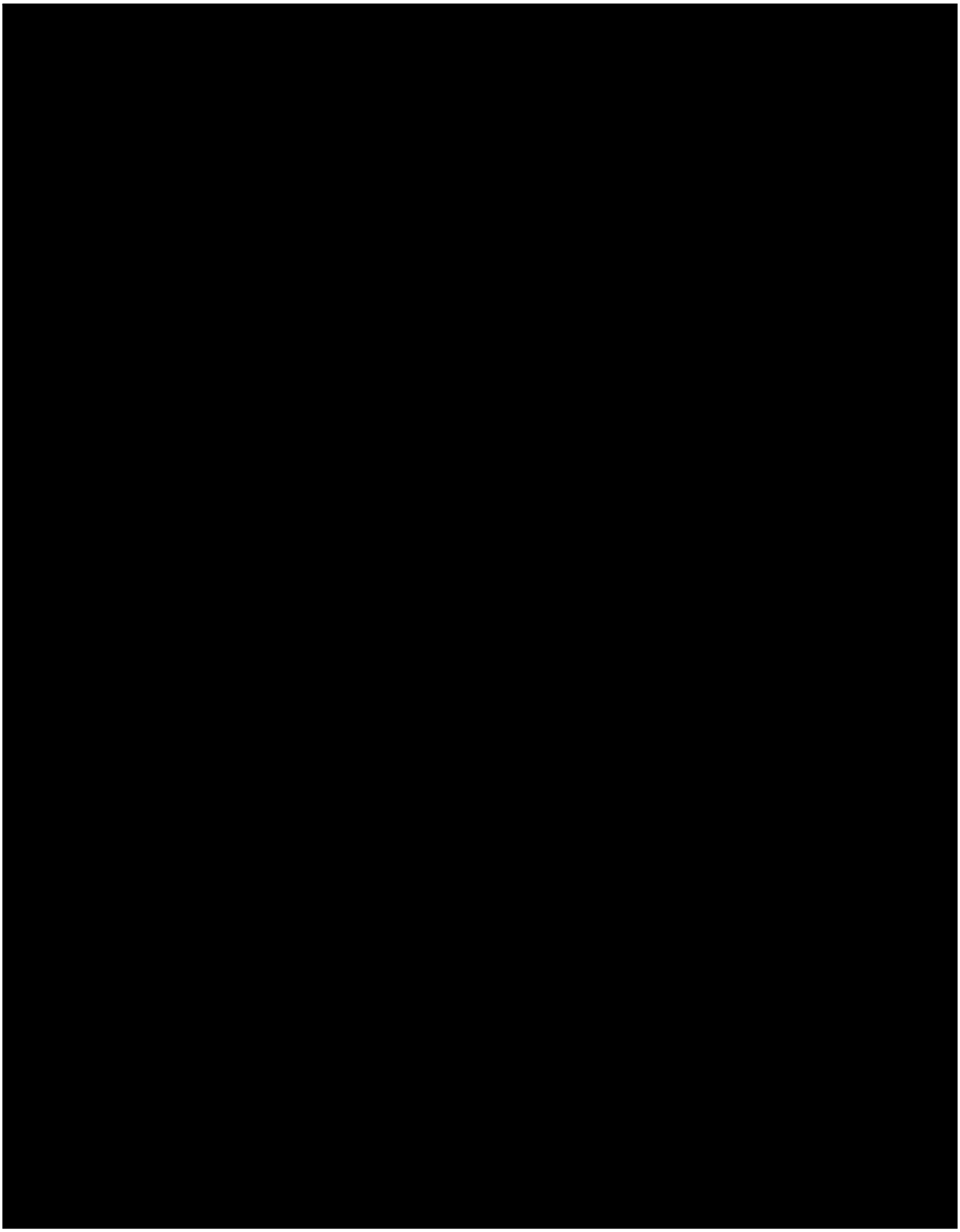

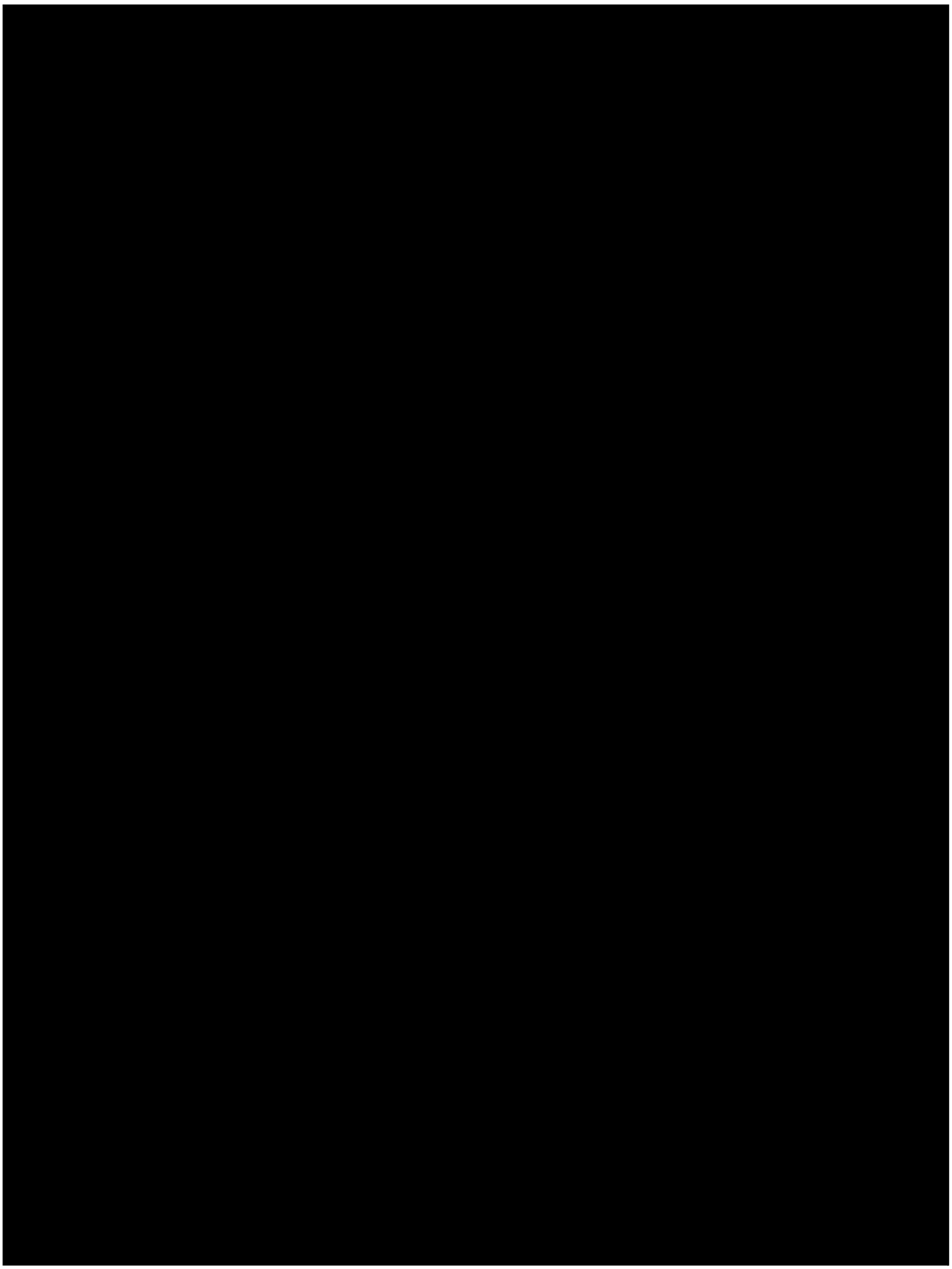

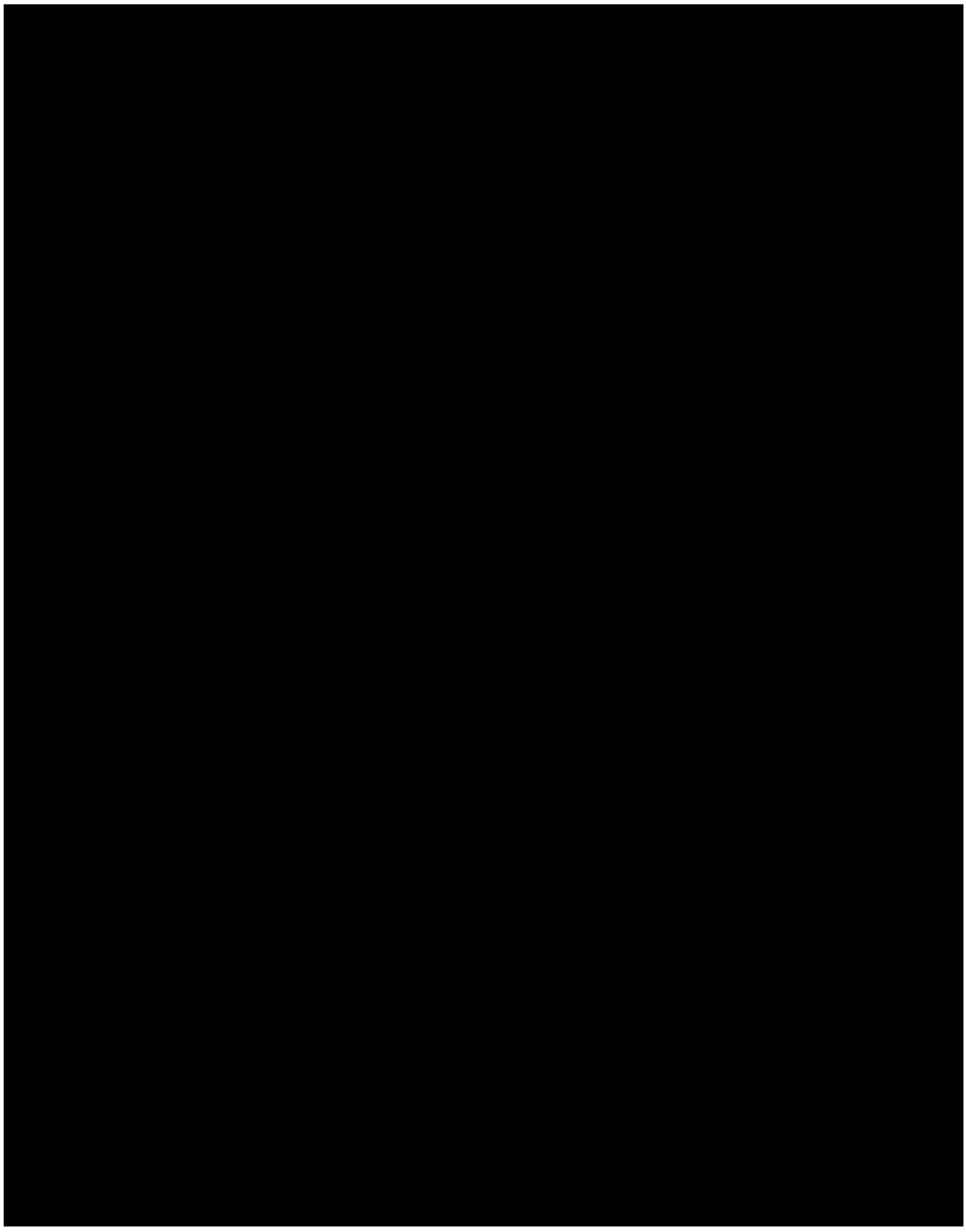

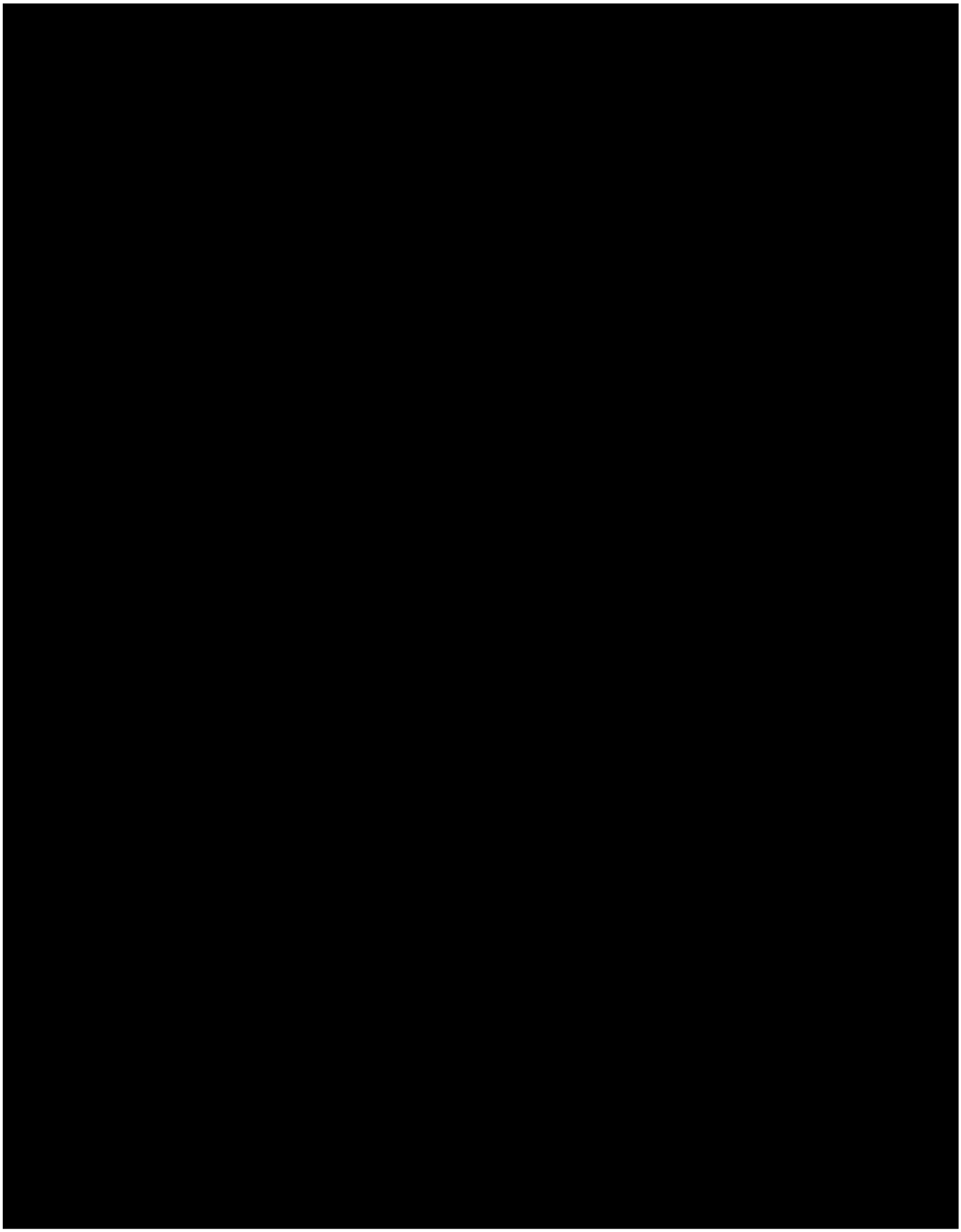

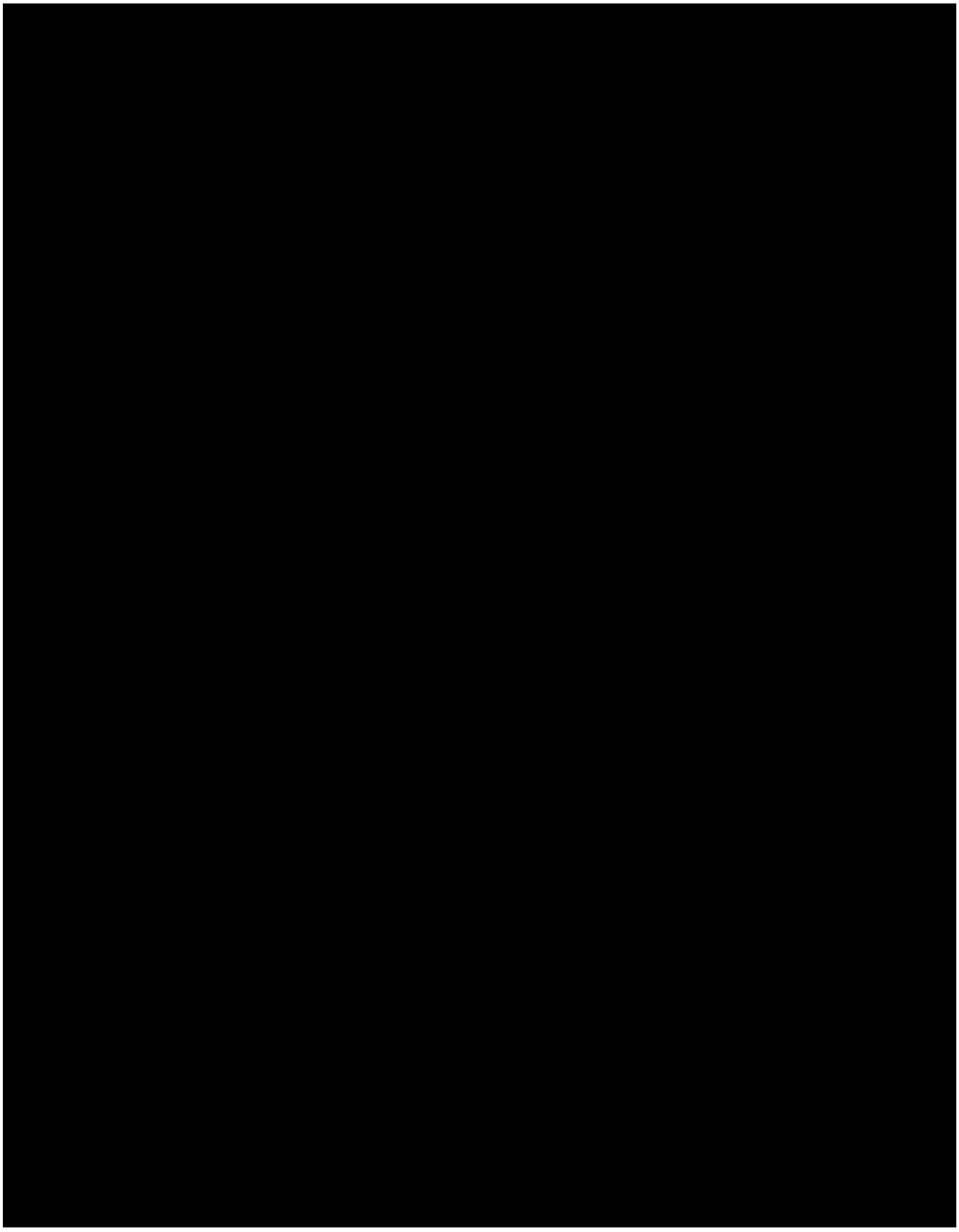

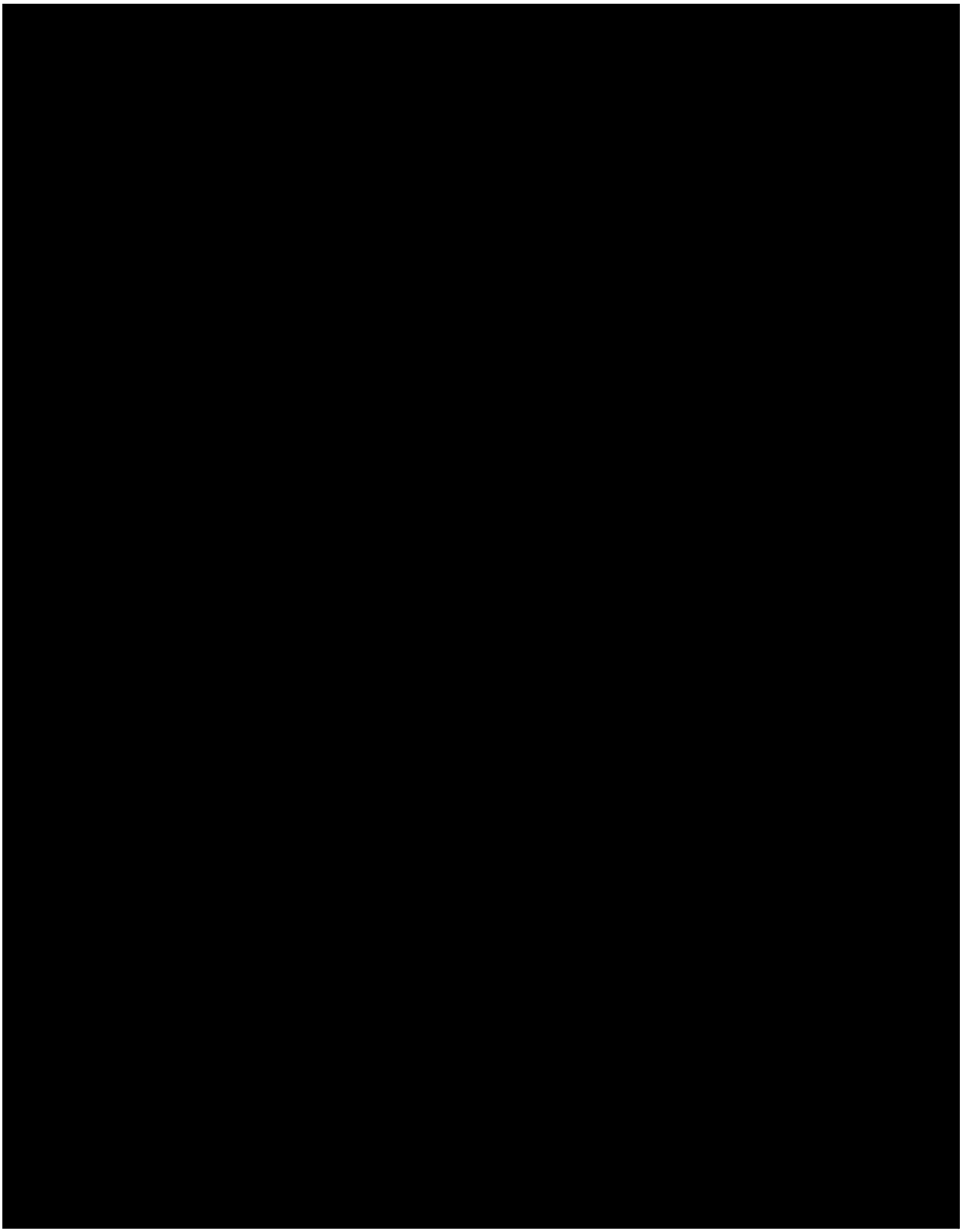

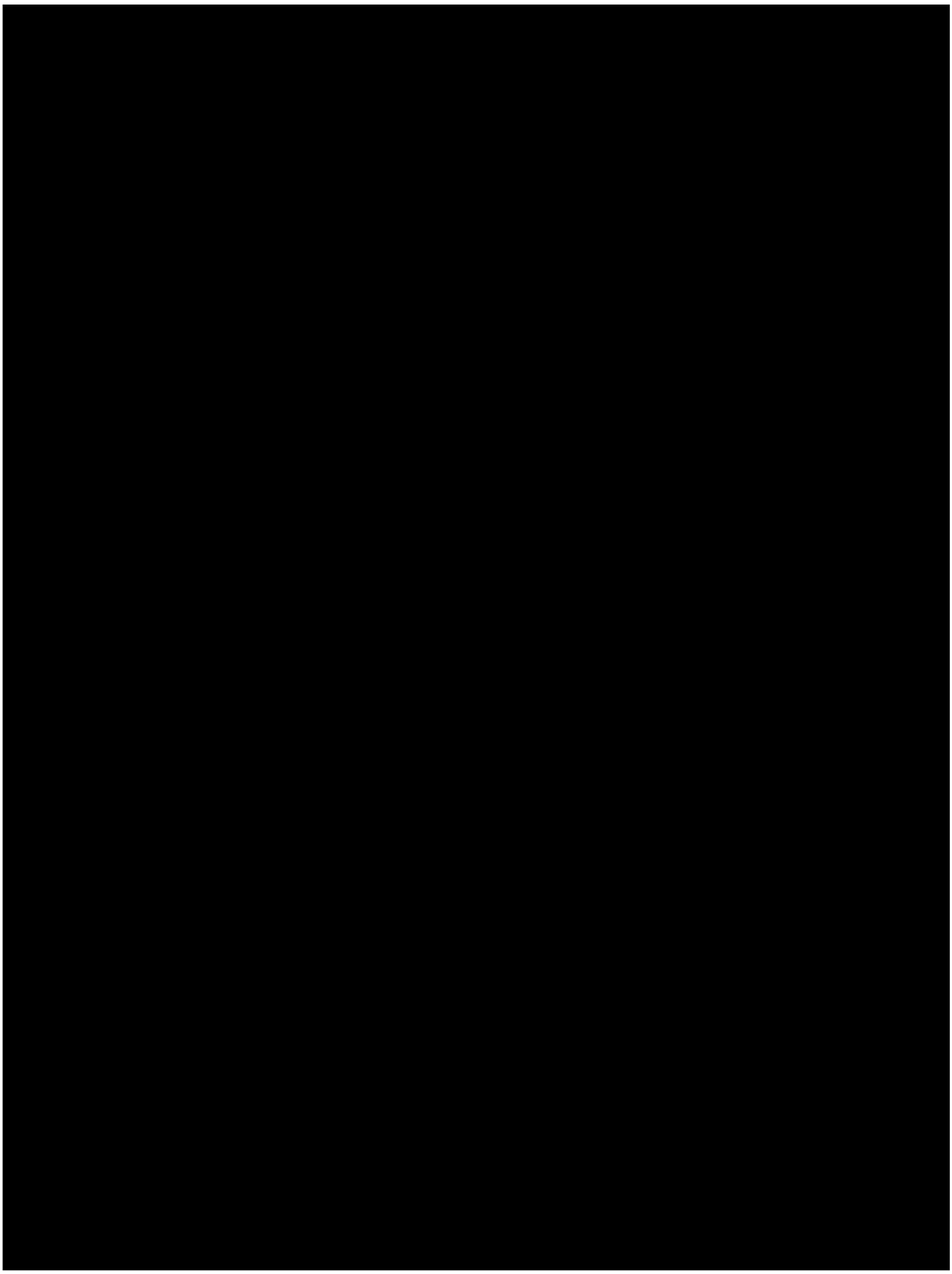

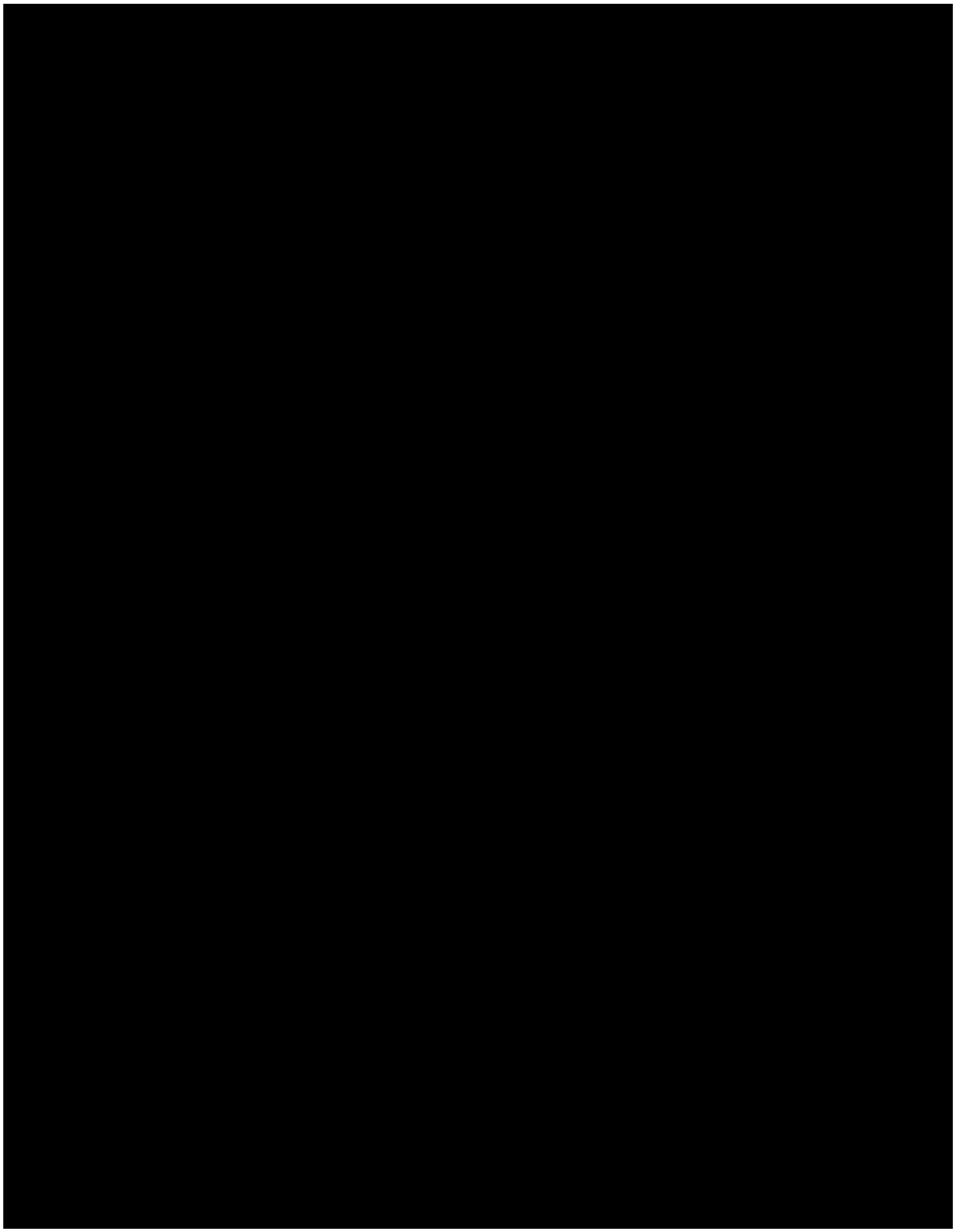

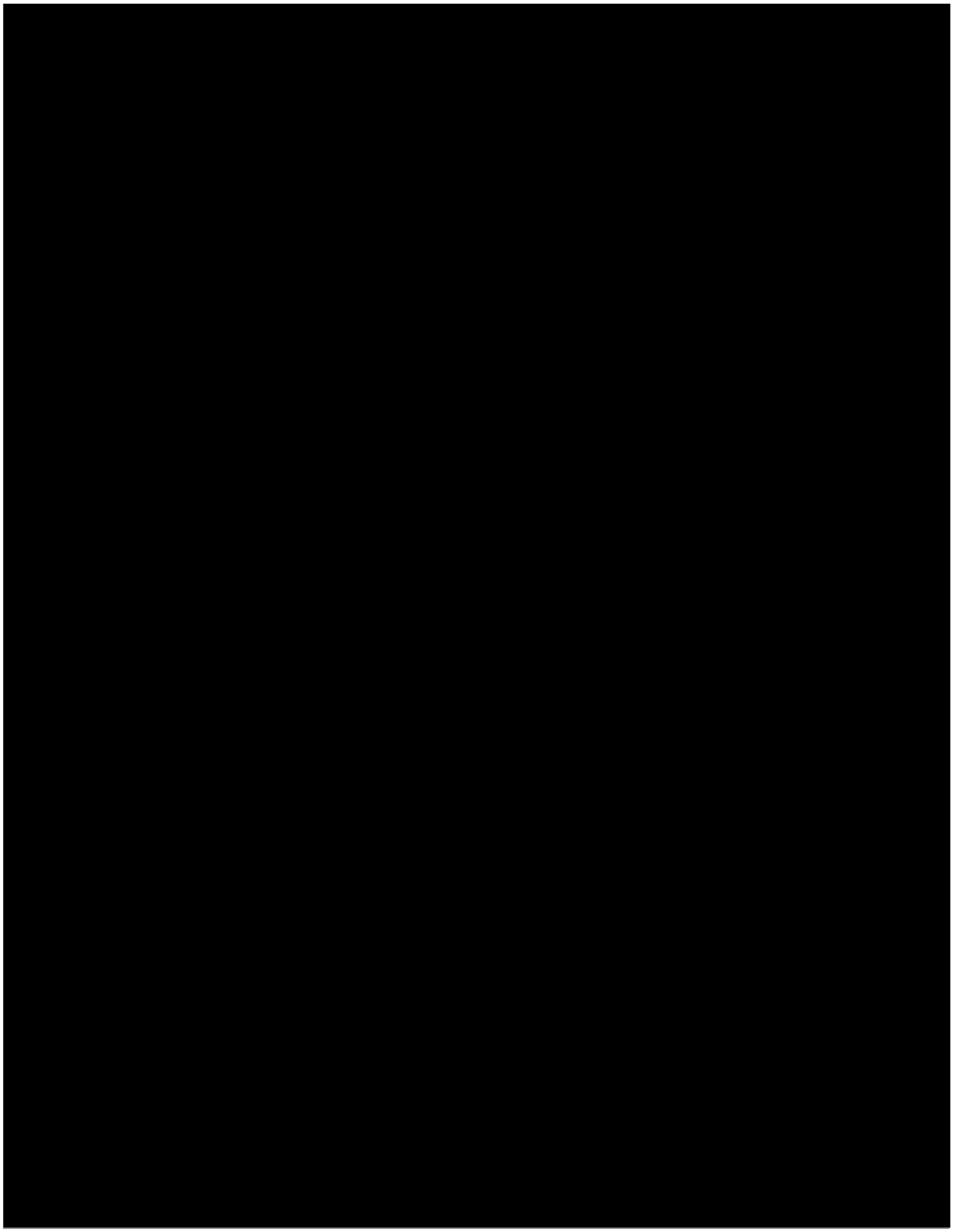

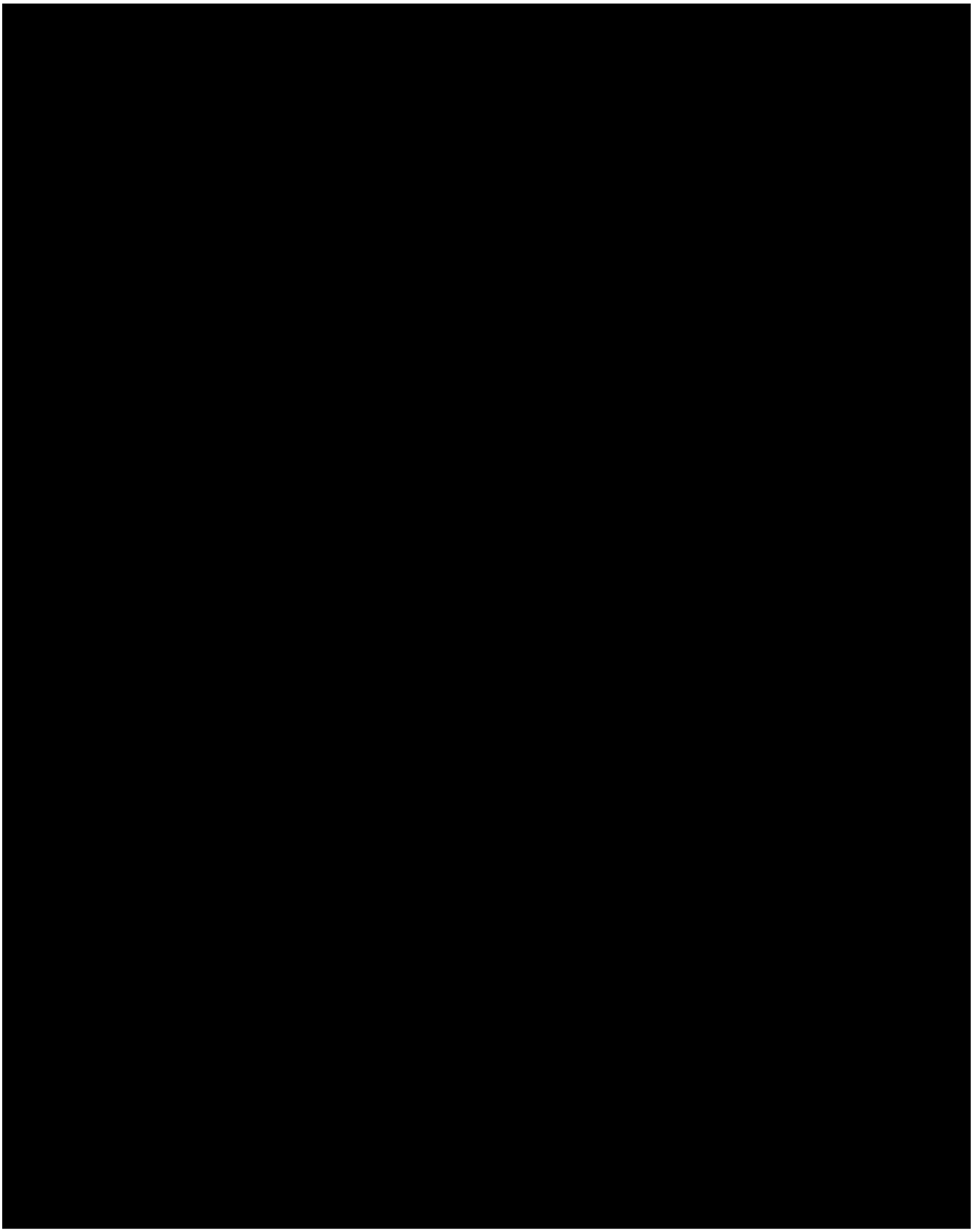

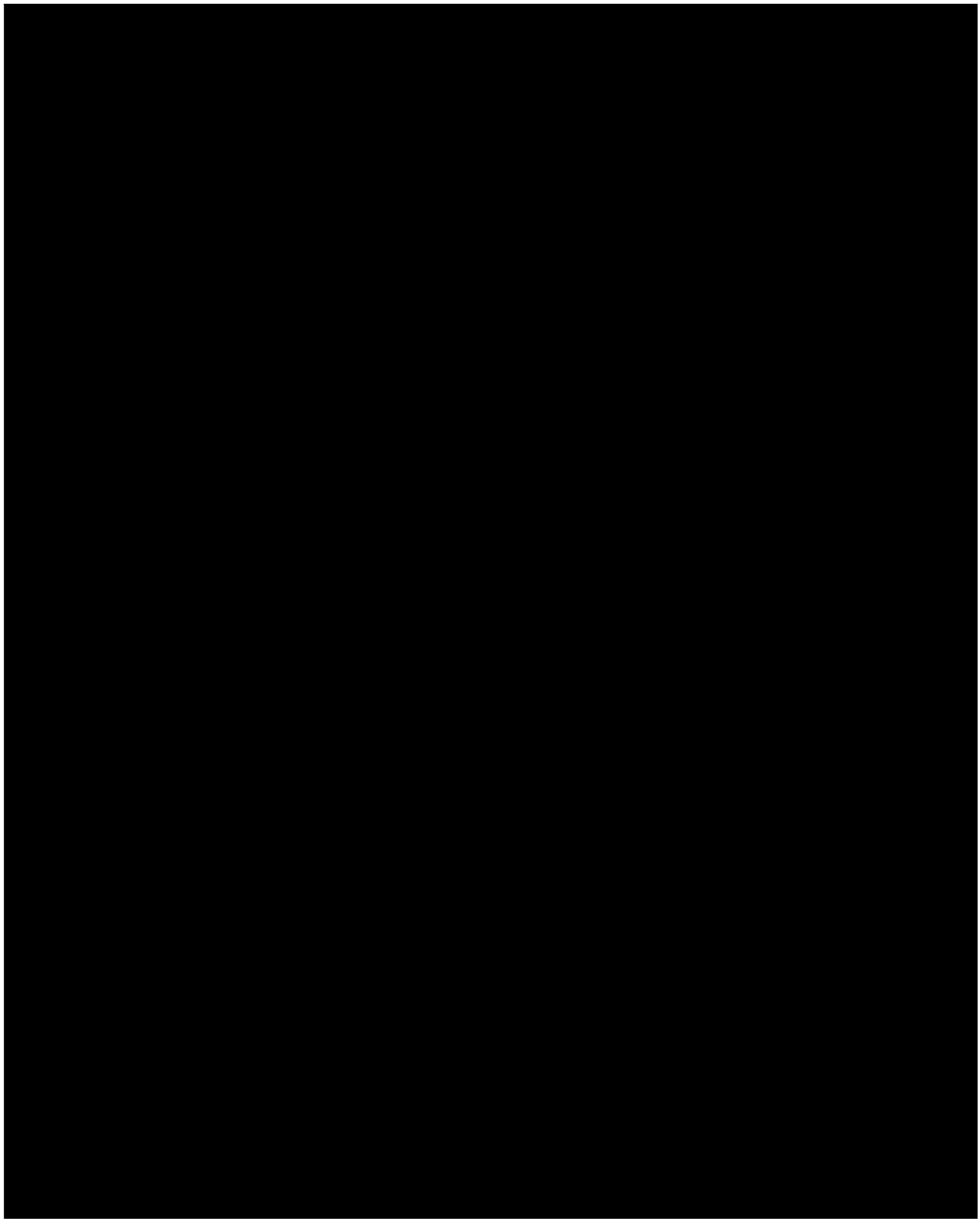

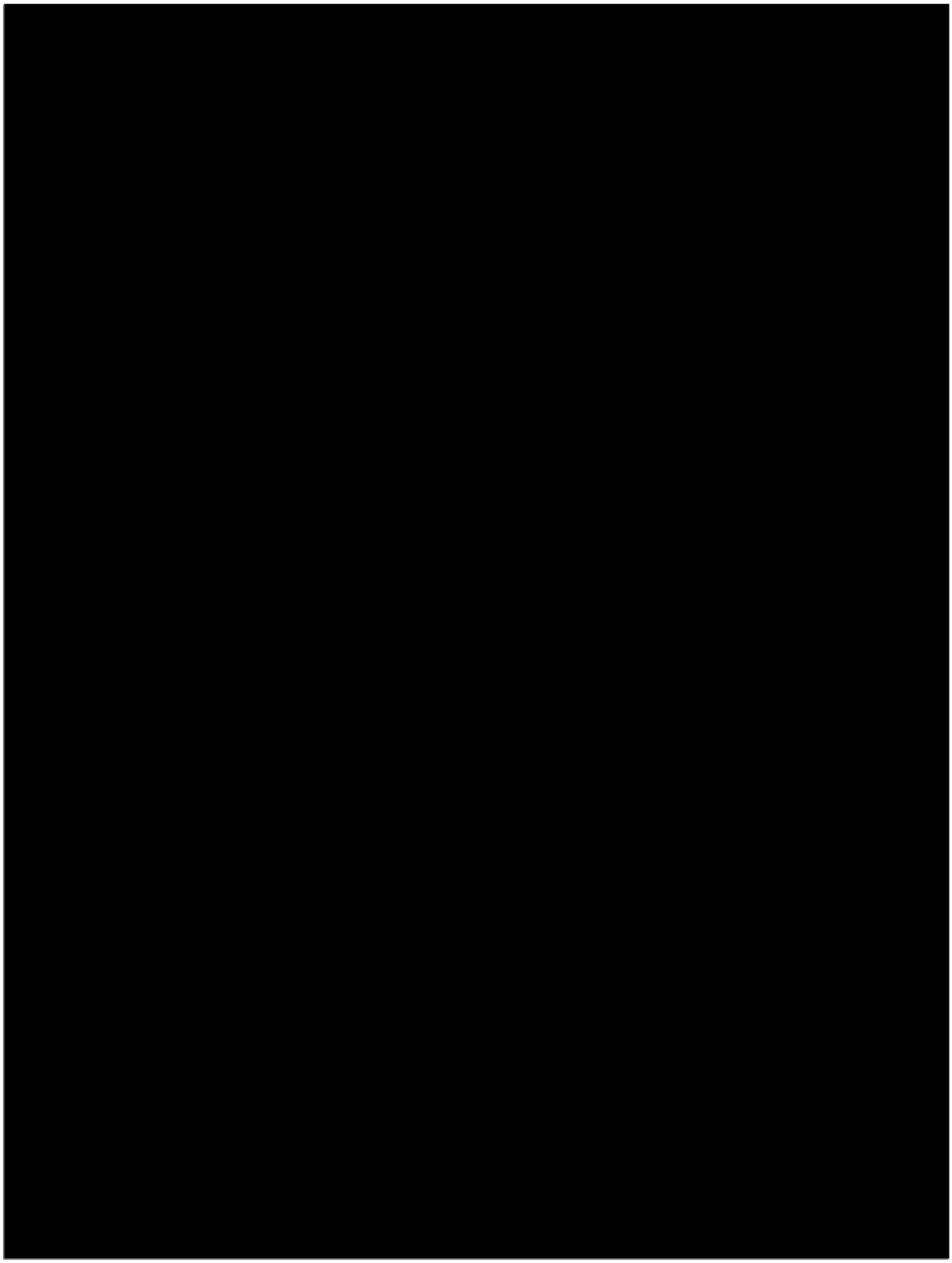

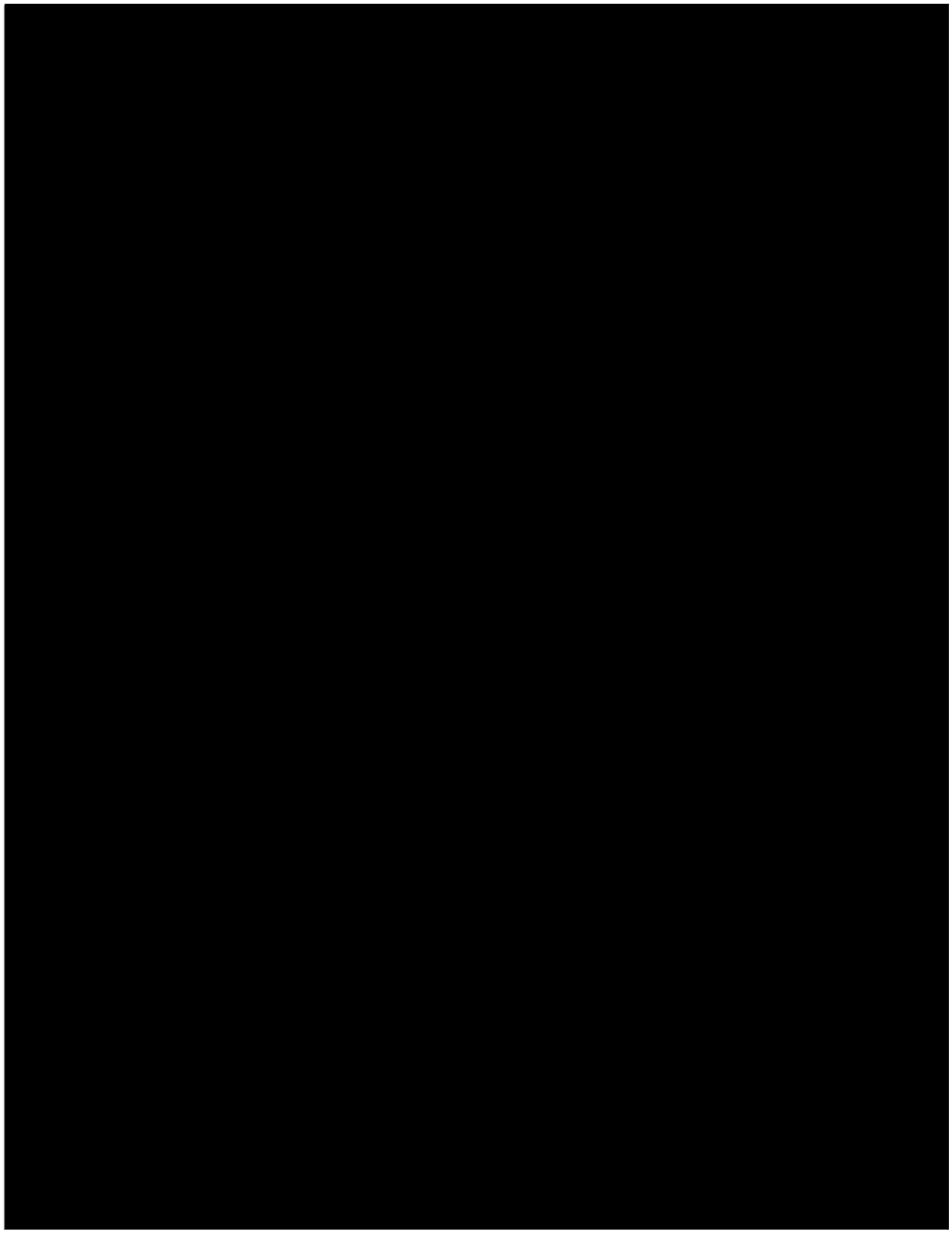

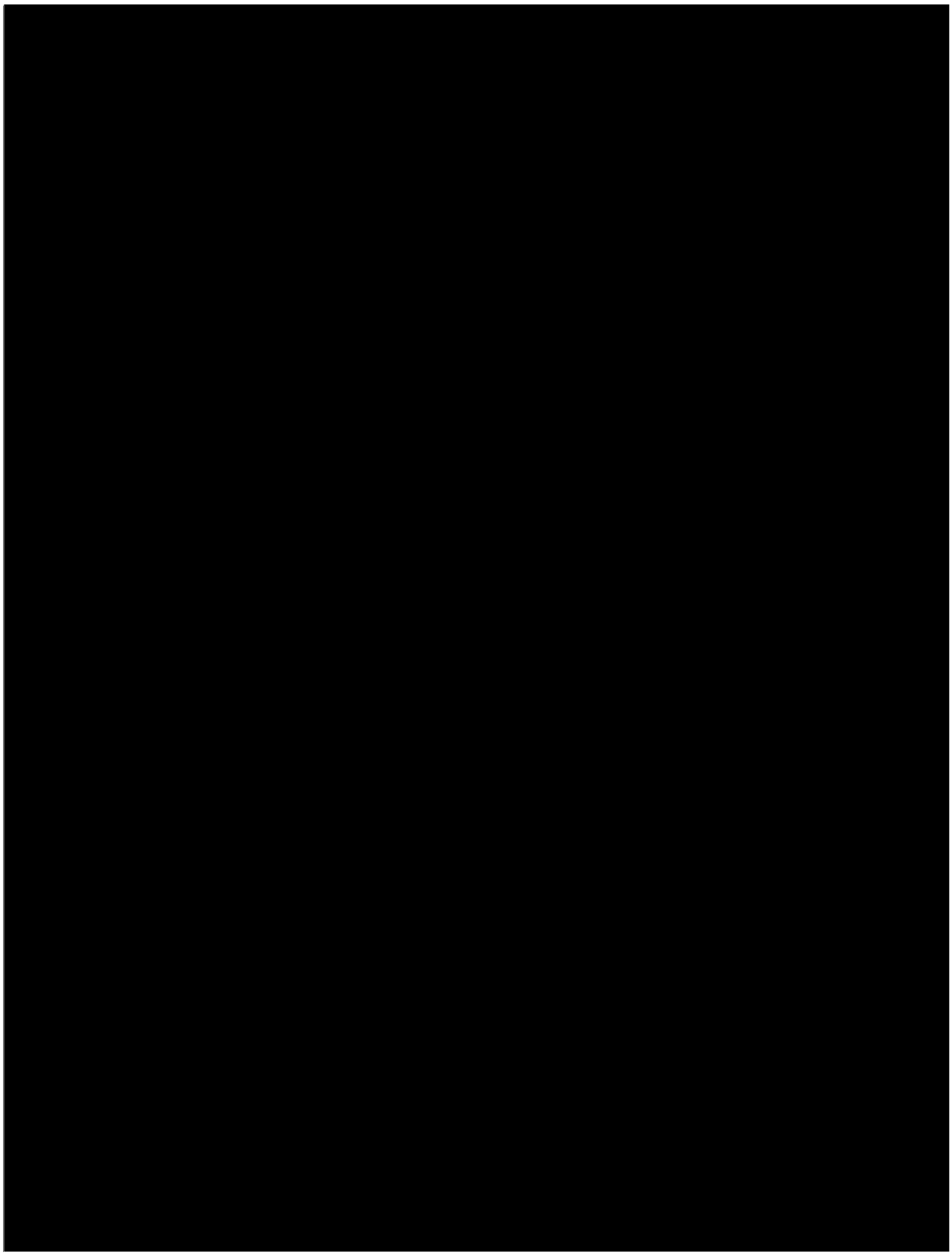

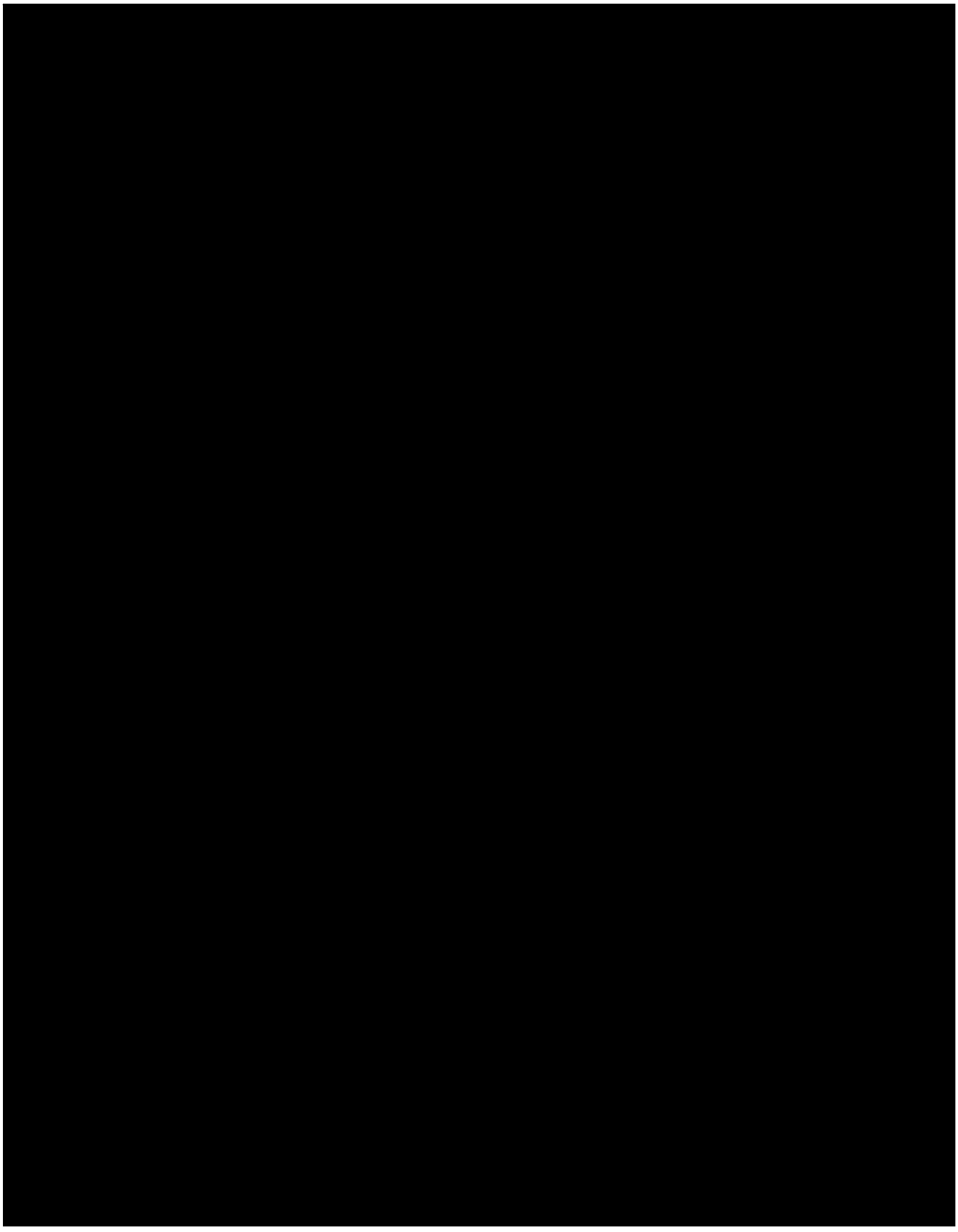

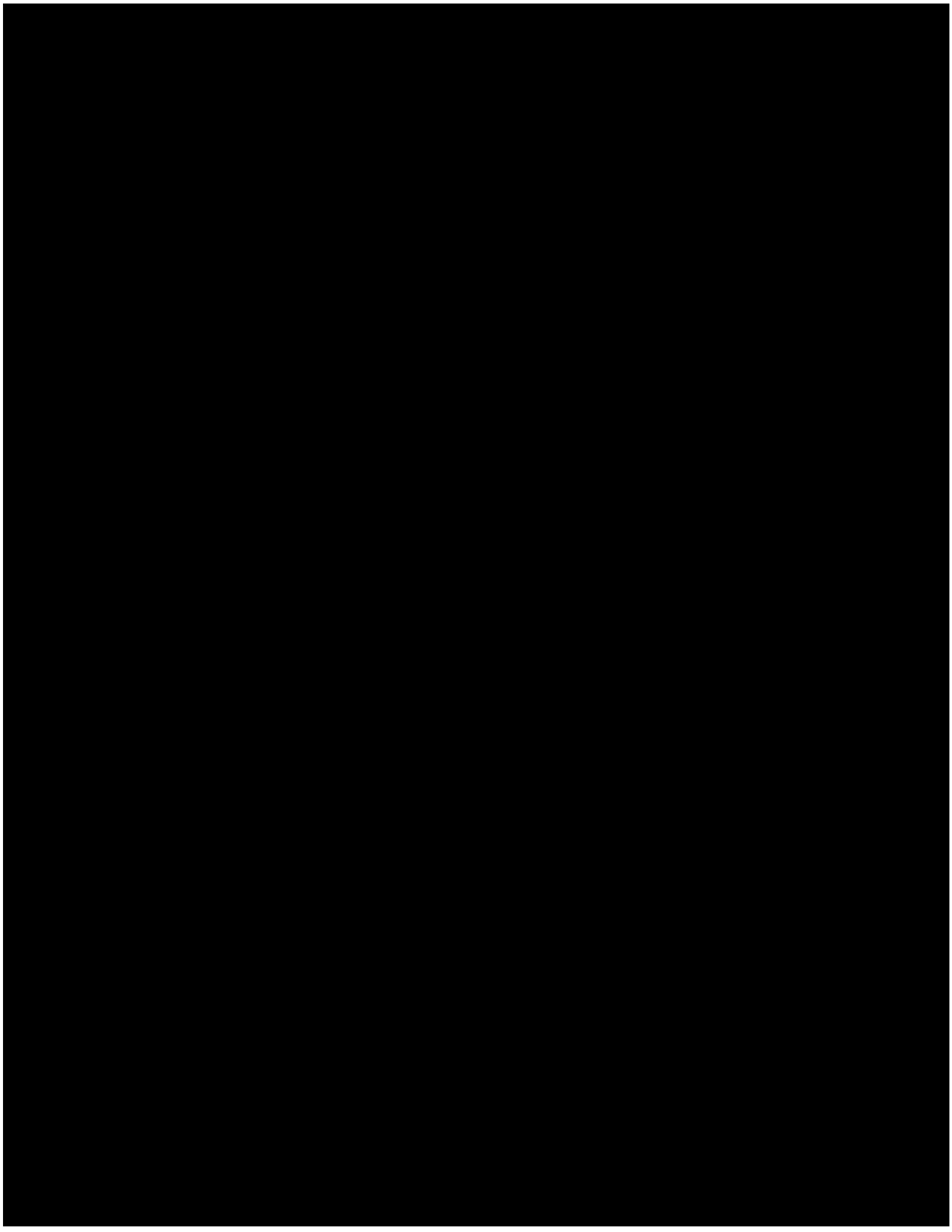

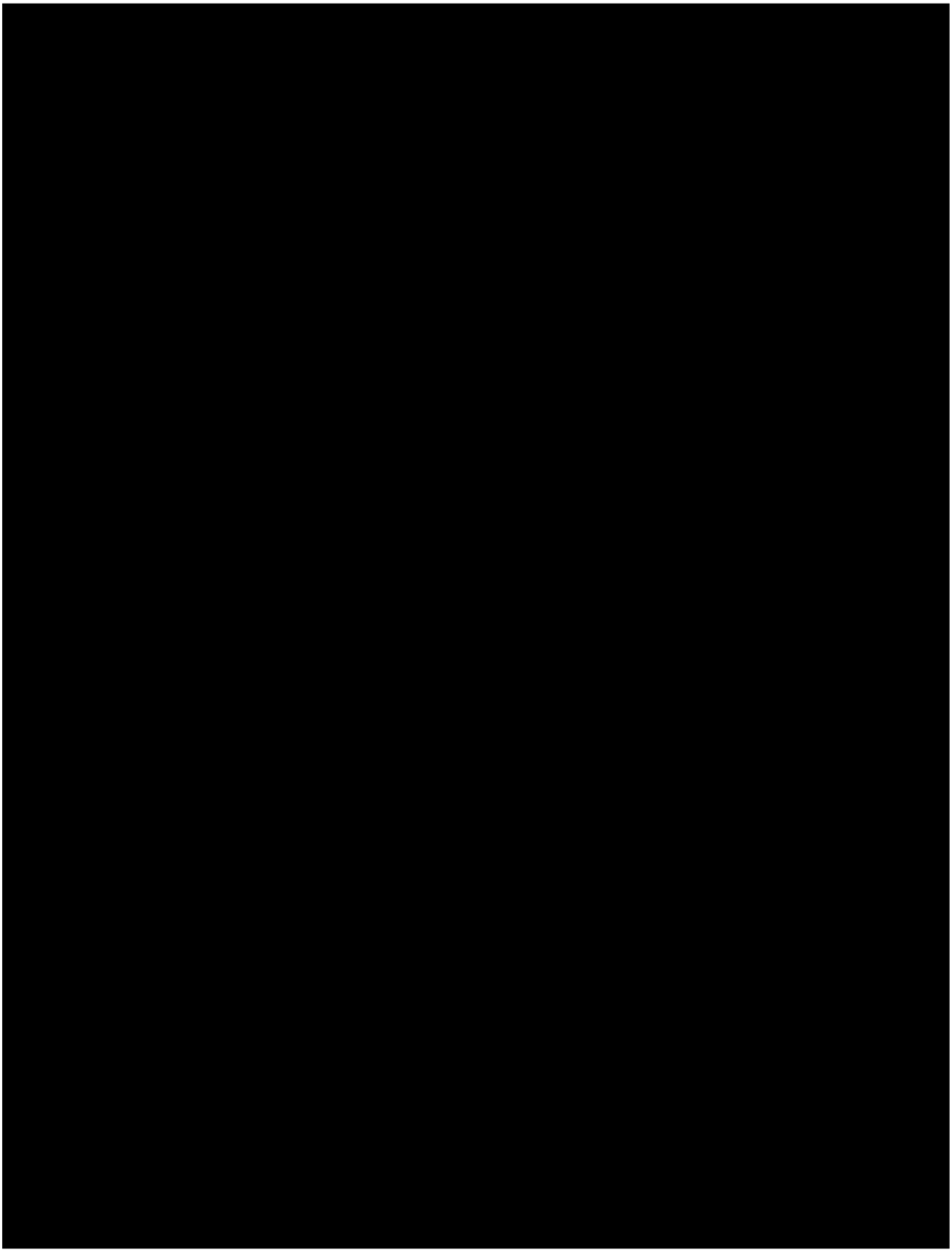

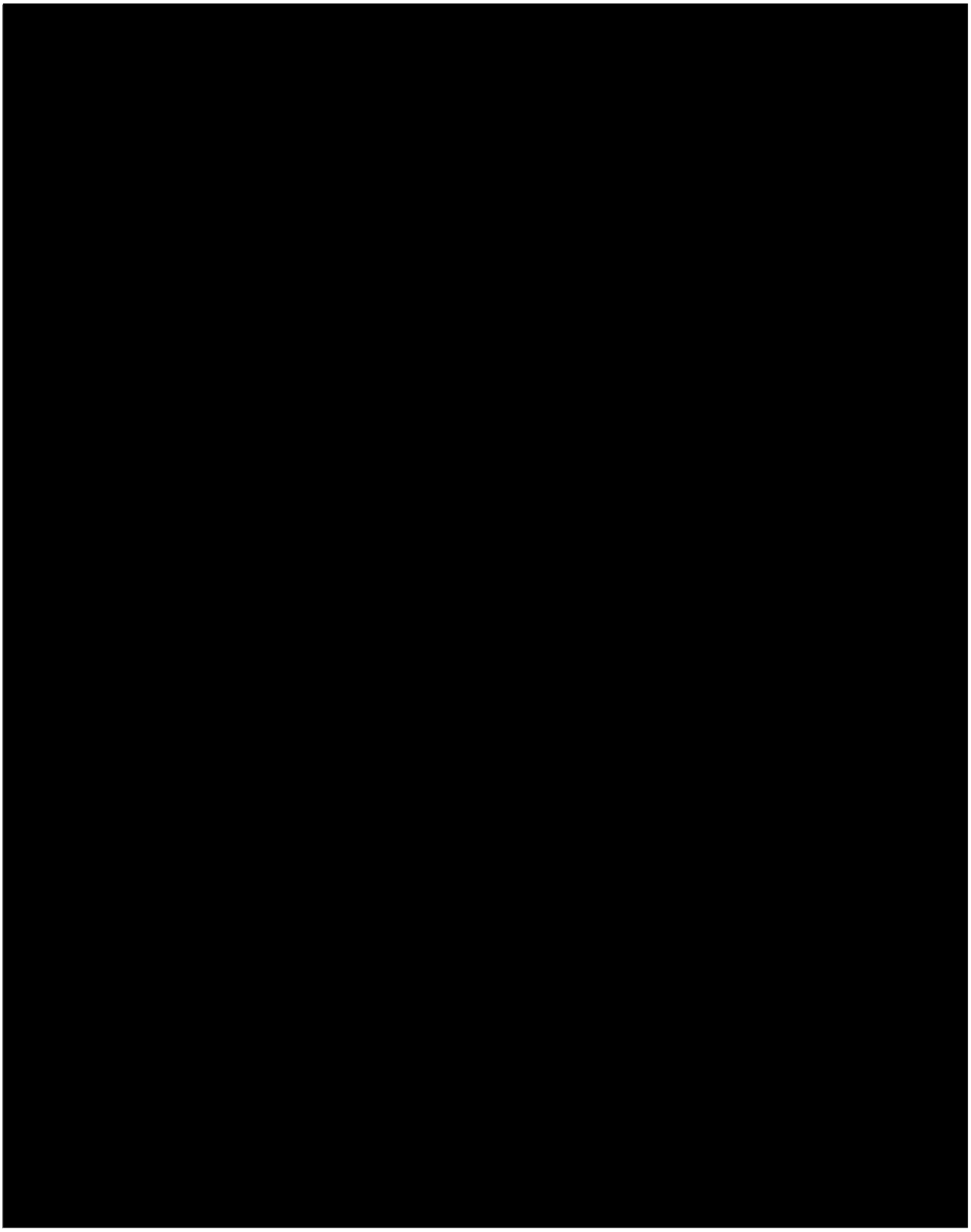

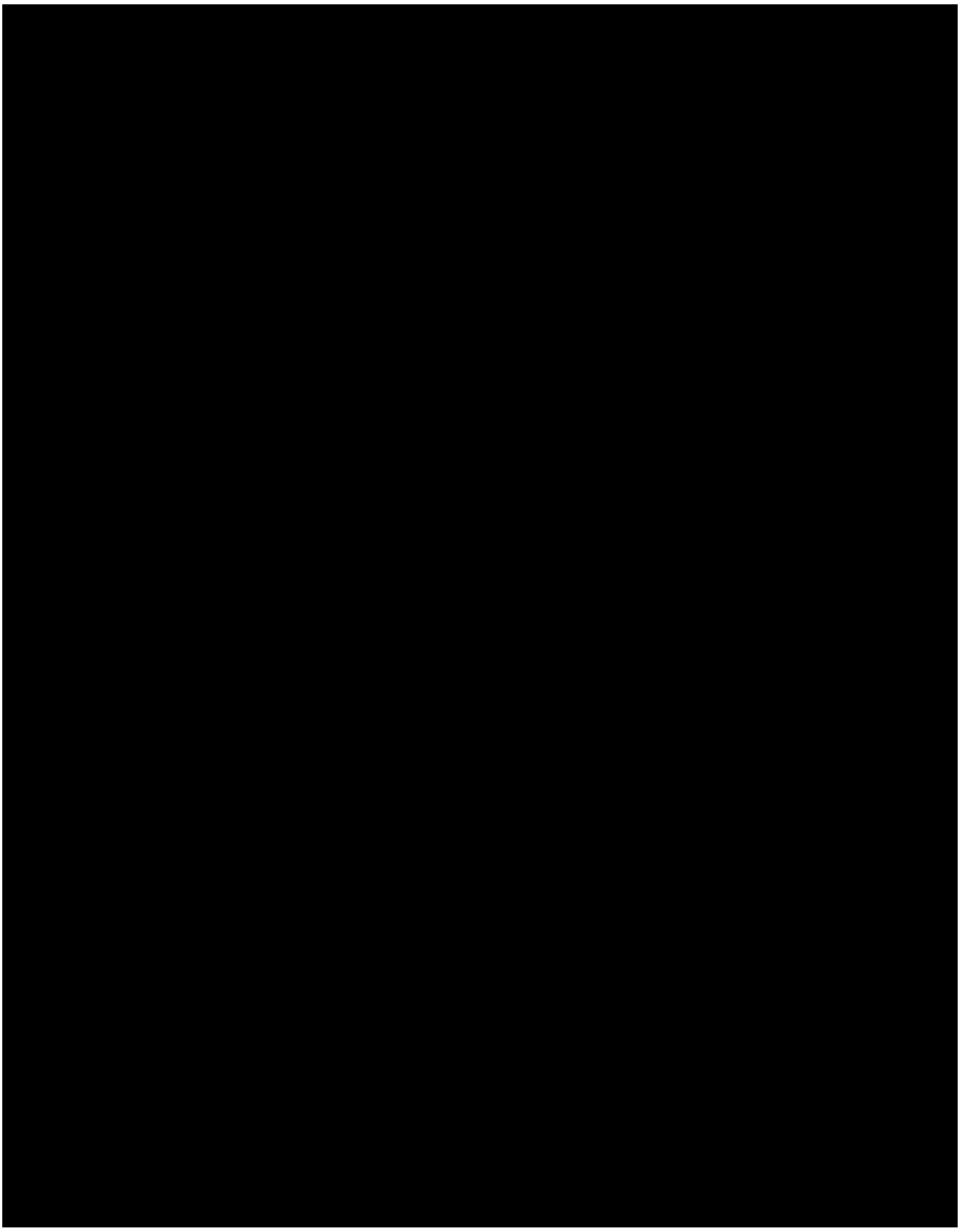

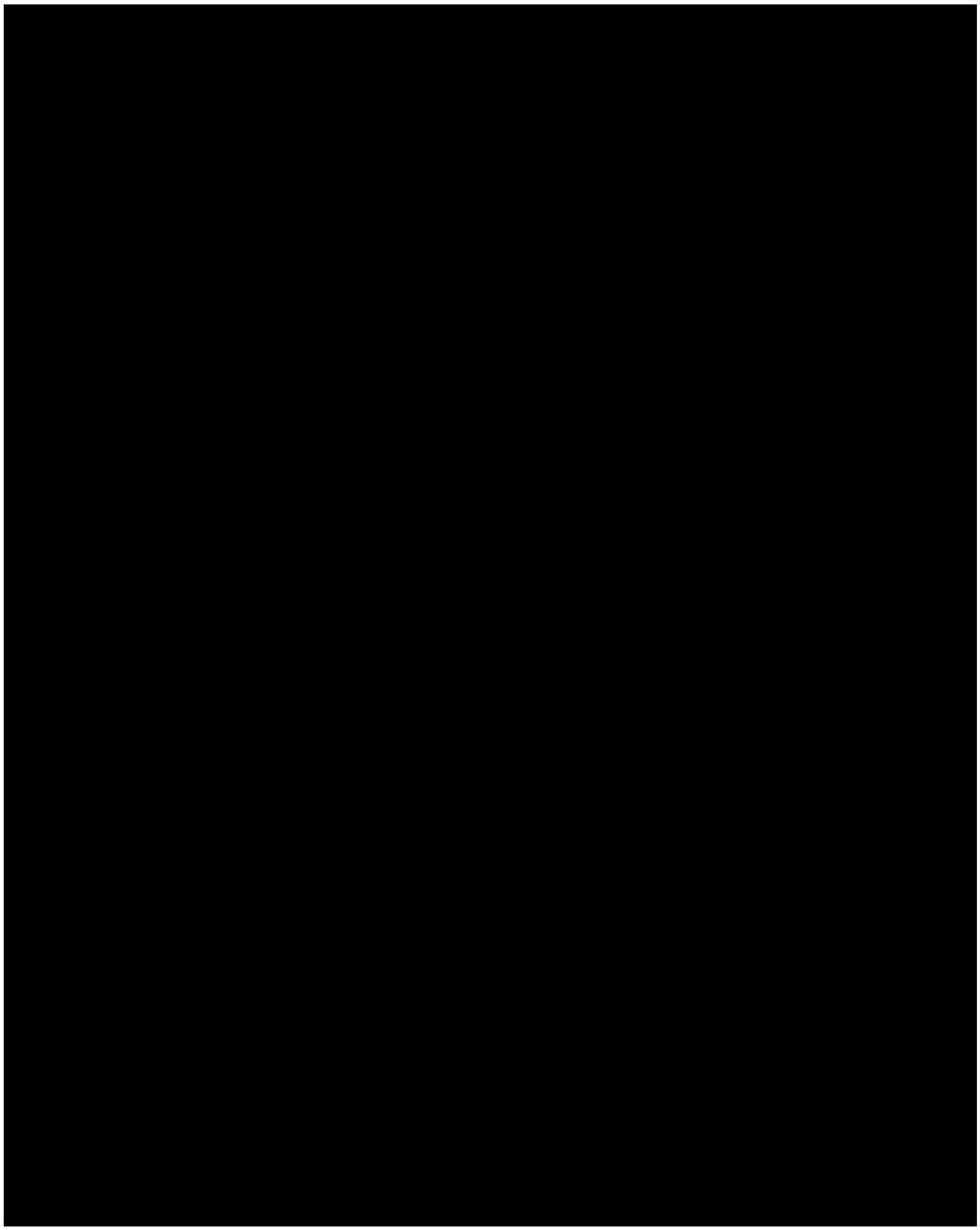

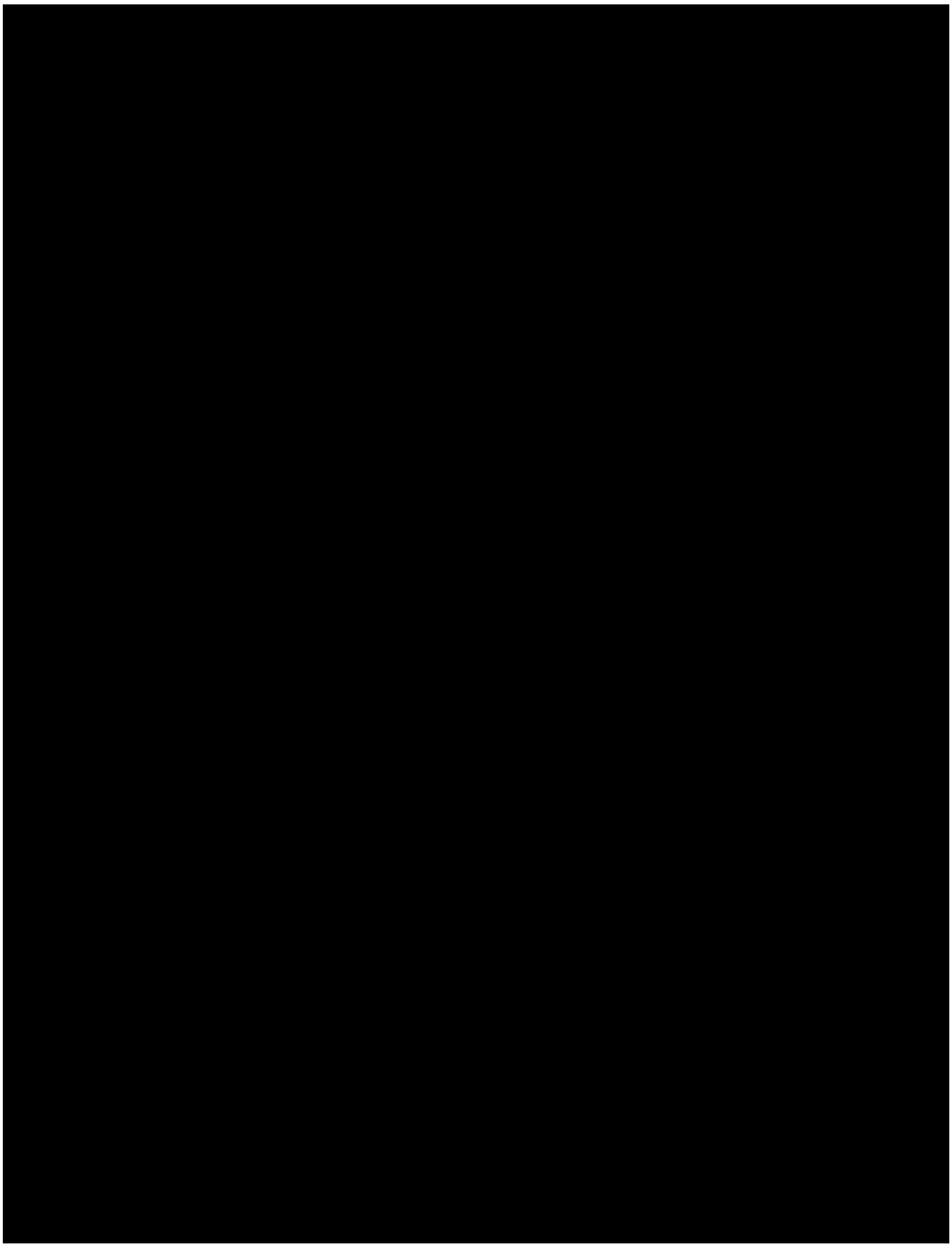

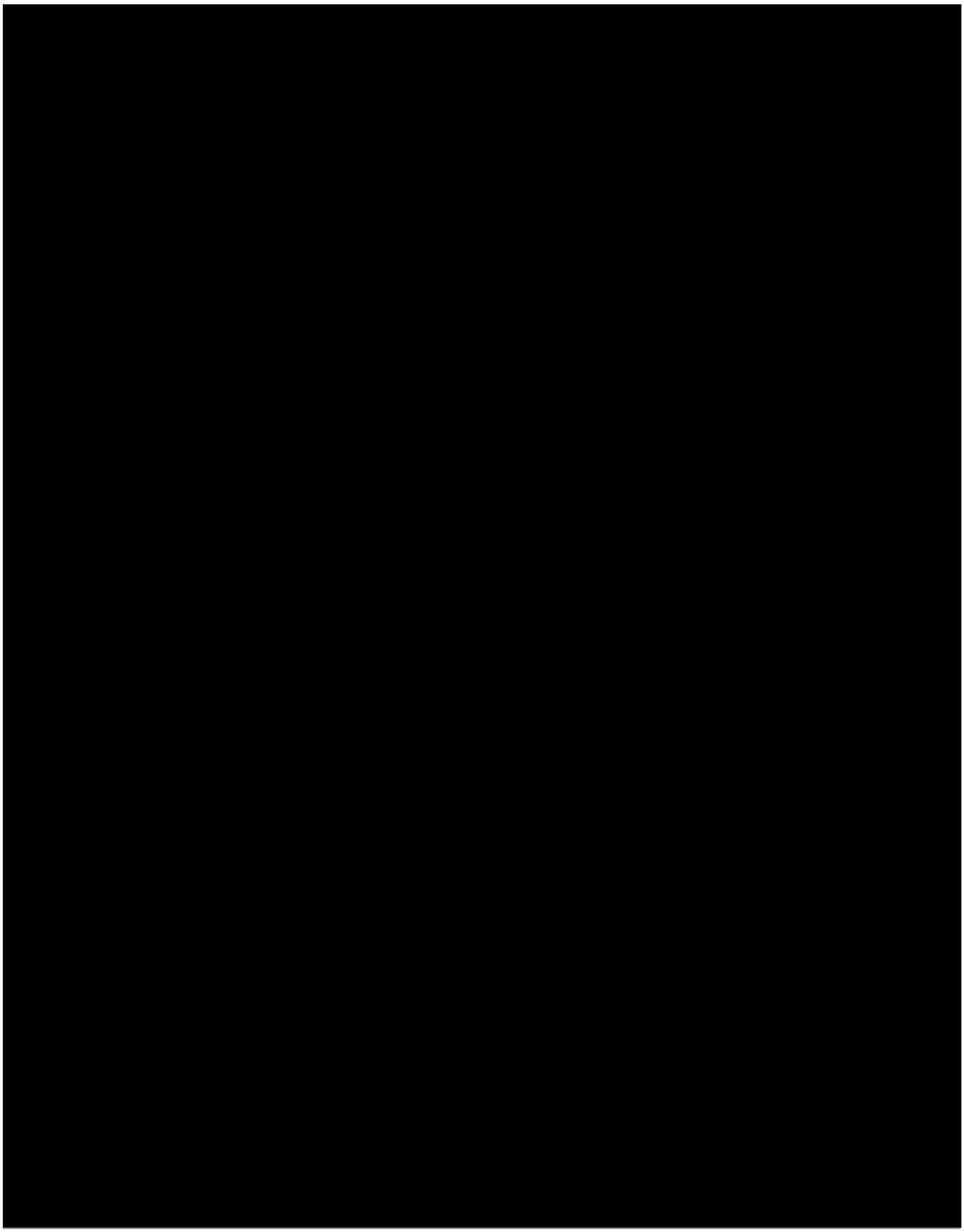

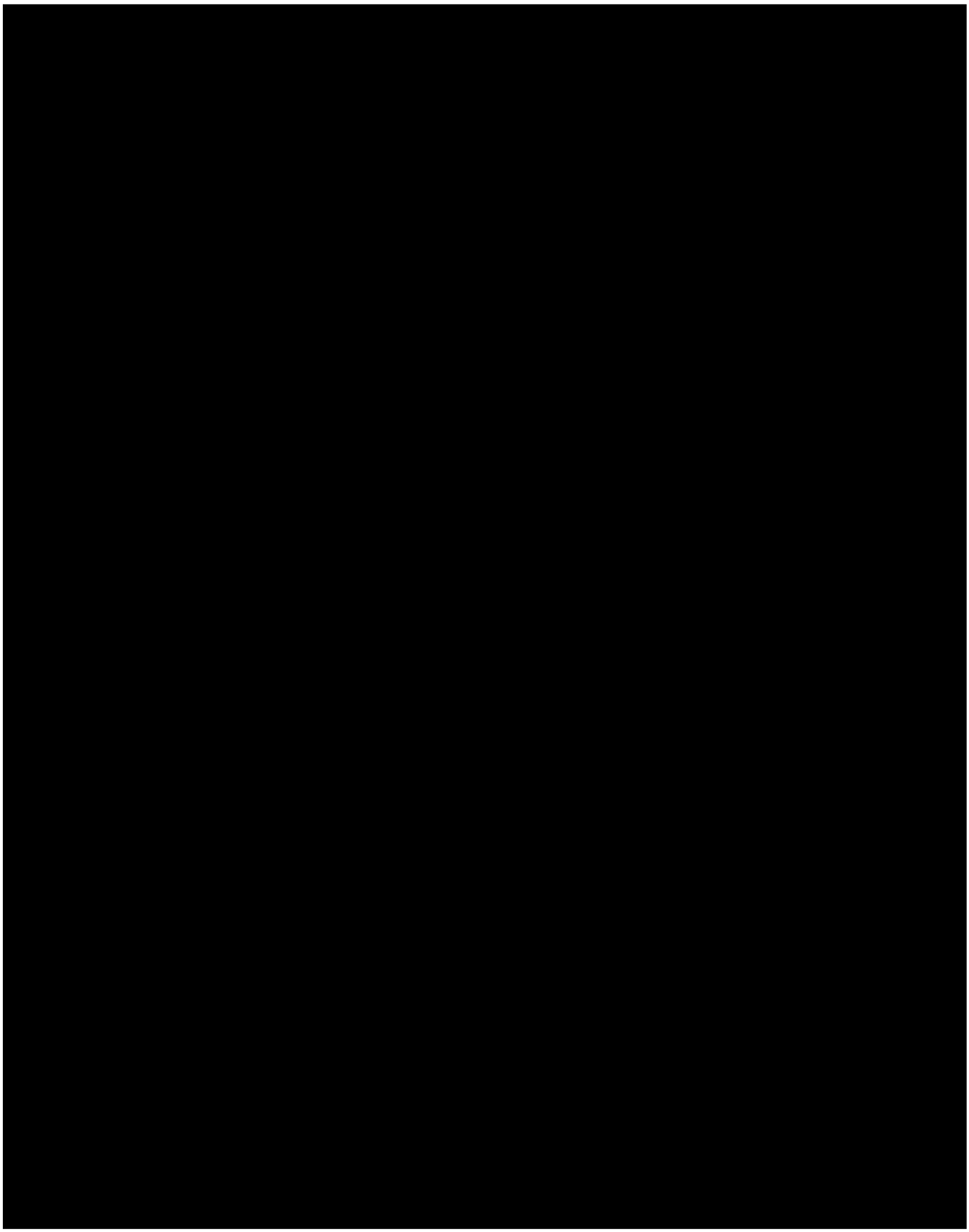

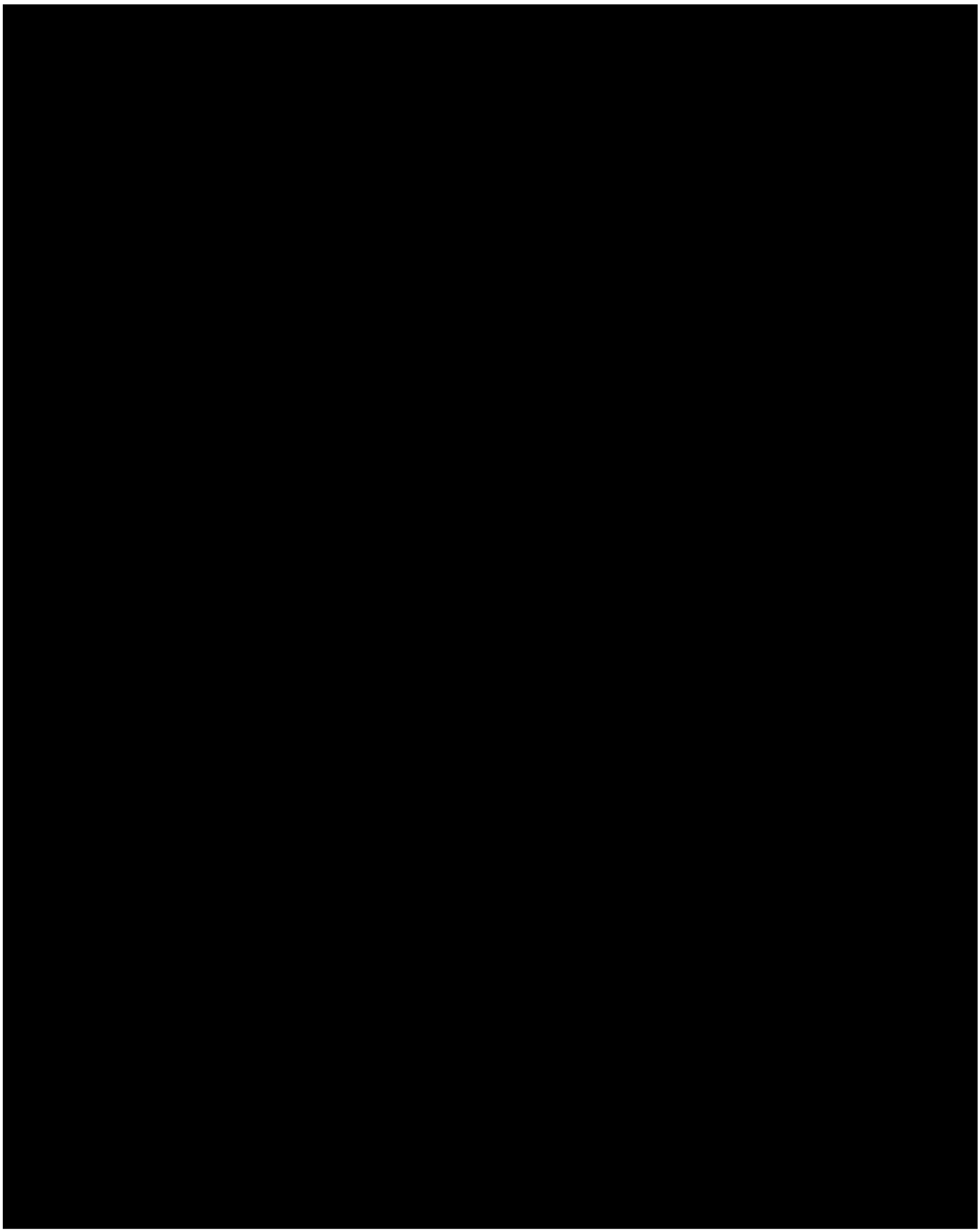

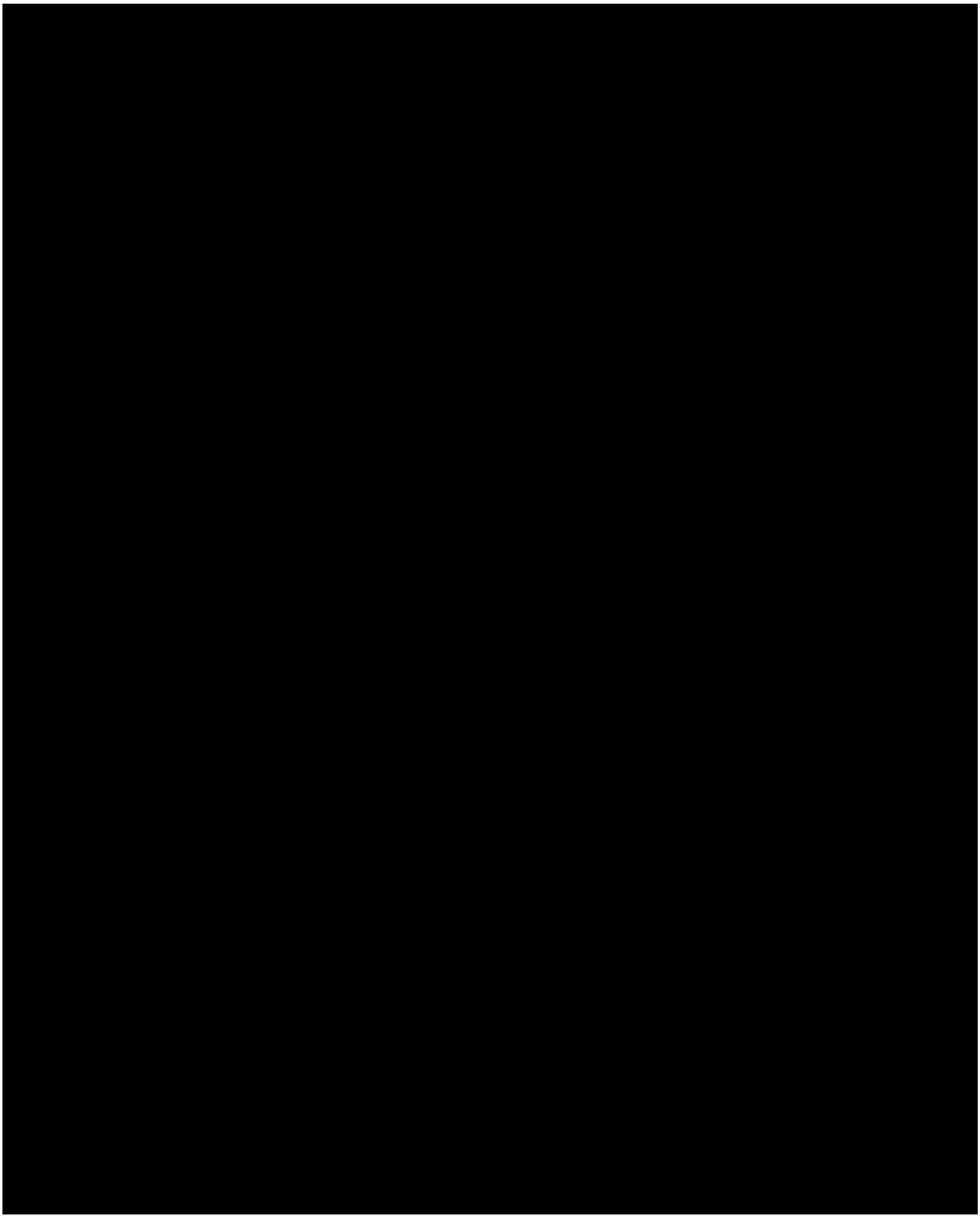

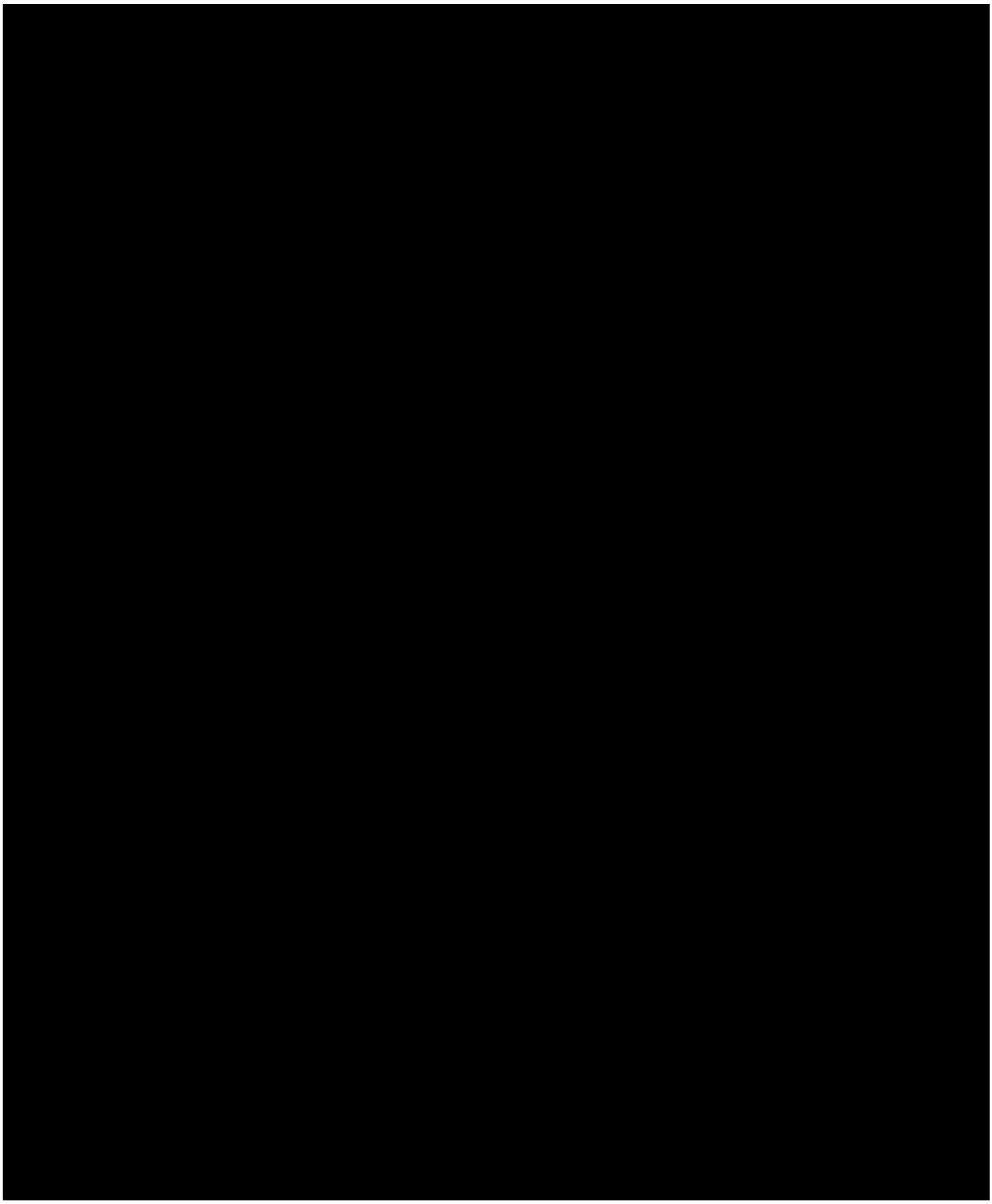

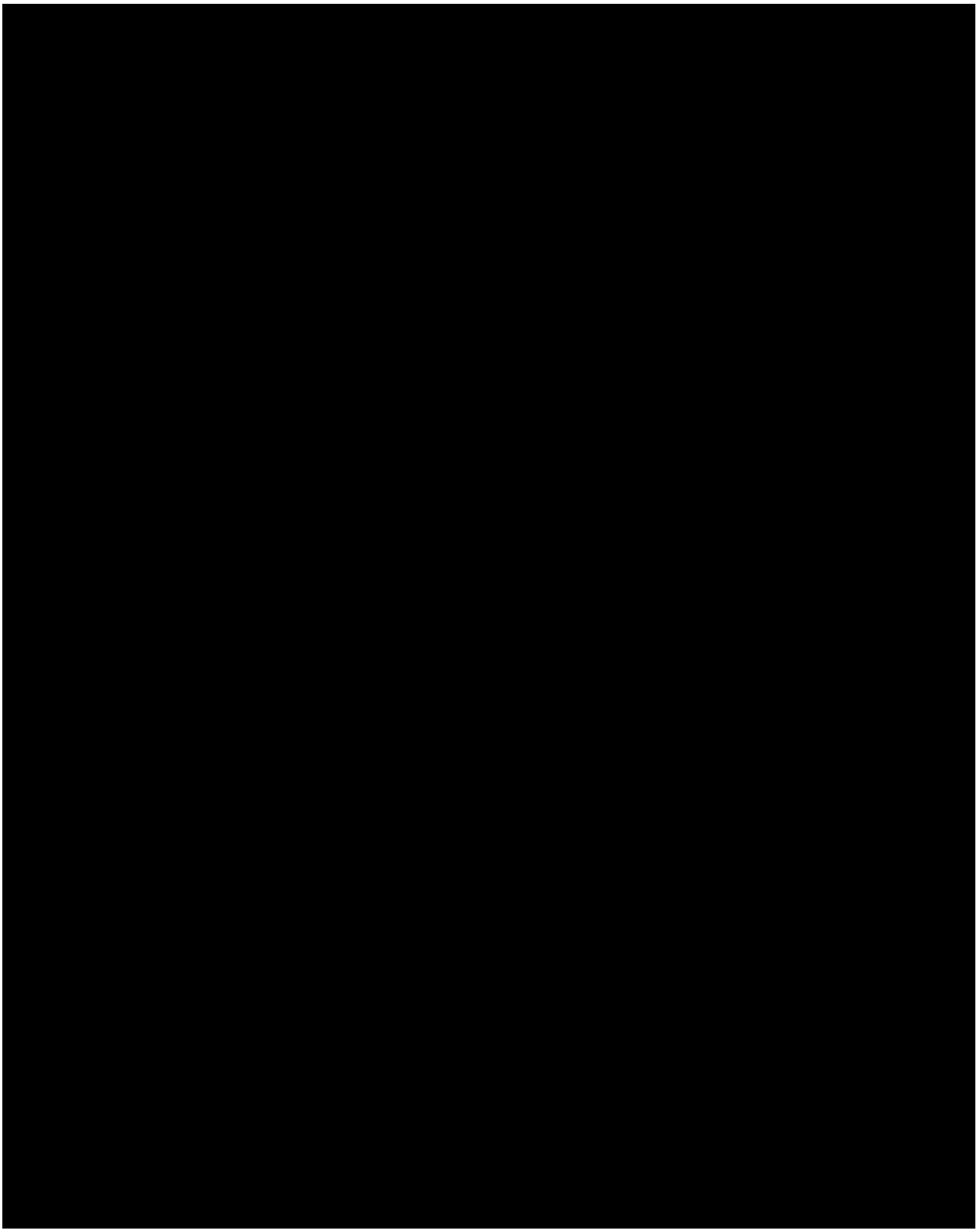

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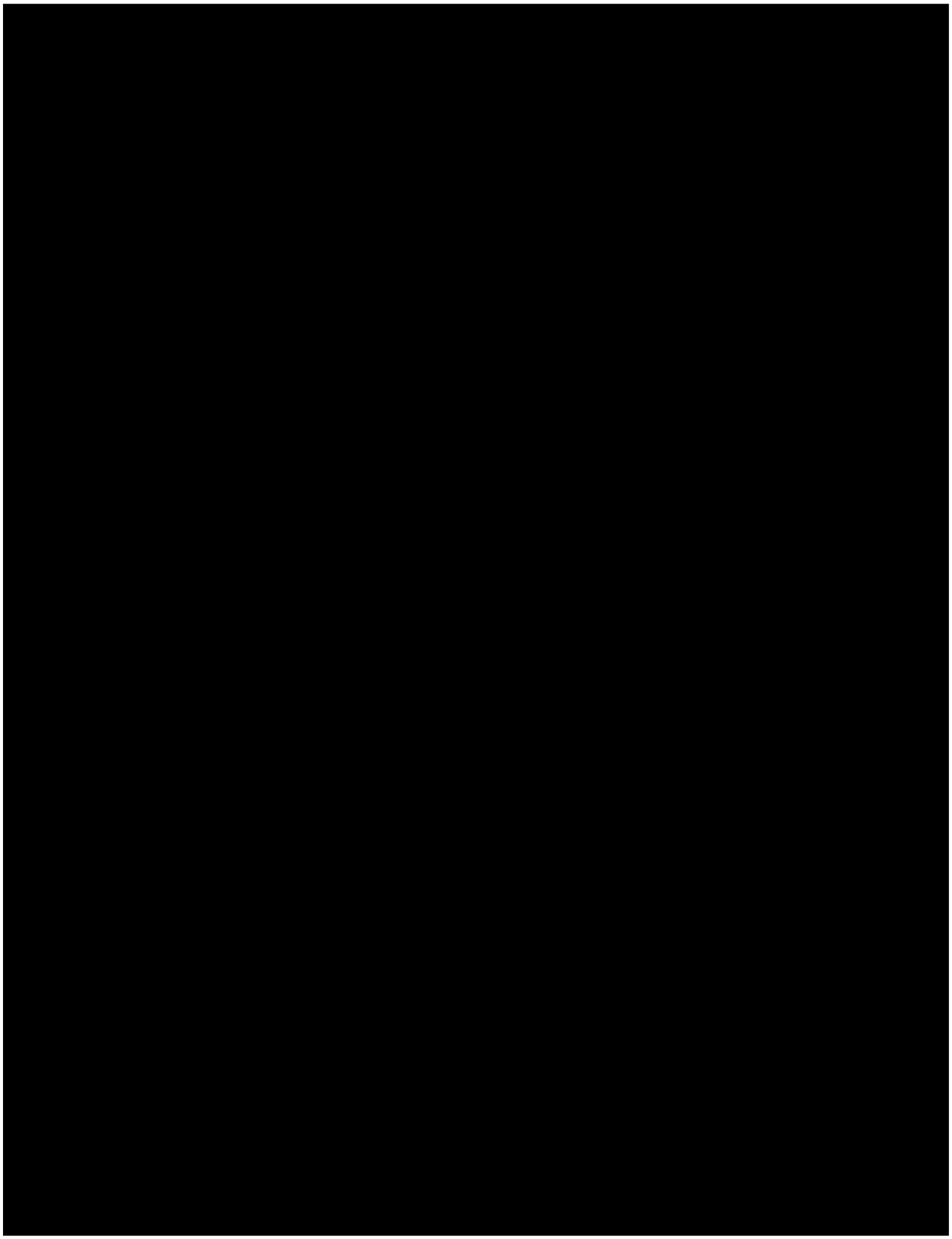
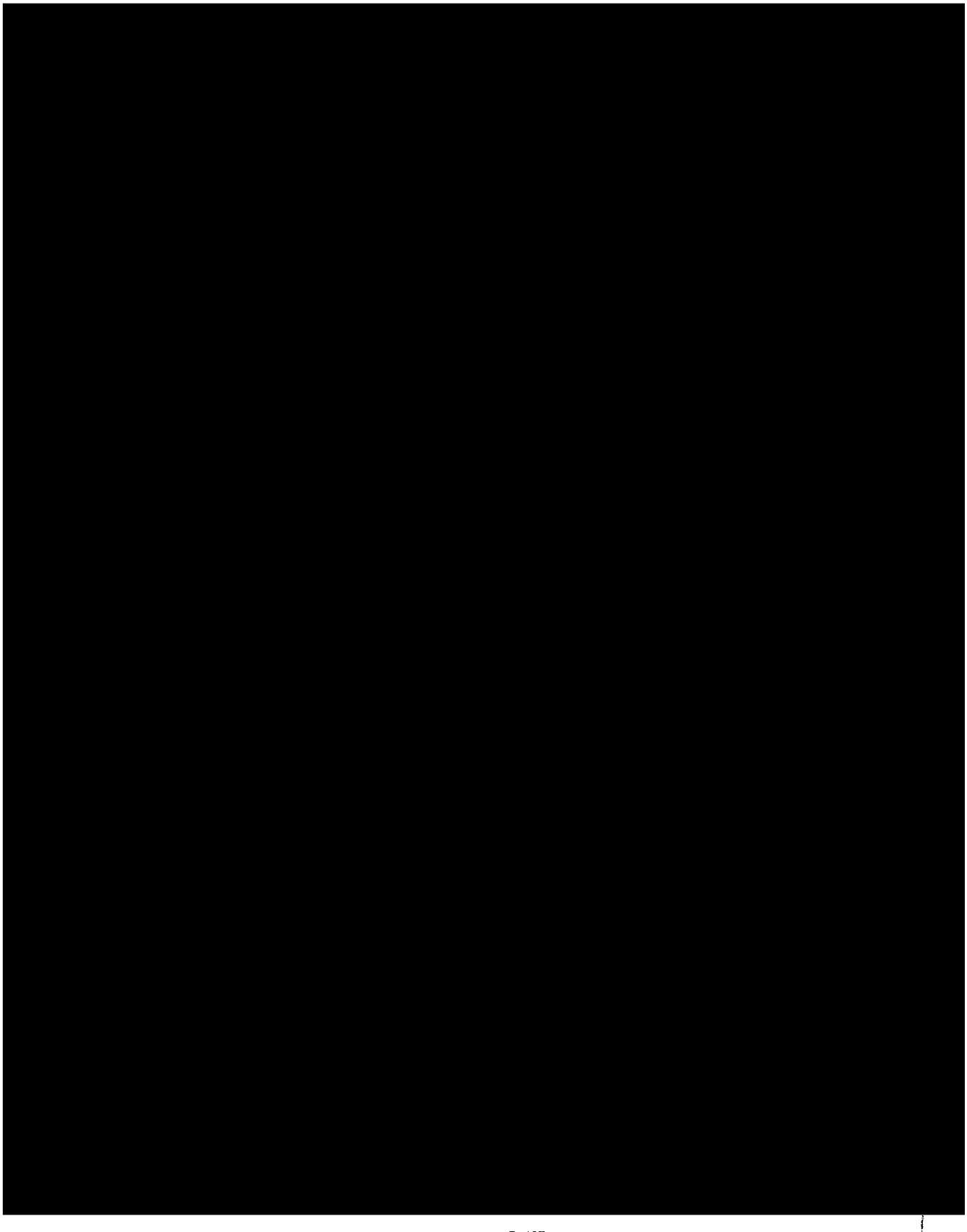
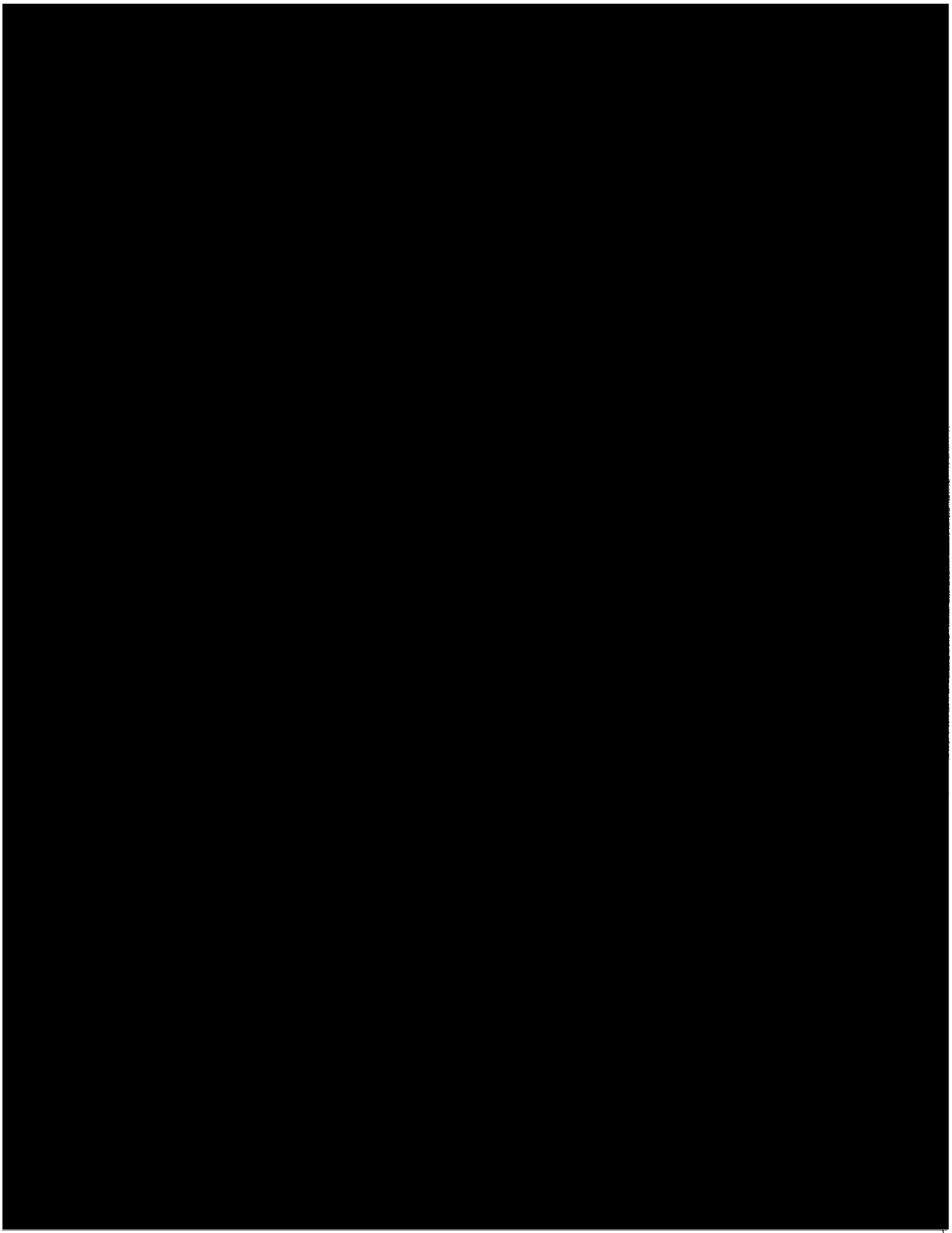
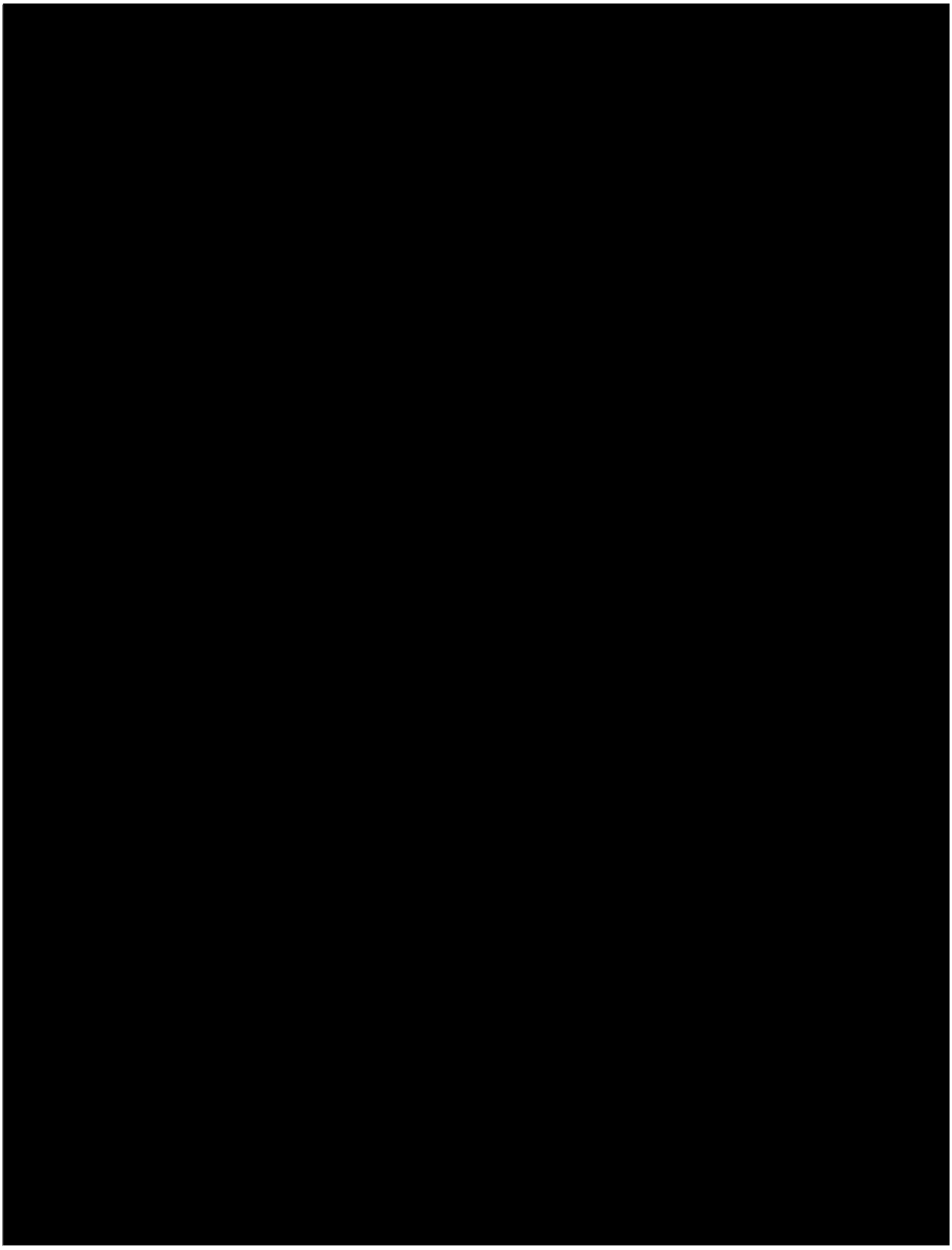
Impurities (HPLC)	Compound	Specifications	
	1AU90	Not more than 0.40%	ND
	2AU90	Not more than 0.10%	ND
	3AU90	Not more than 1.00%	ND
	750W93	Not more than 0.50%	0.06 % w/w
	751W93	Not more than 0.30%	< 0.05 % w/w
	97W86 (Benzindene Triol)	Not more than 0.20%	ND
	Treprostinil Ethyl Ester	Not more than 0.50%	0.13 % w/w
	Treprostinil Methyl Ester	Not more than 0.20%	ND
Impurities (HPLC) [Unidentified Impurities]	Not more than 0.10% AUC each		ND
Impurities (HPLC) [Total Related Substances]	Not more than 3.00%		0.2 %

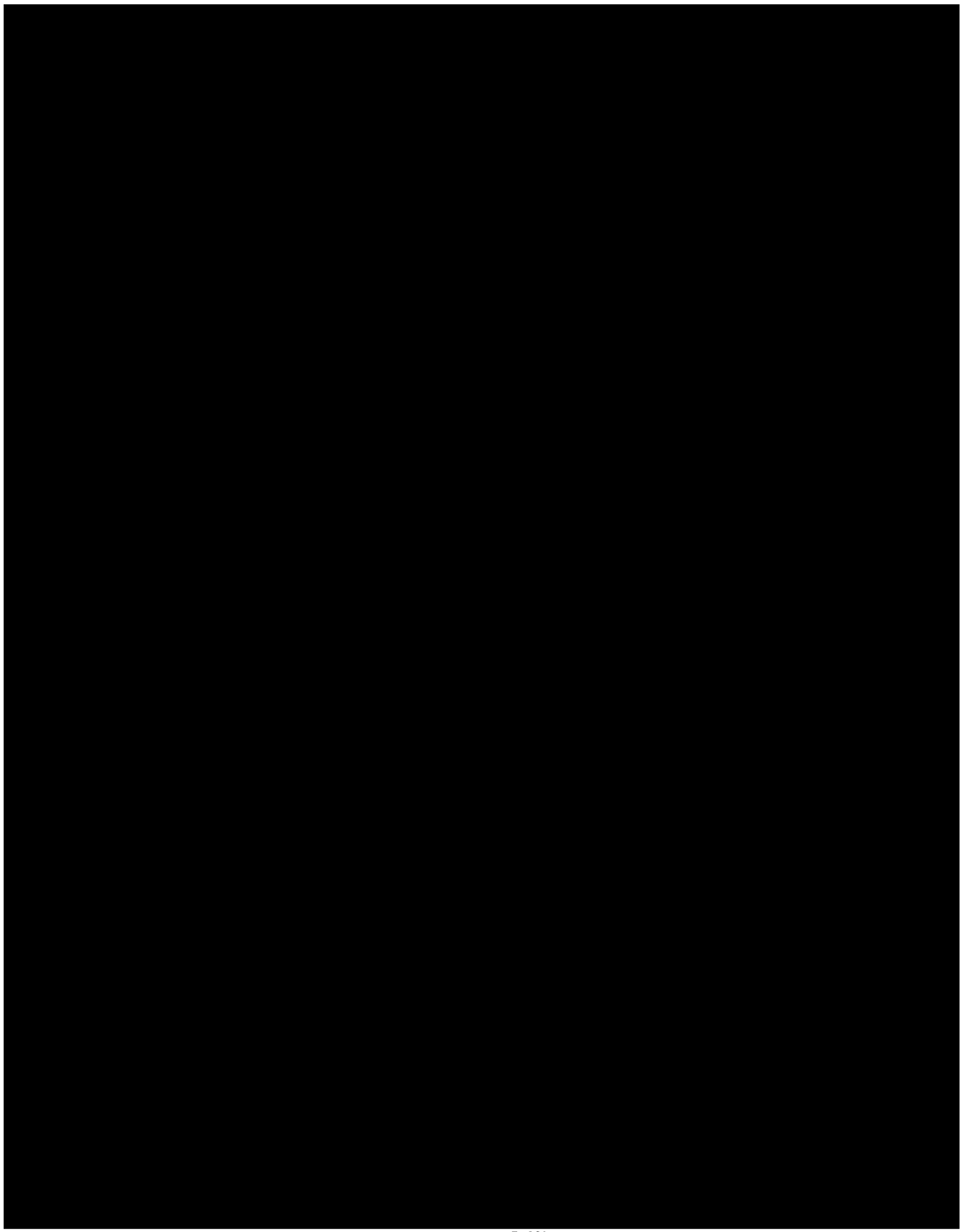
[redacted]

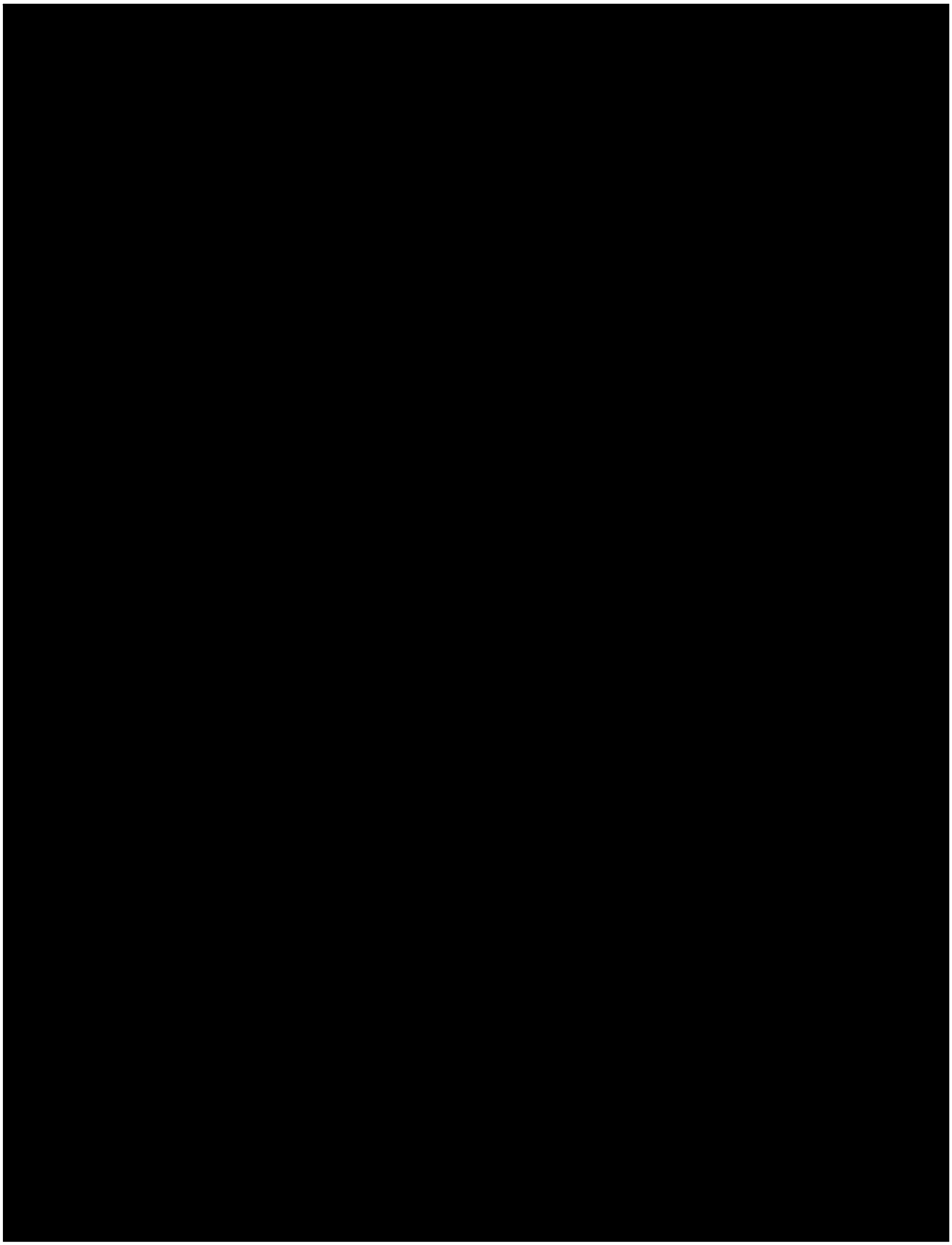


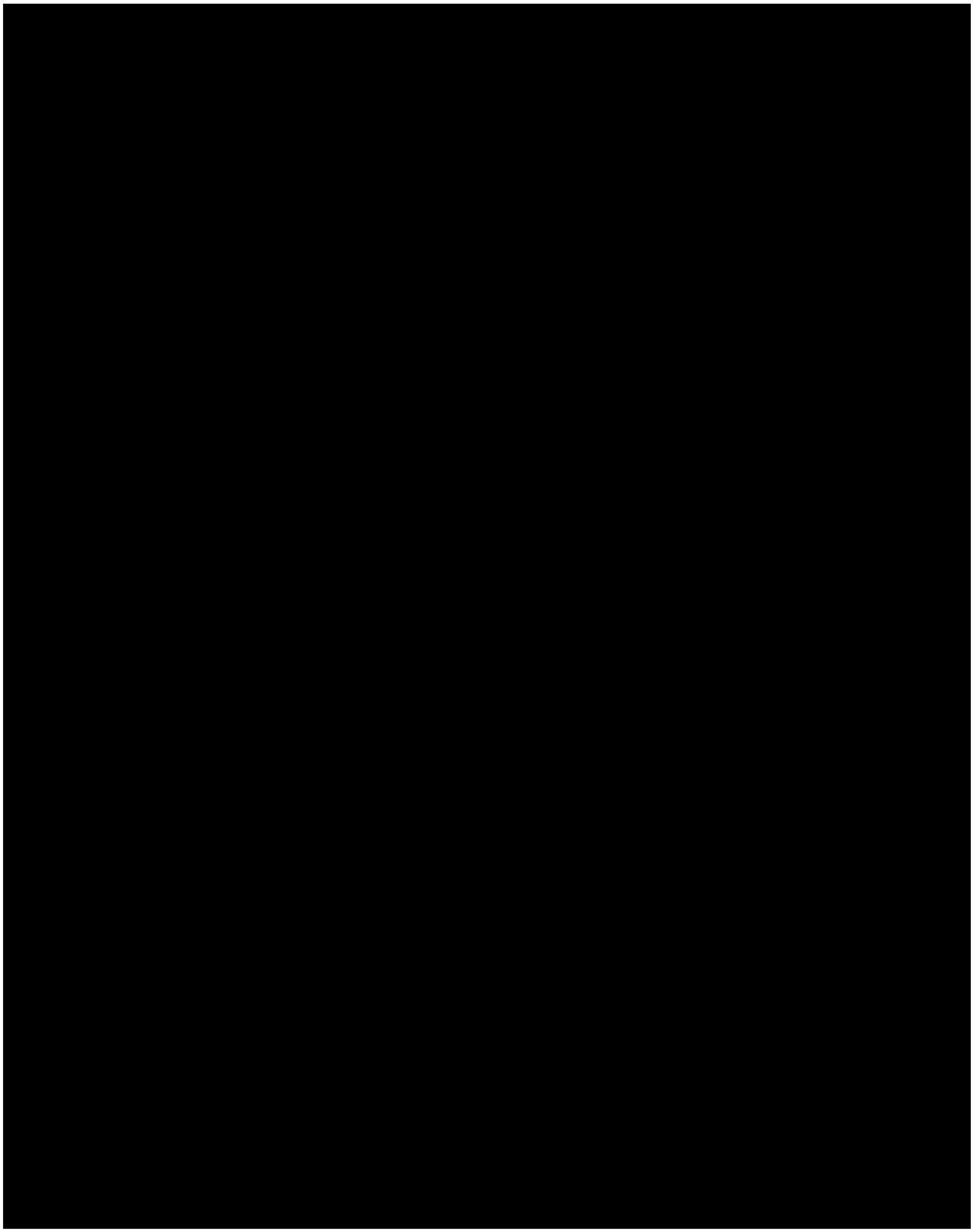


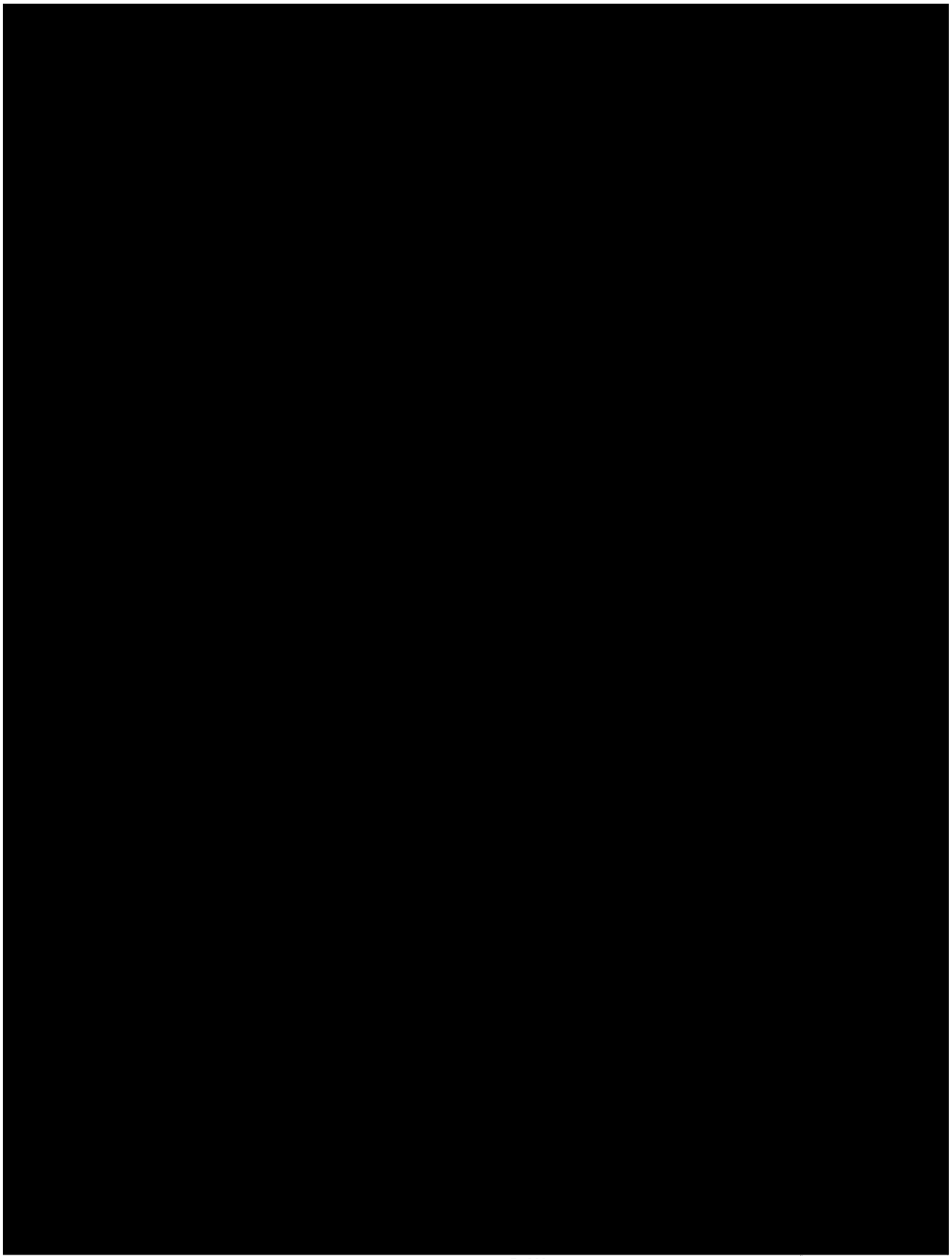


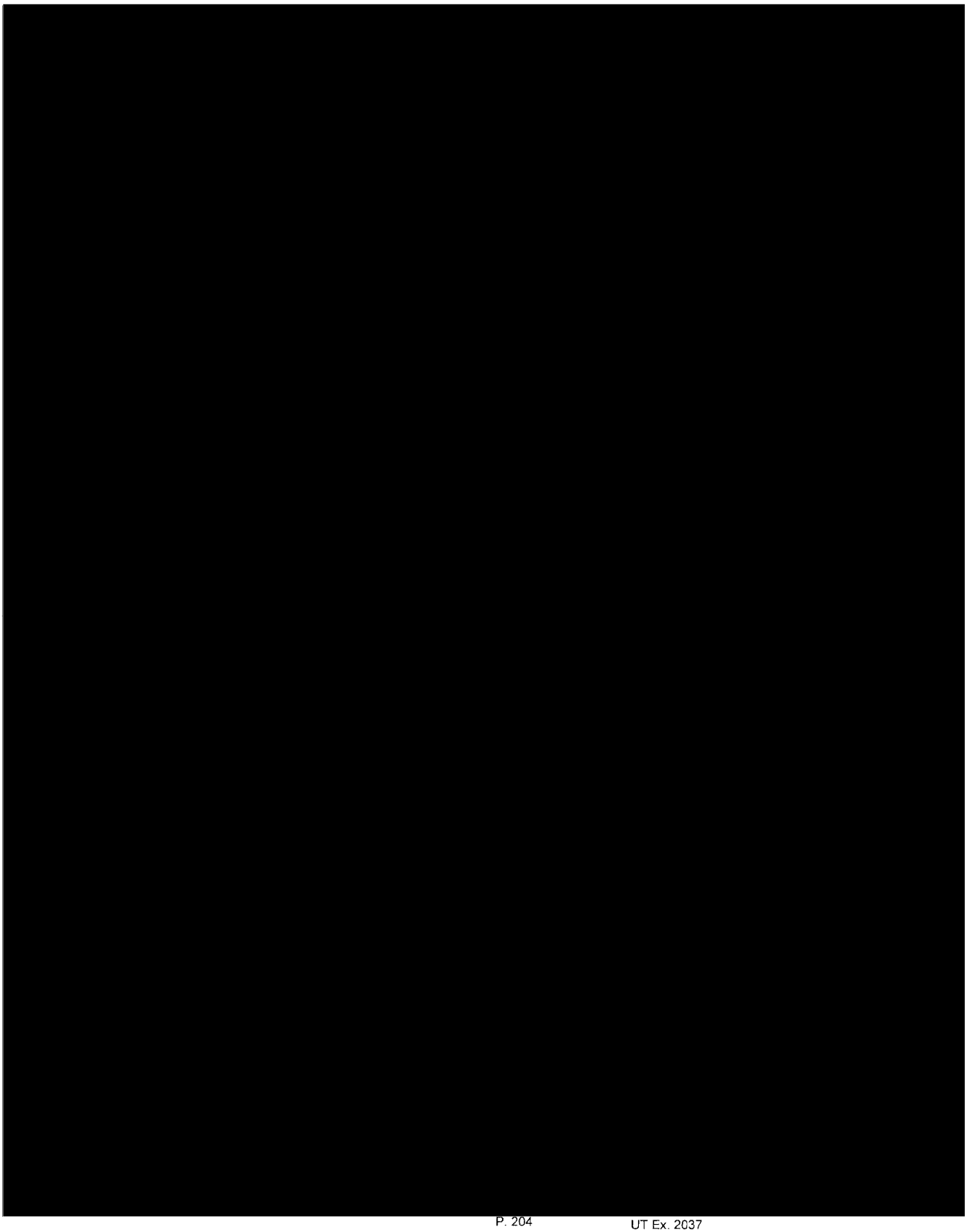


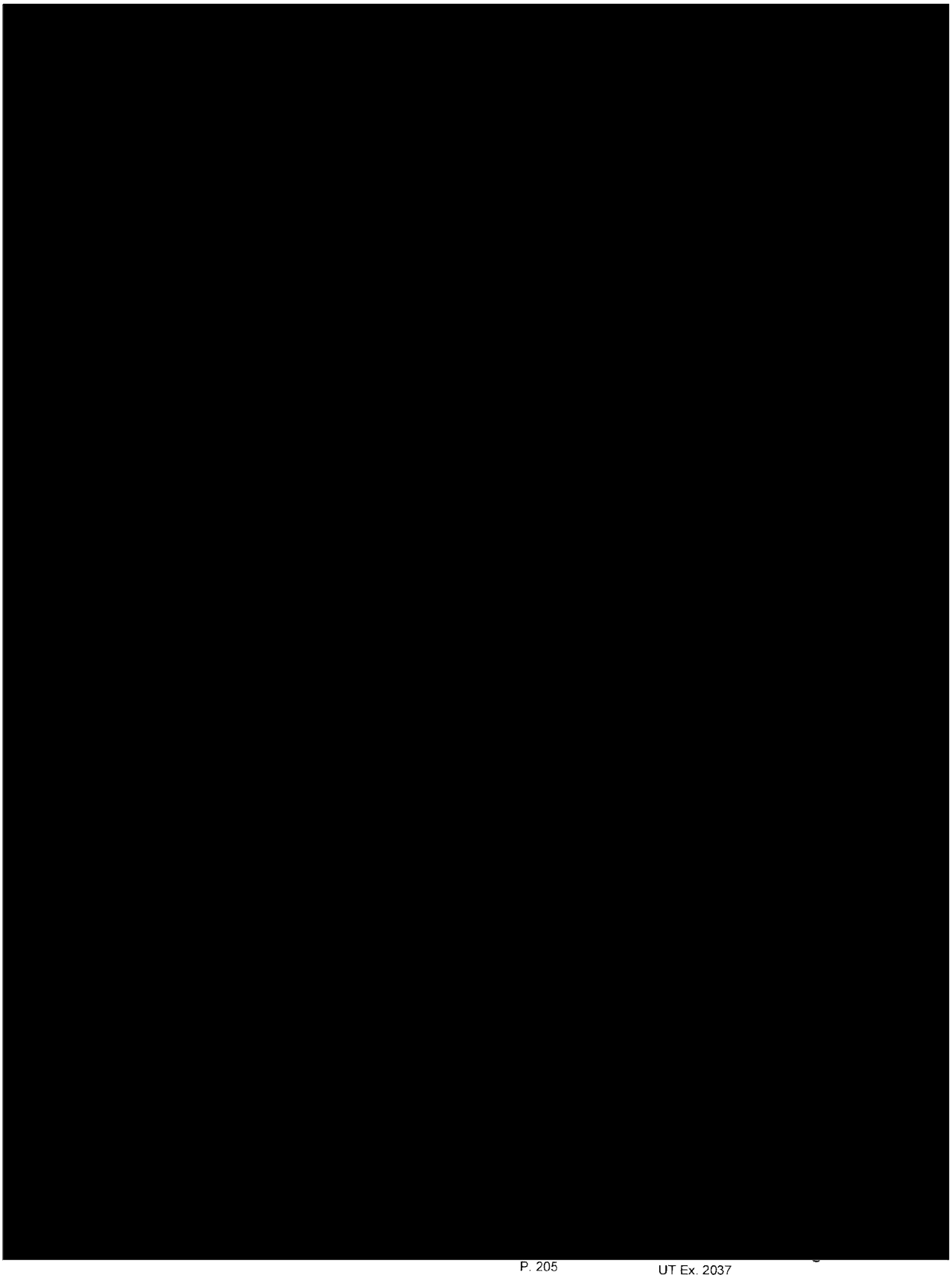


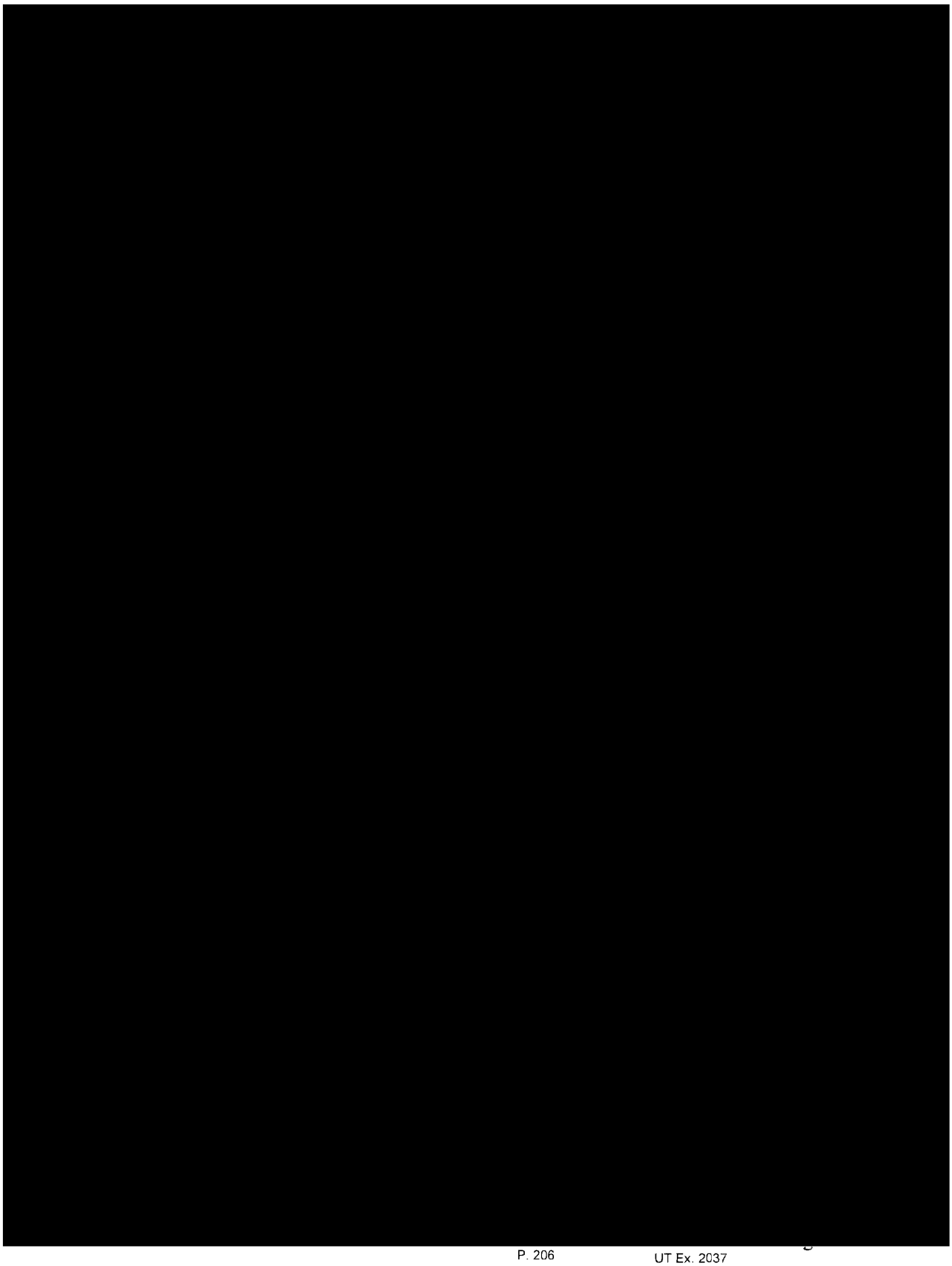


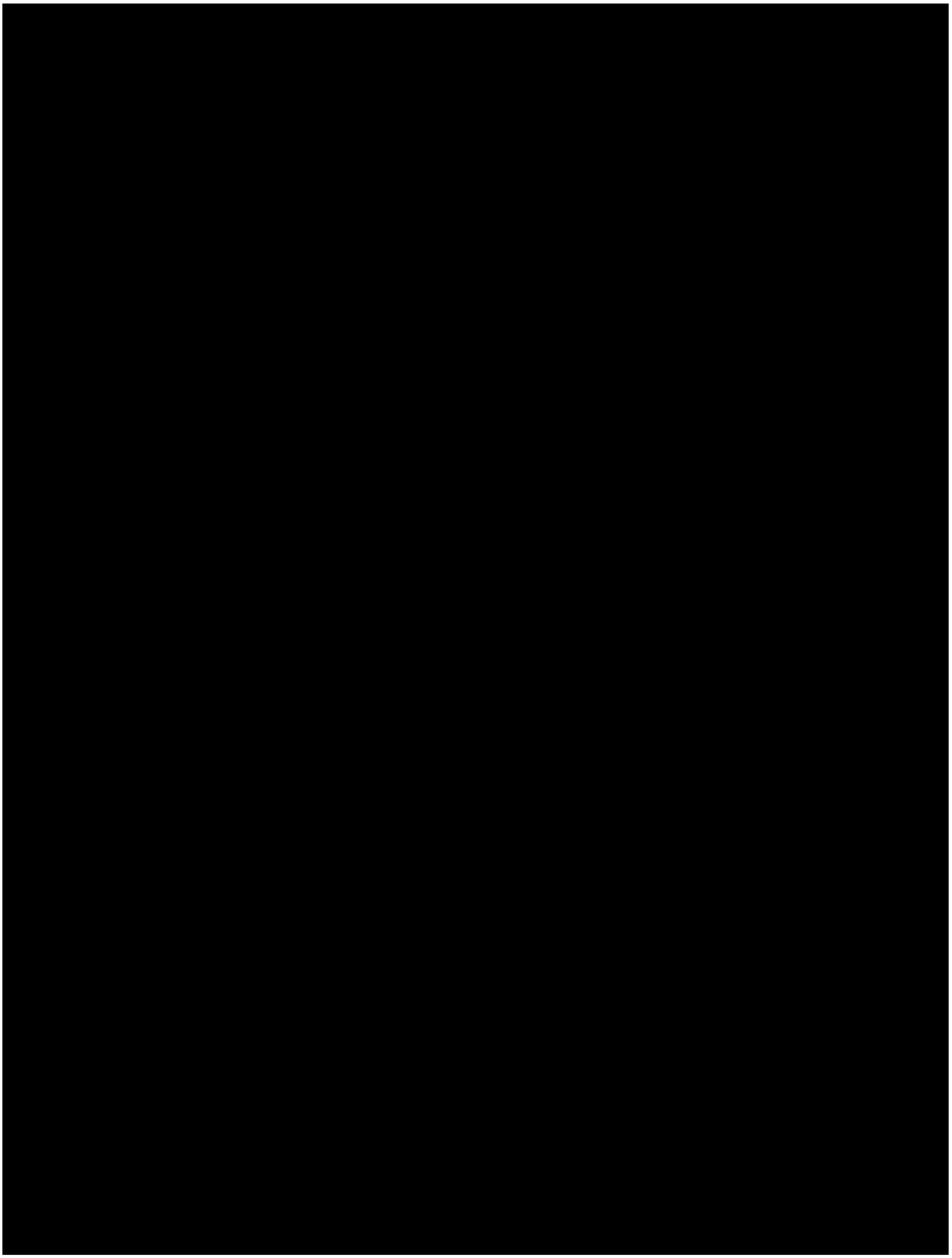


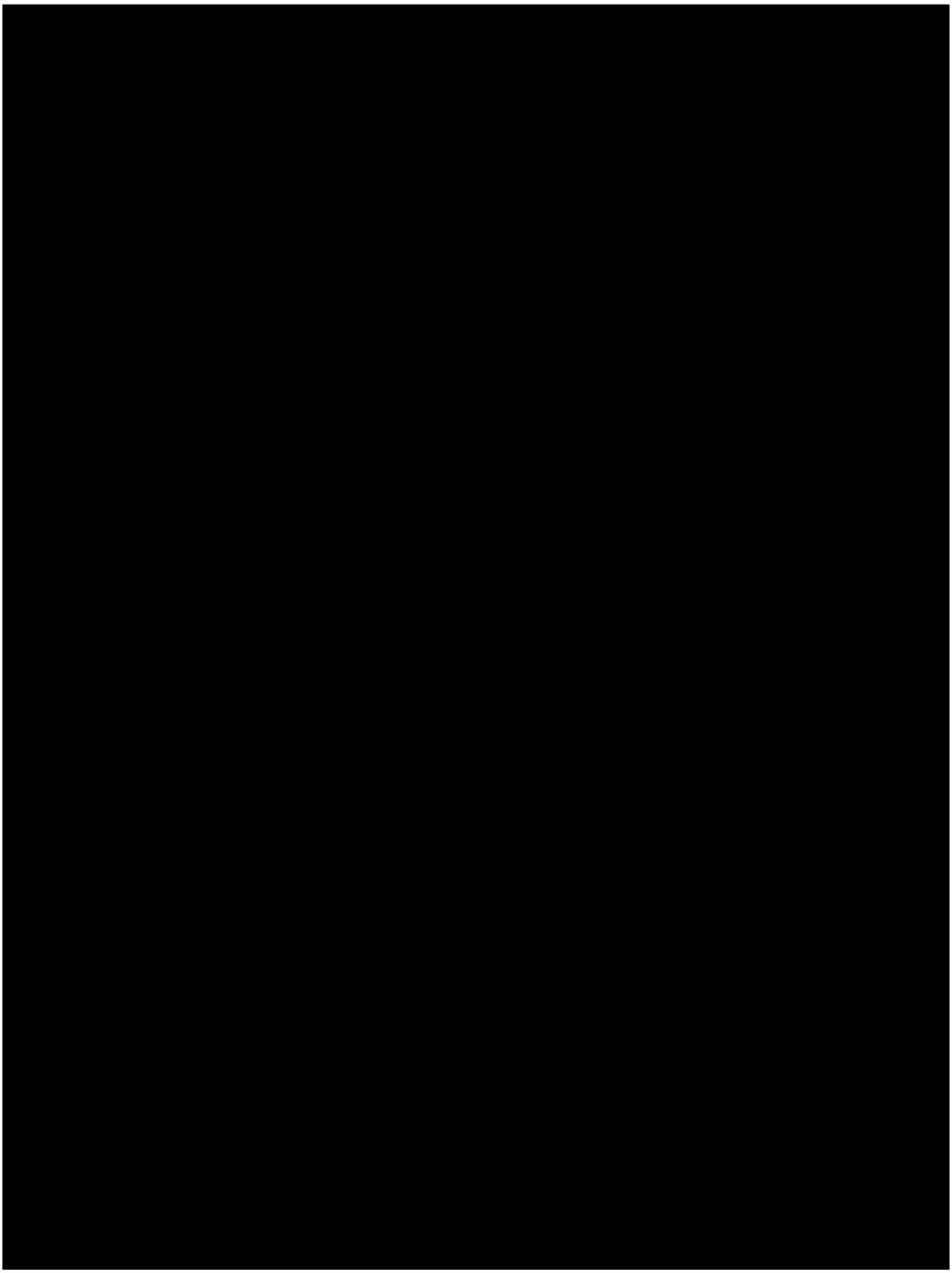


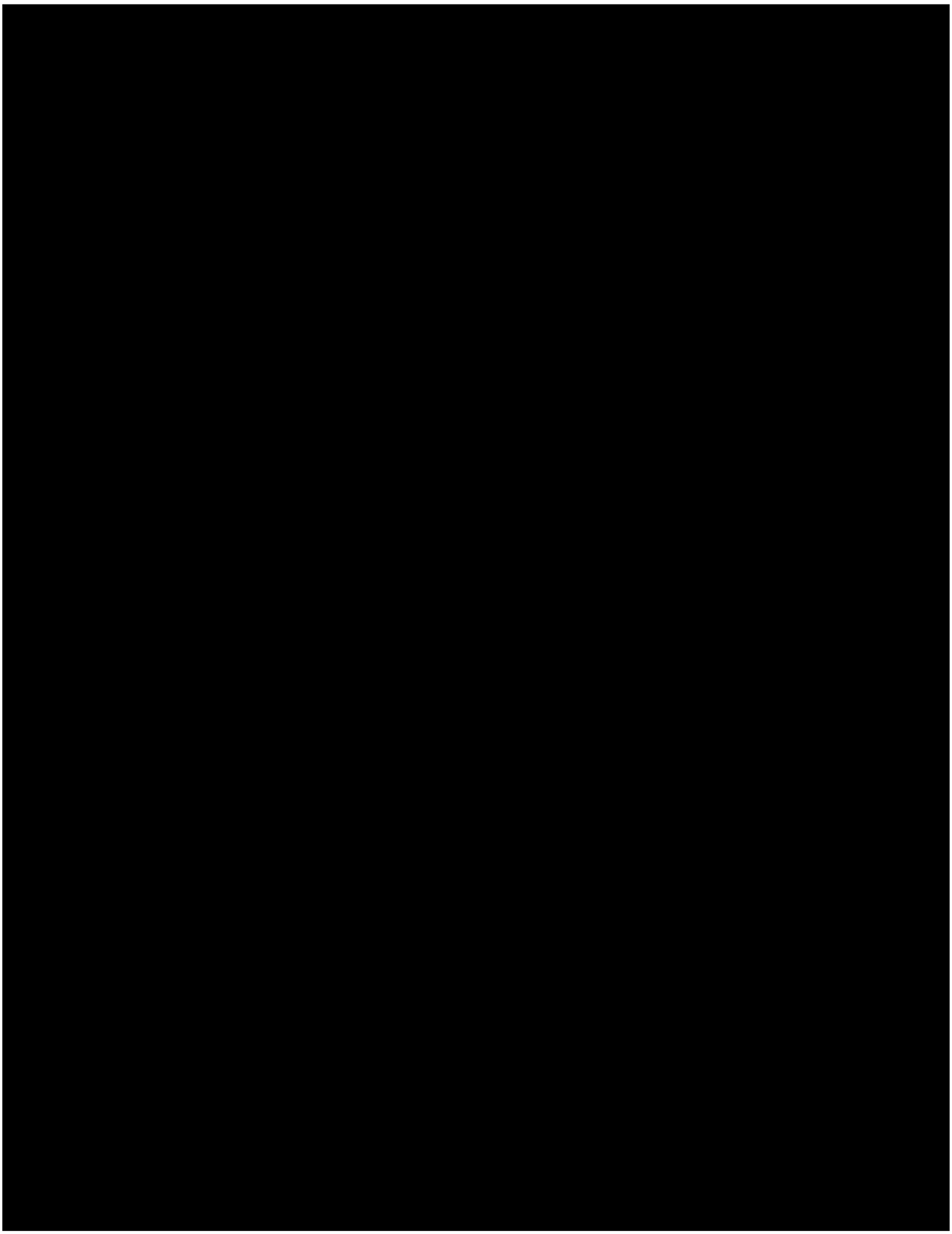


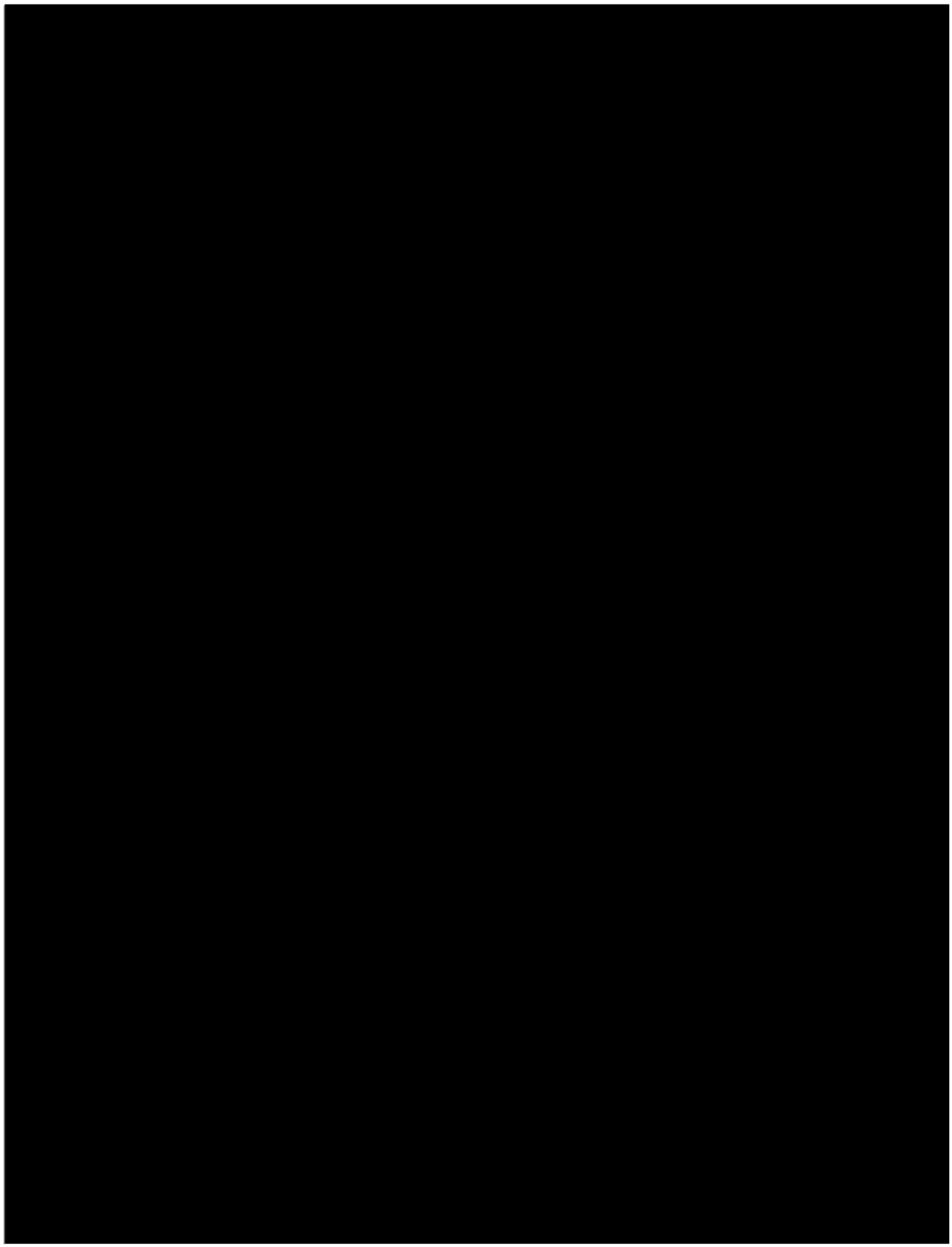


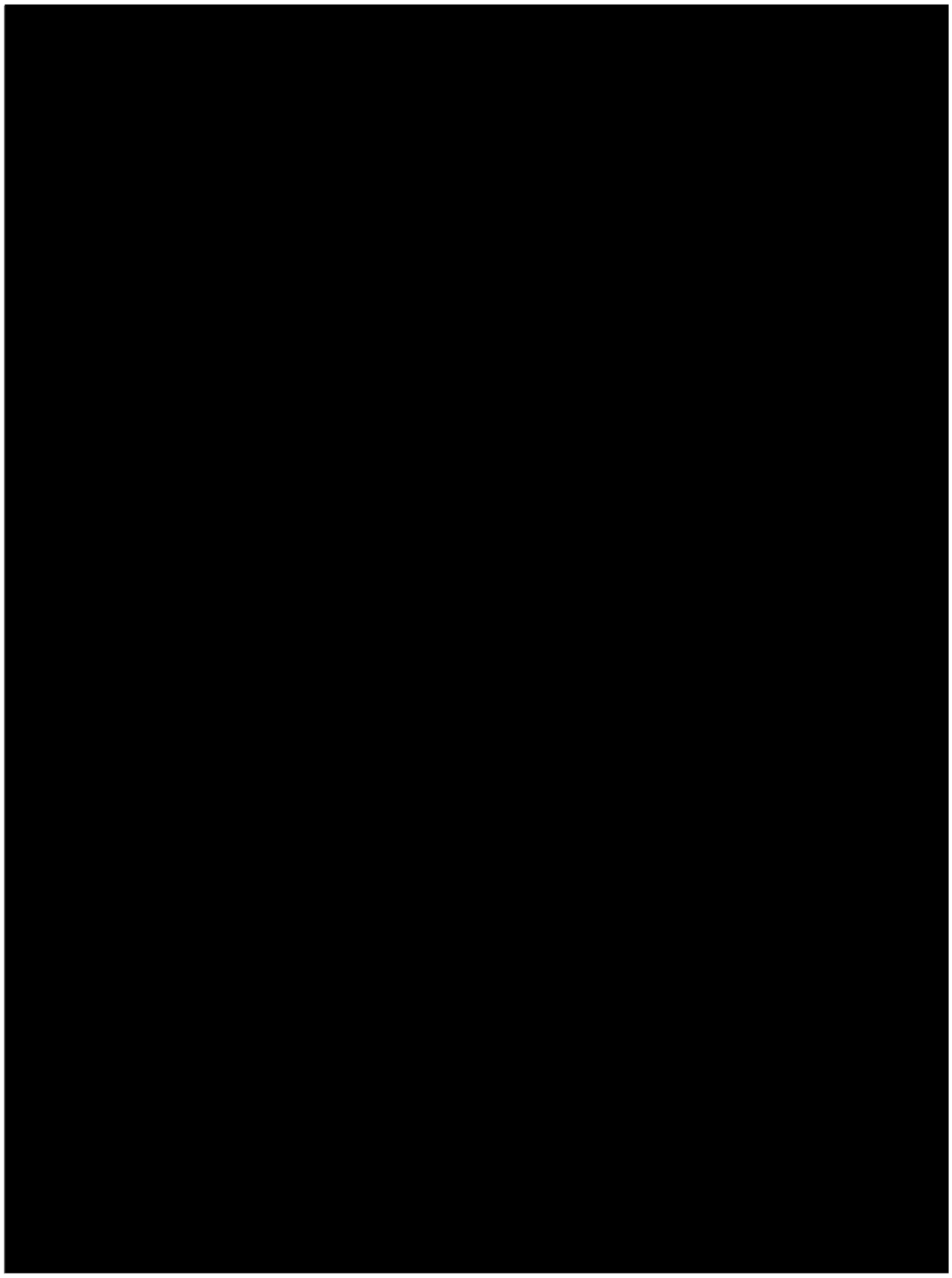


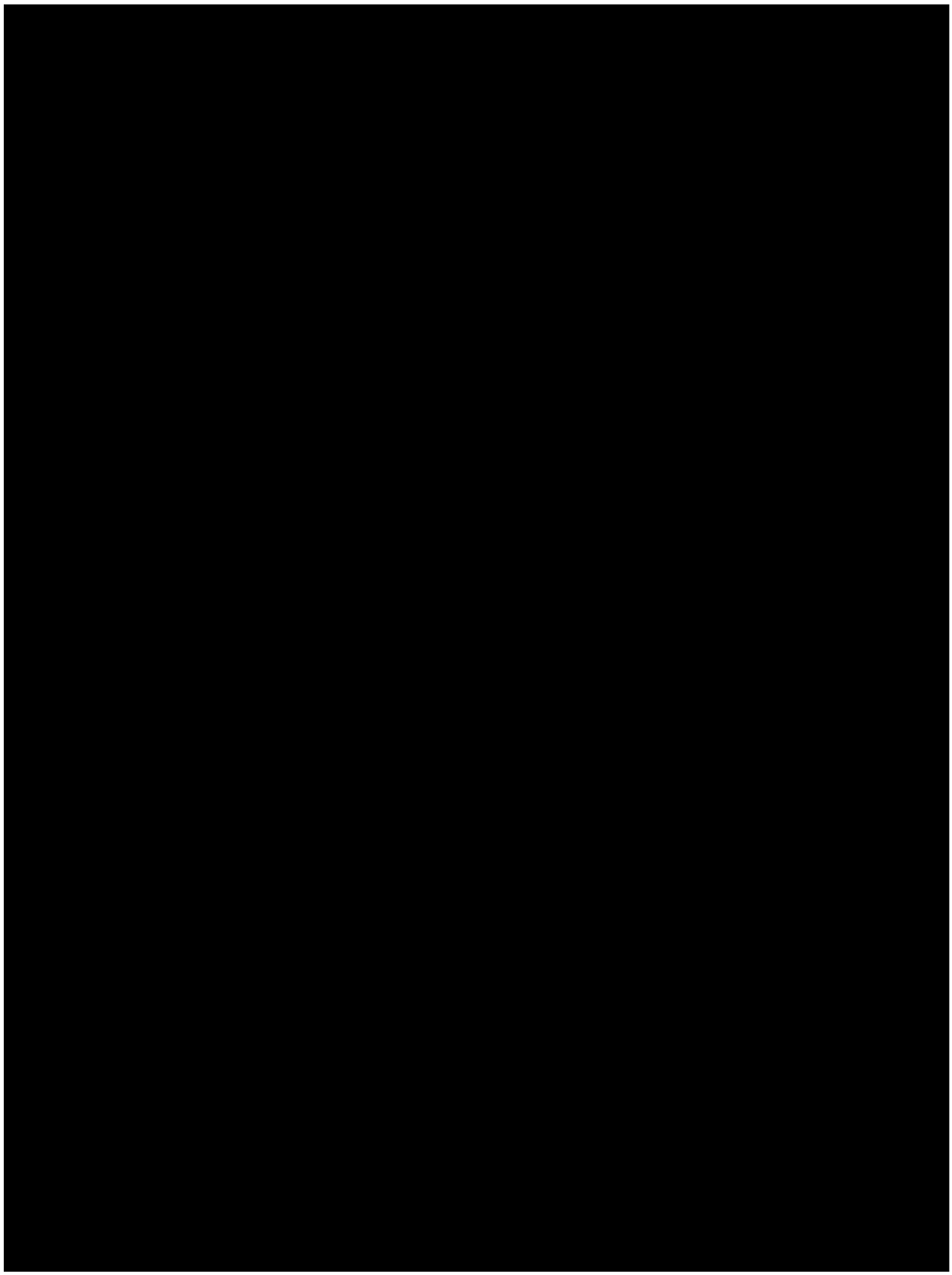


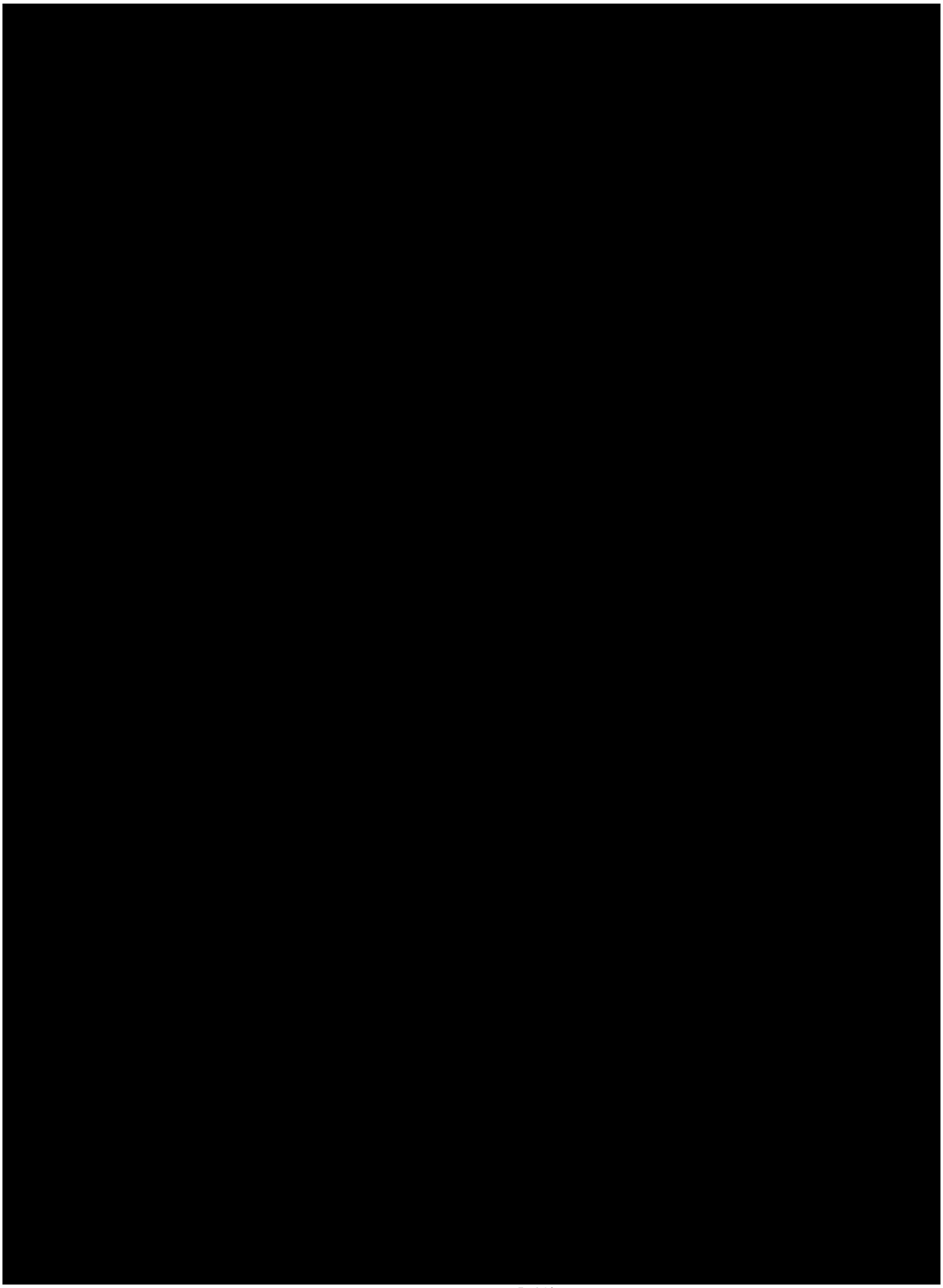


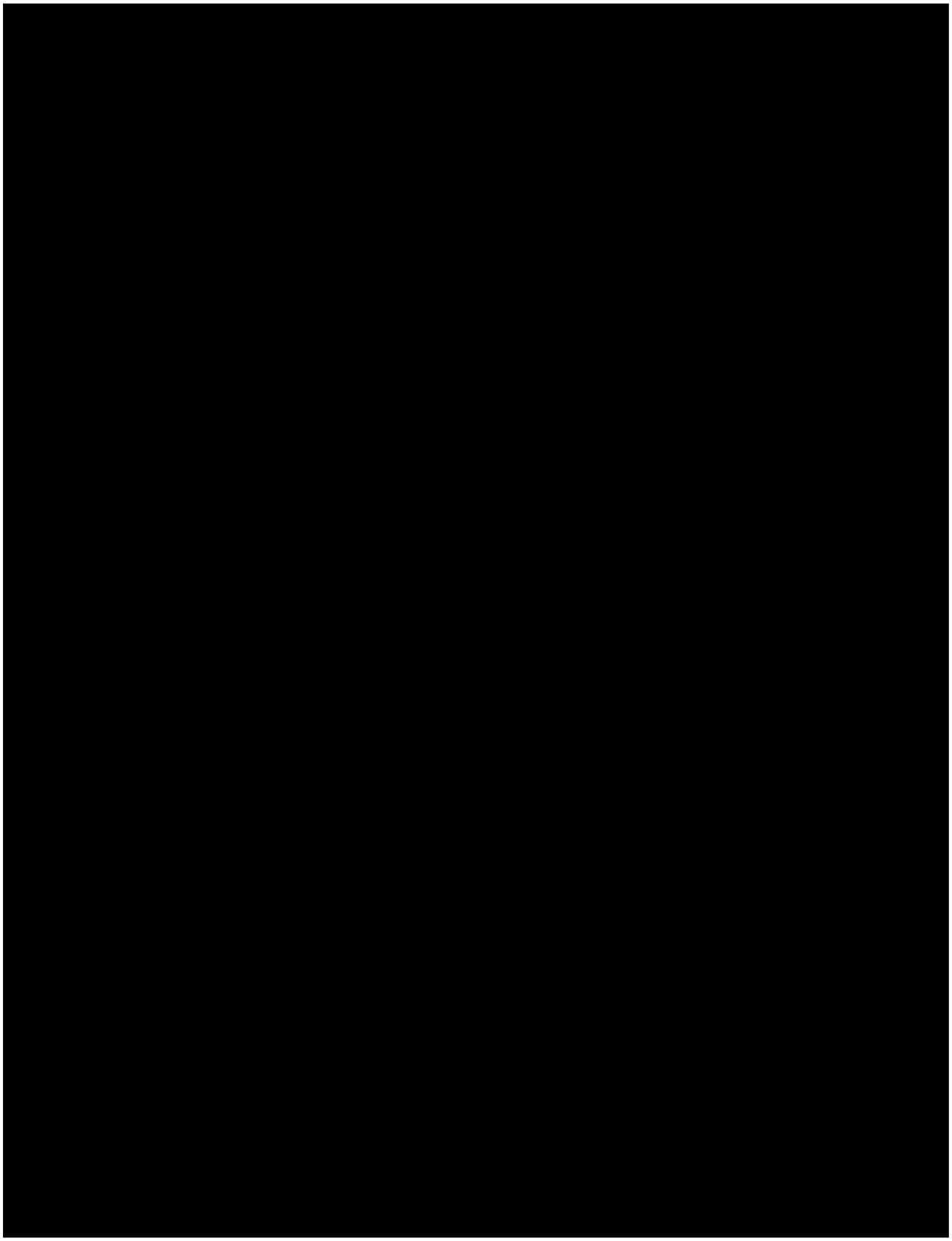


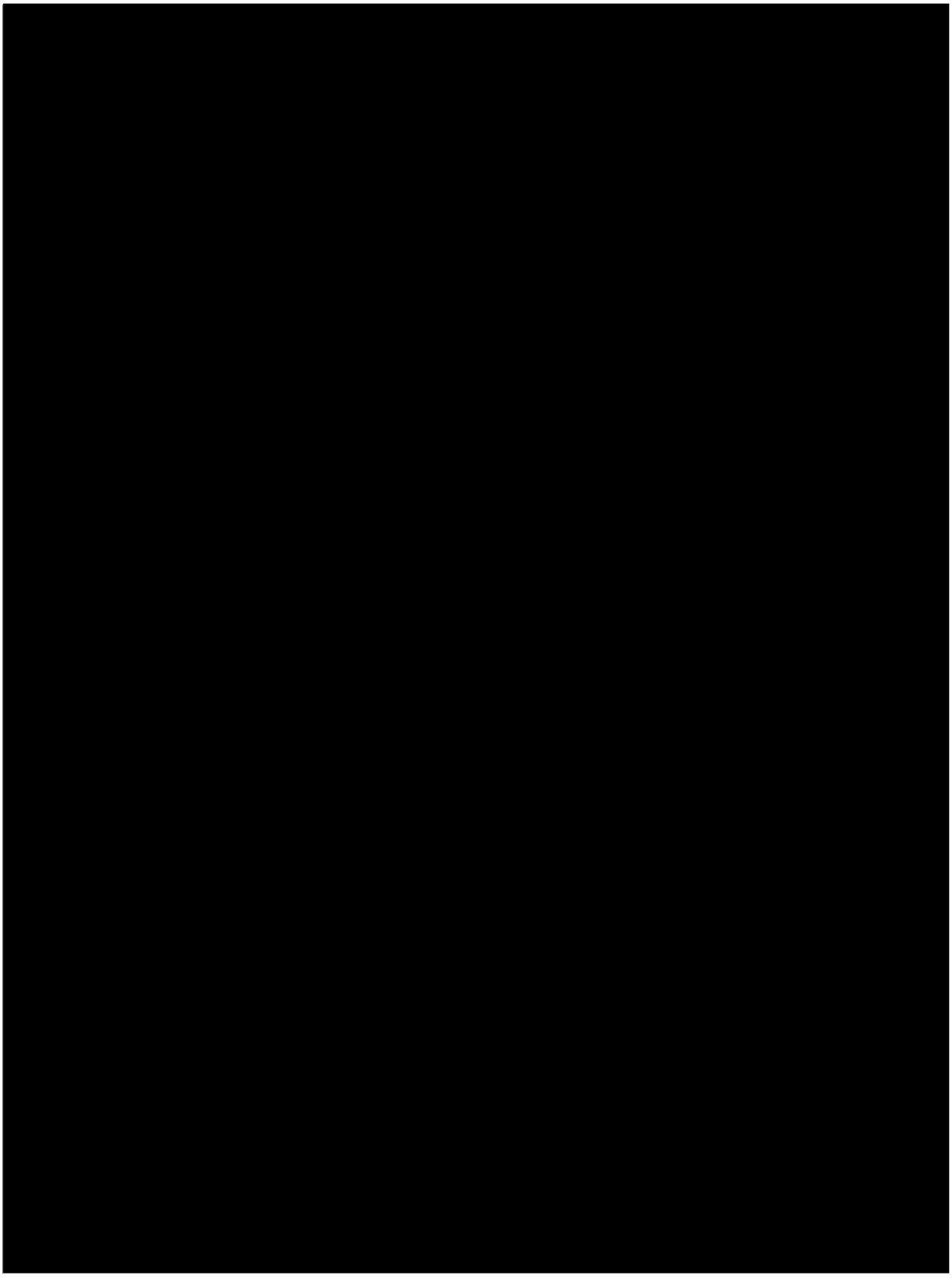


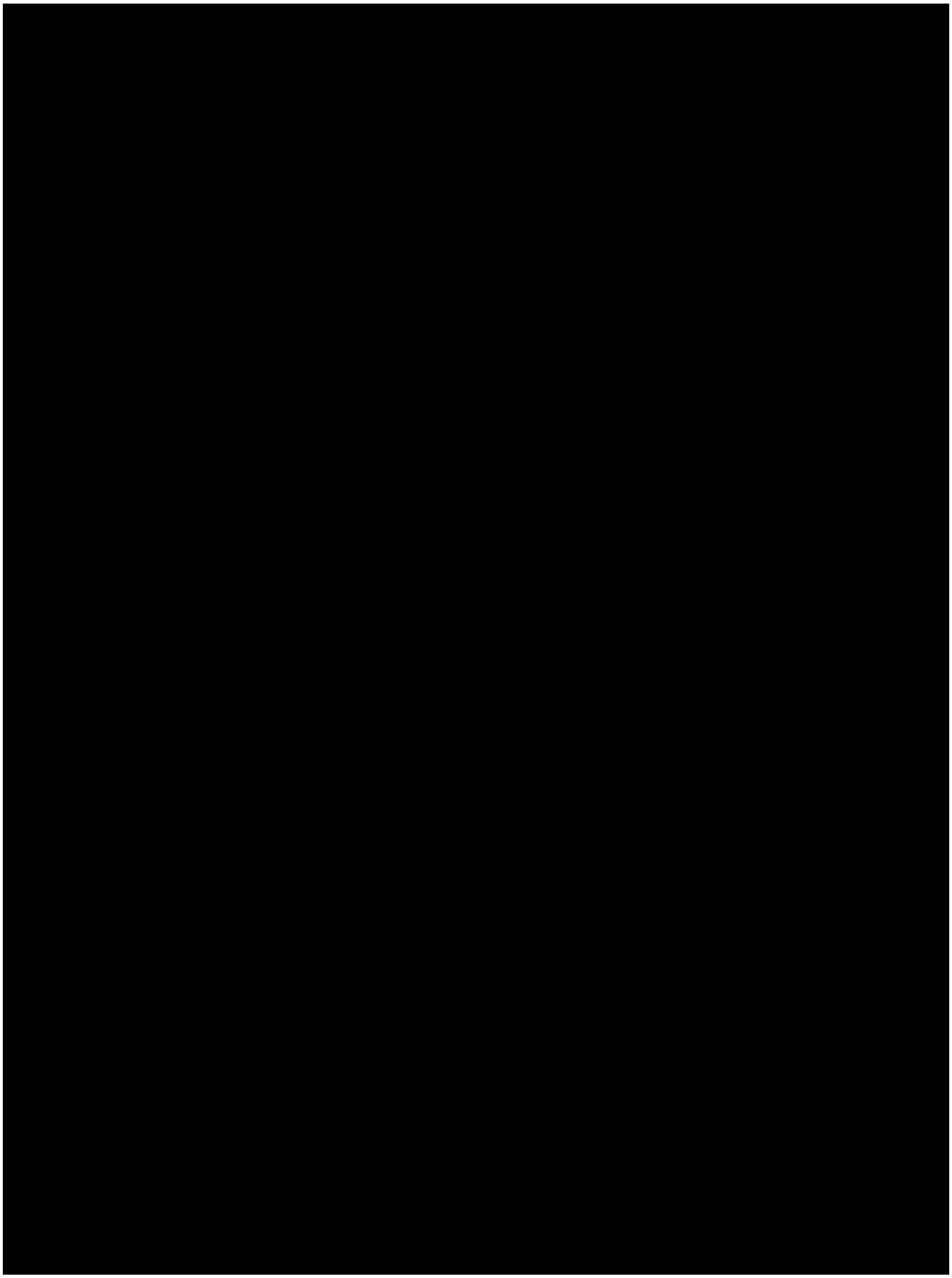


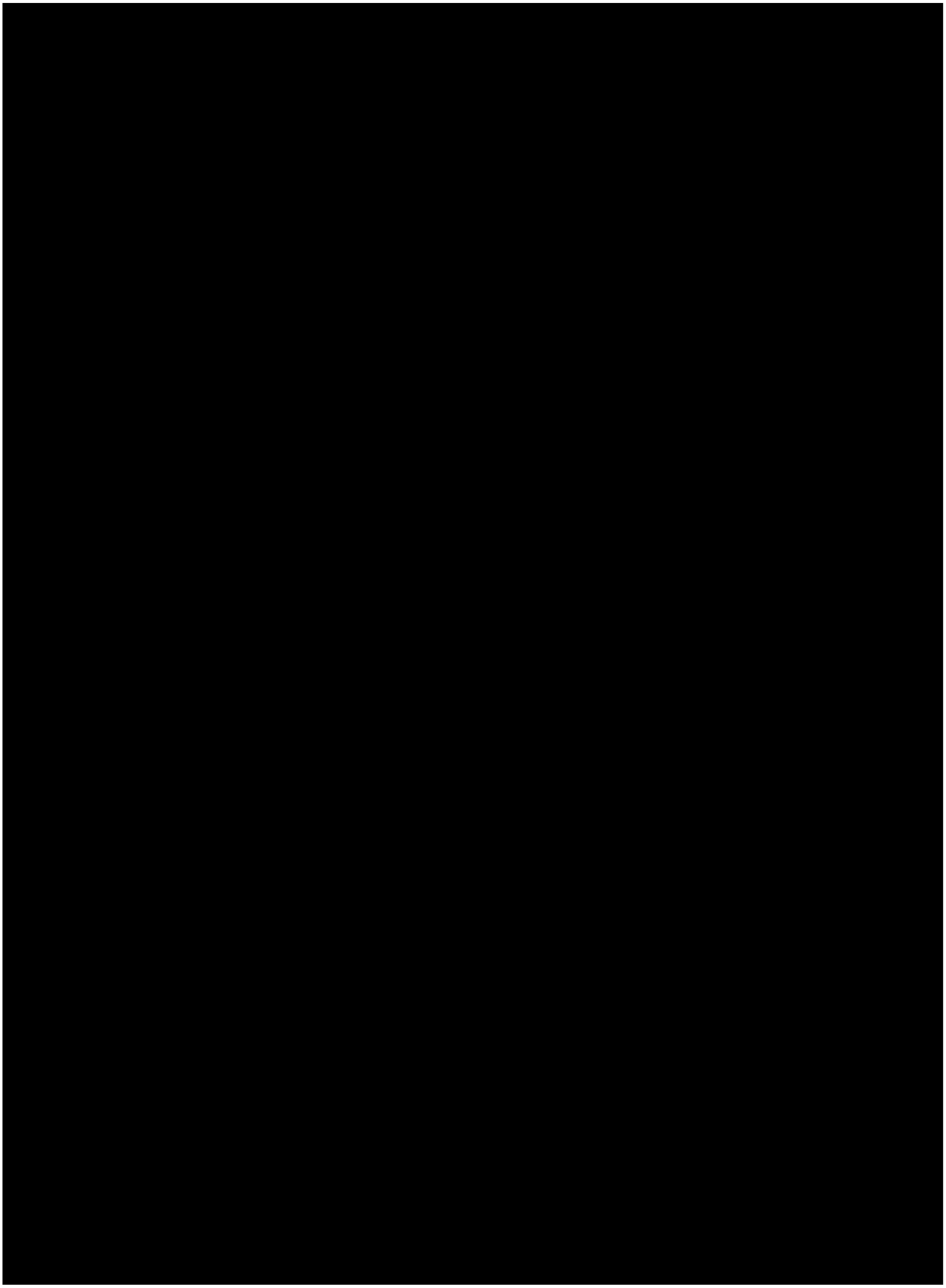


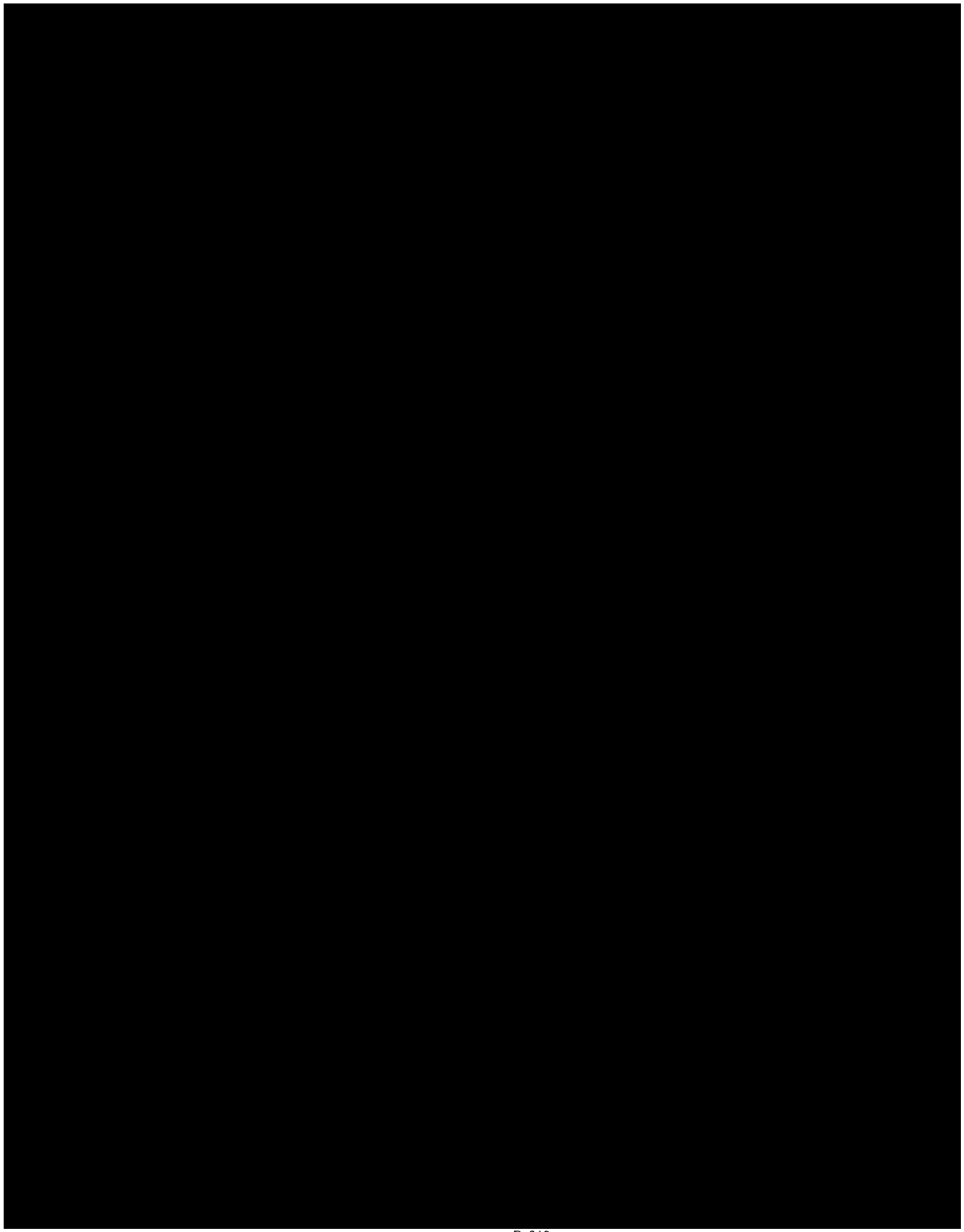


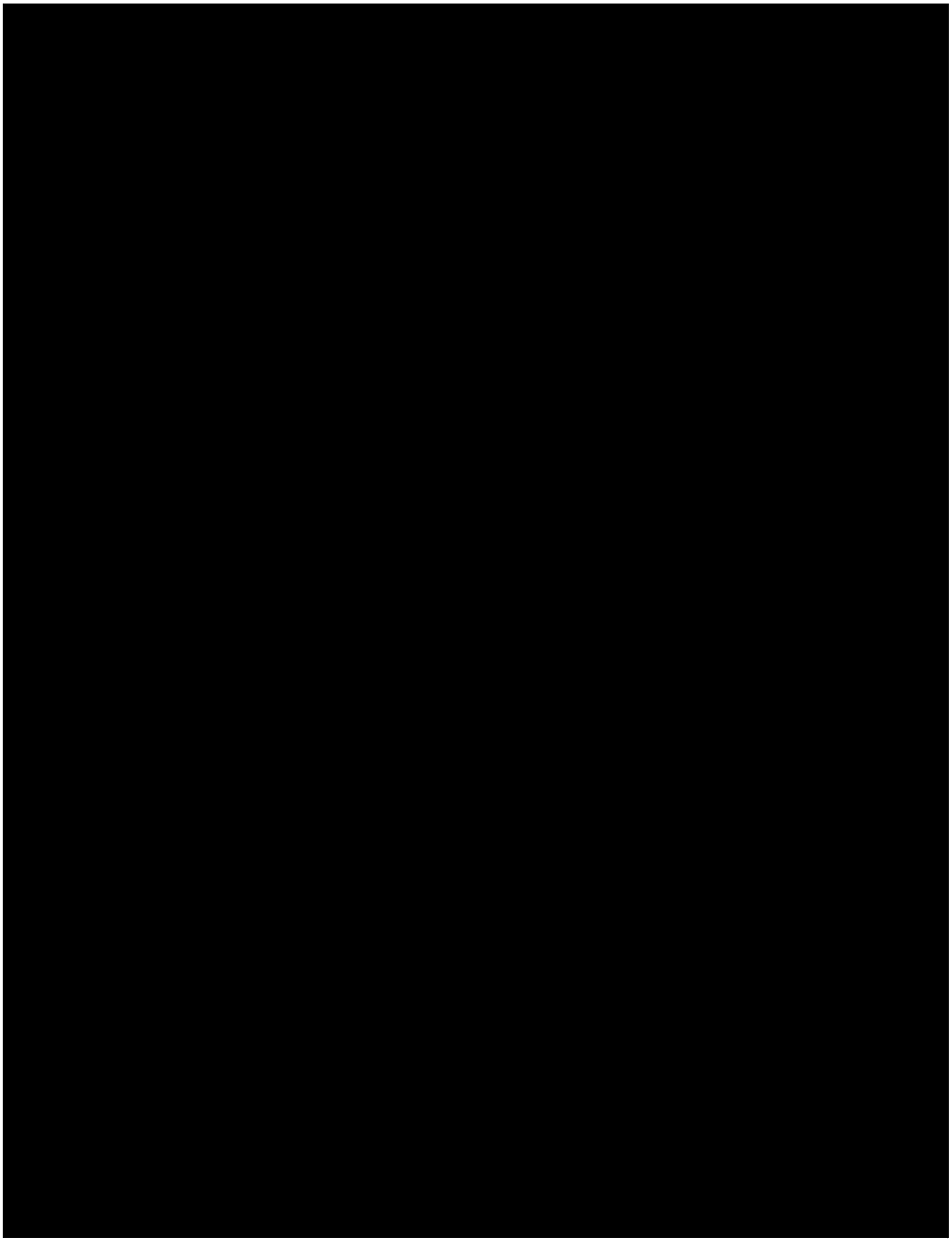


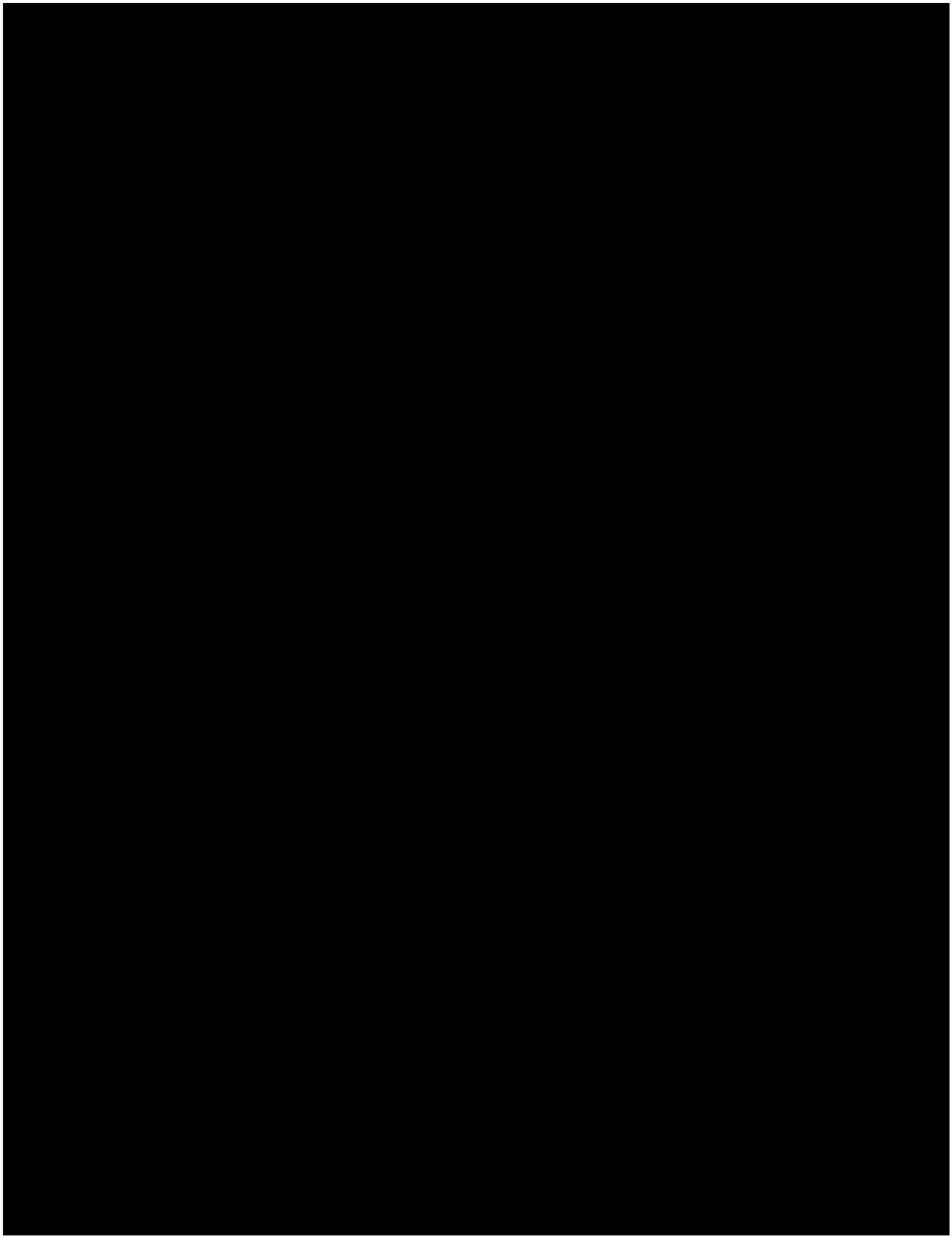


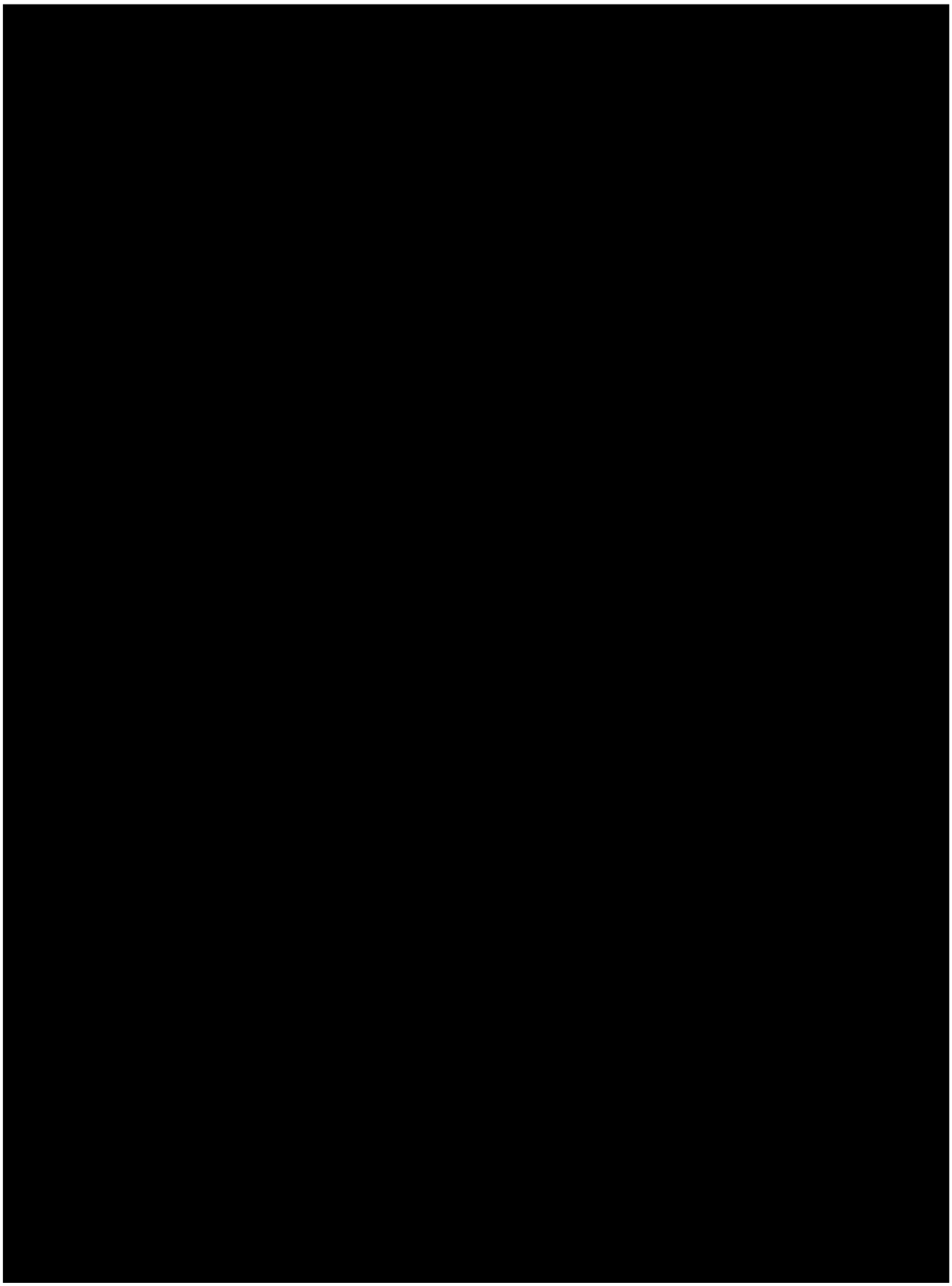


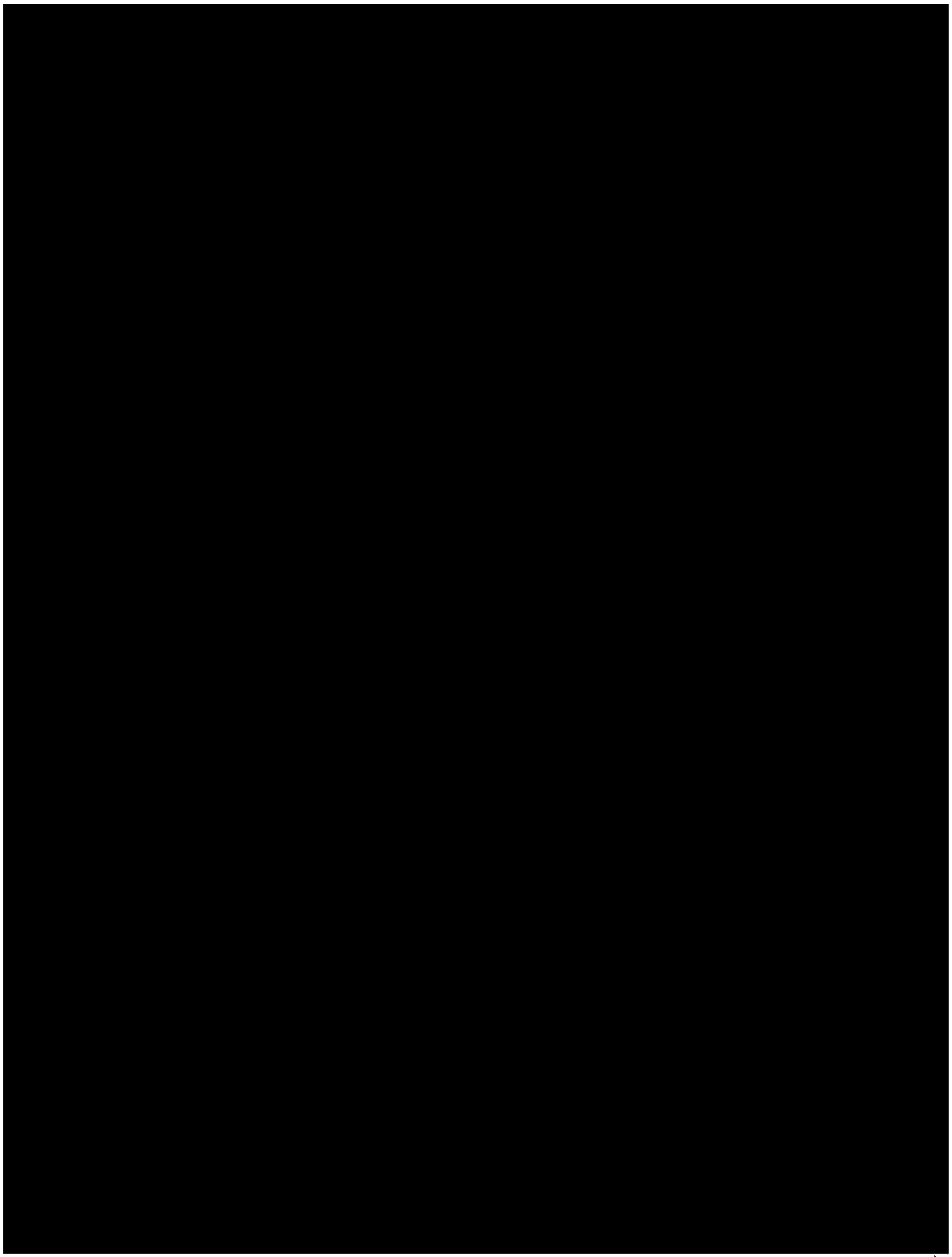


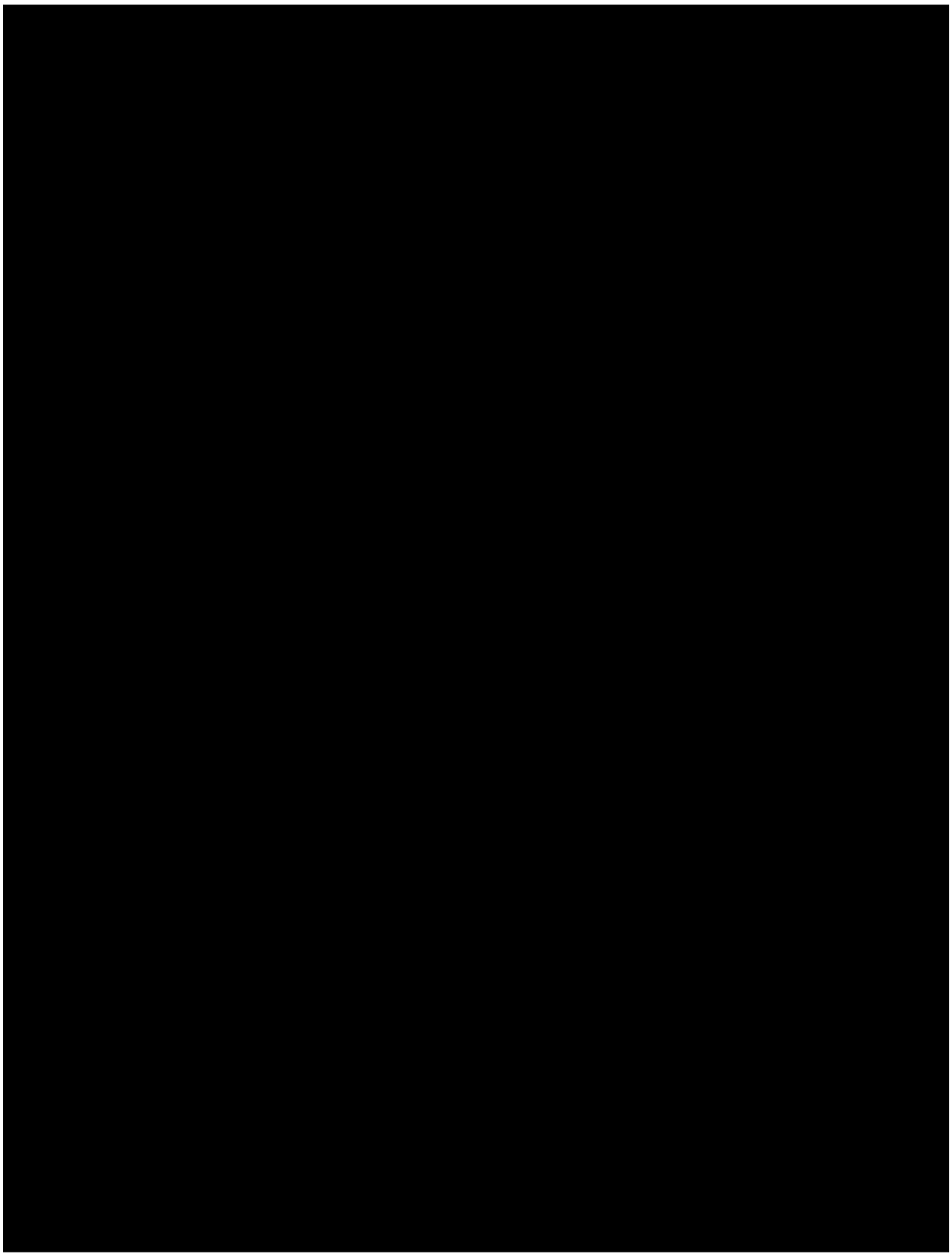


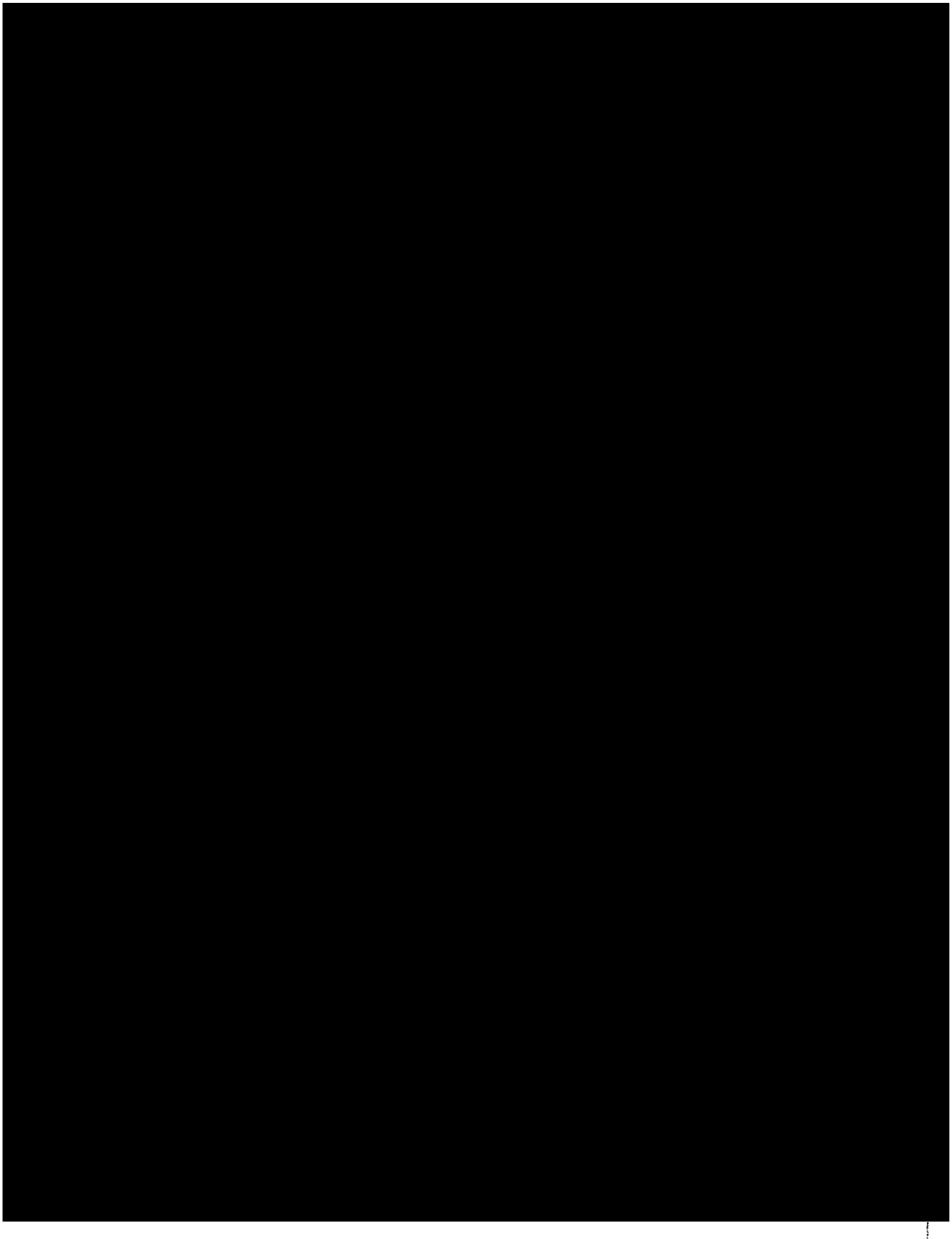


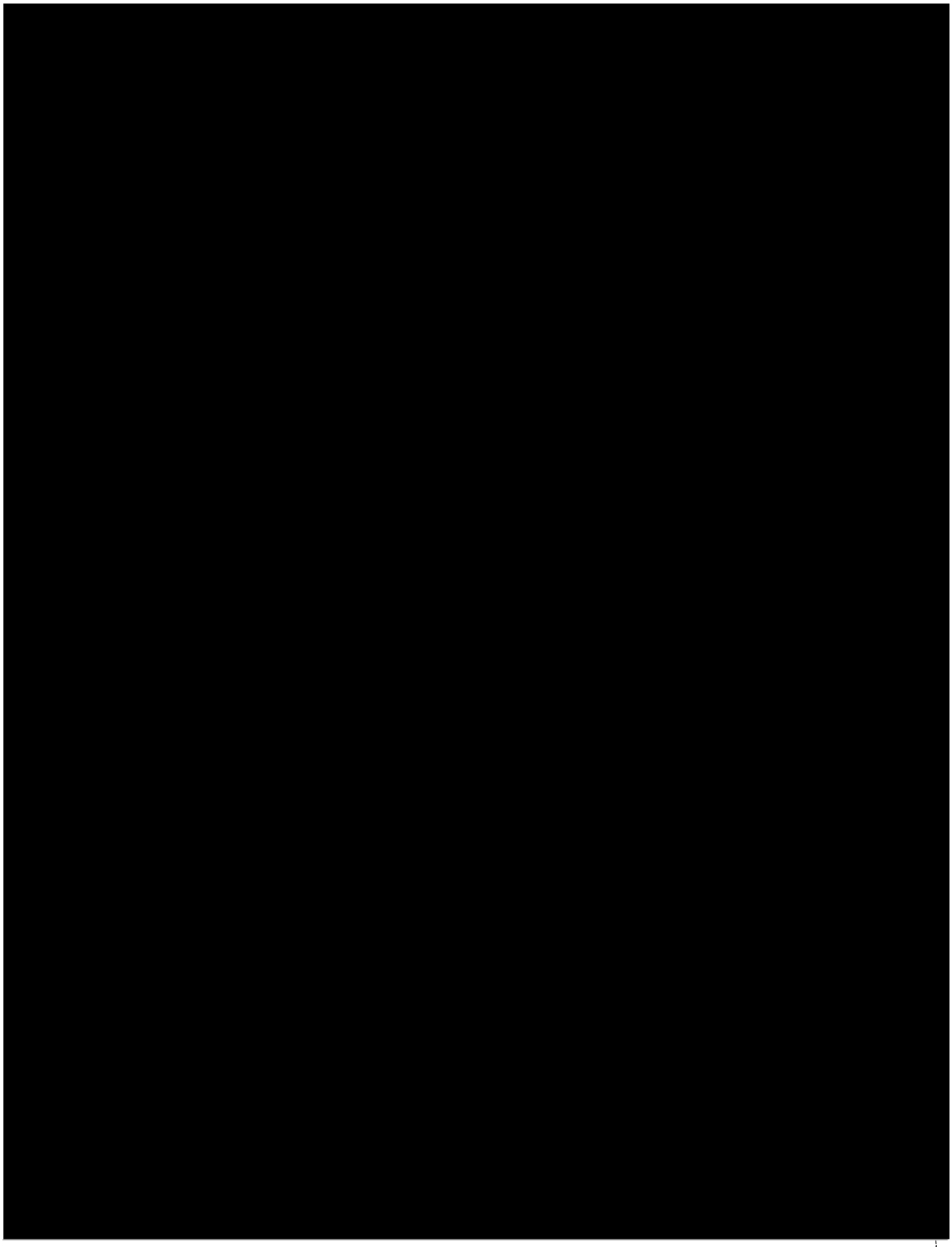


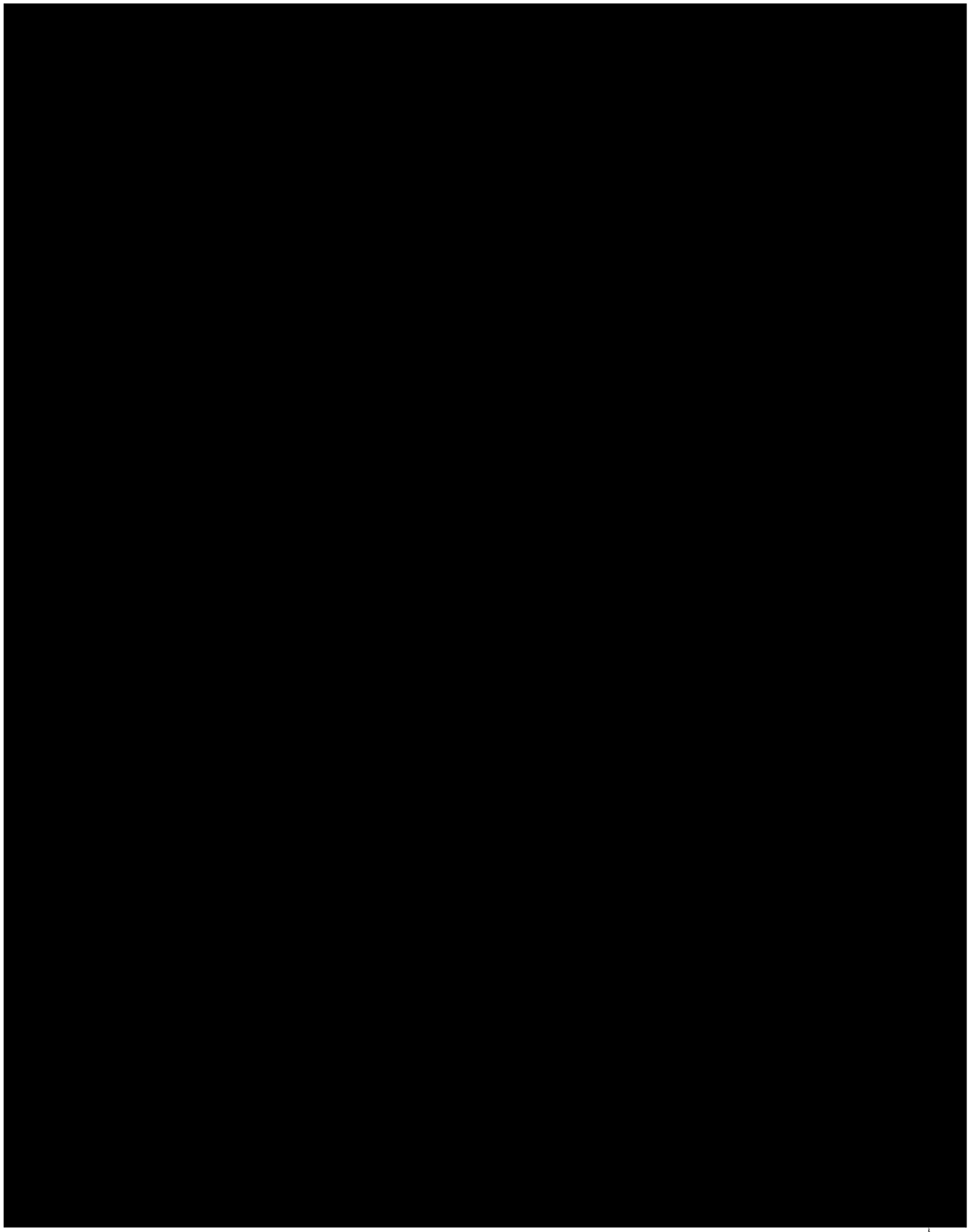


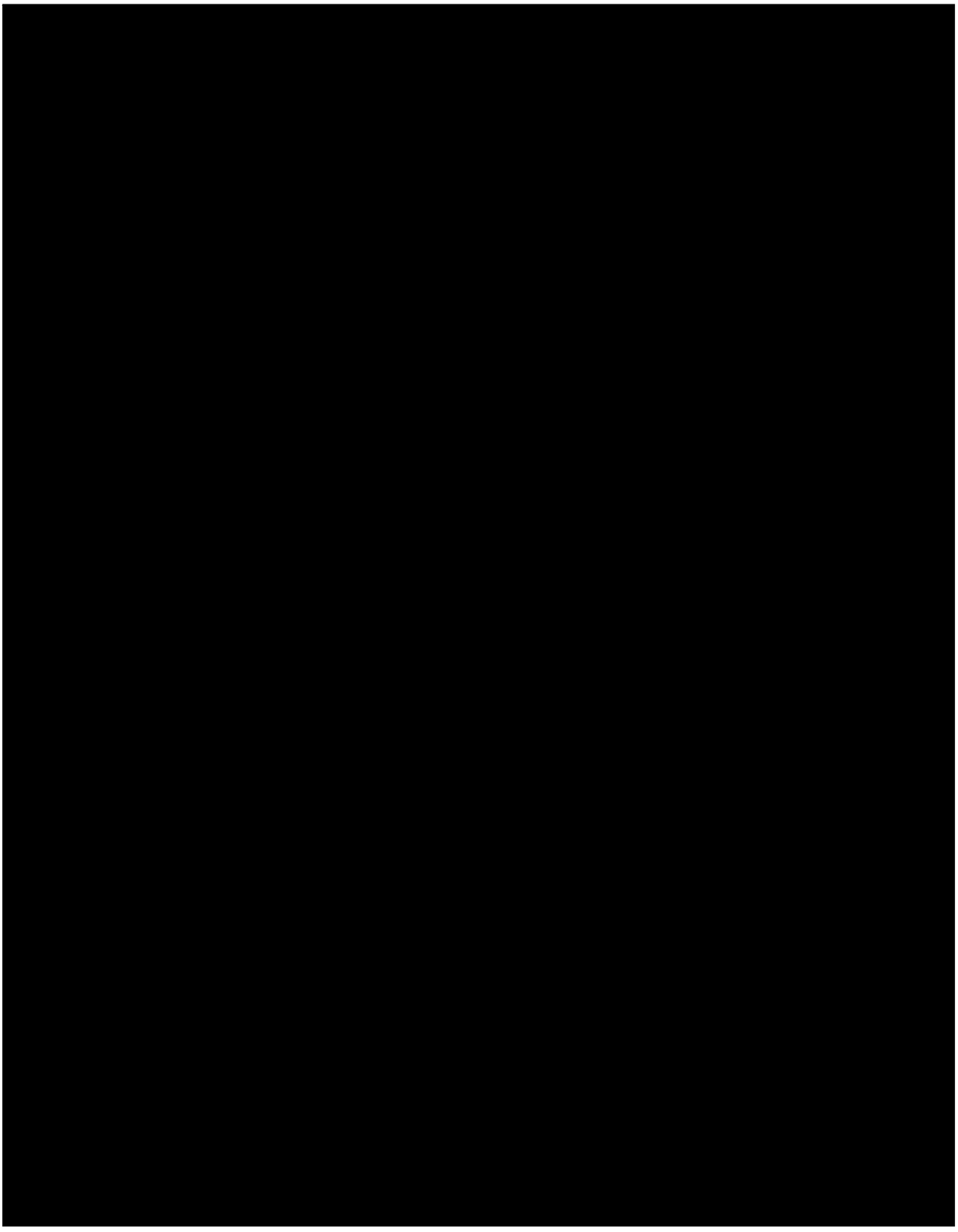


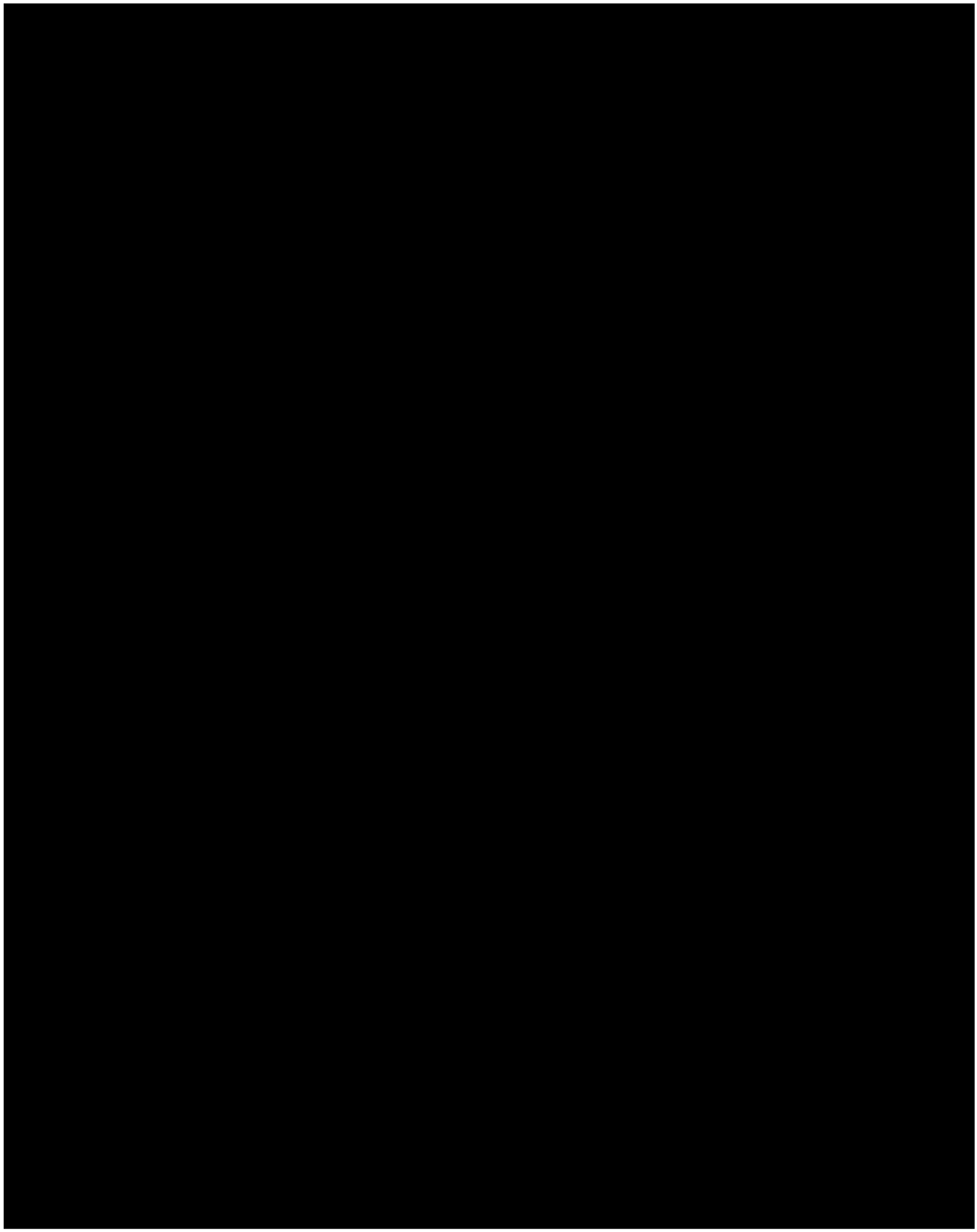












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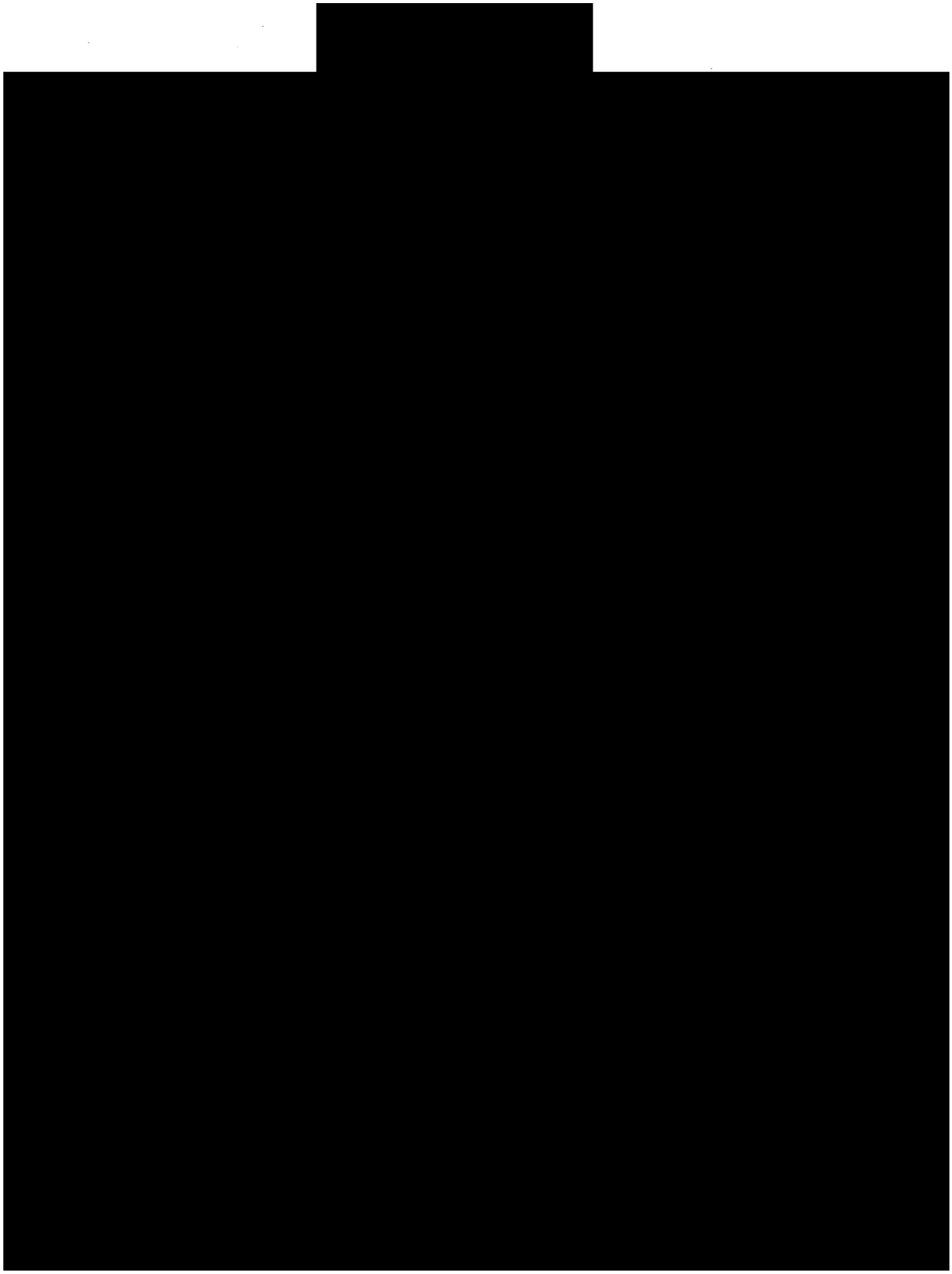

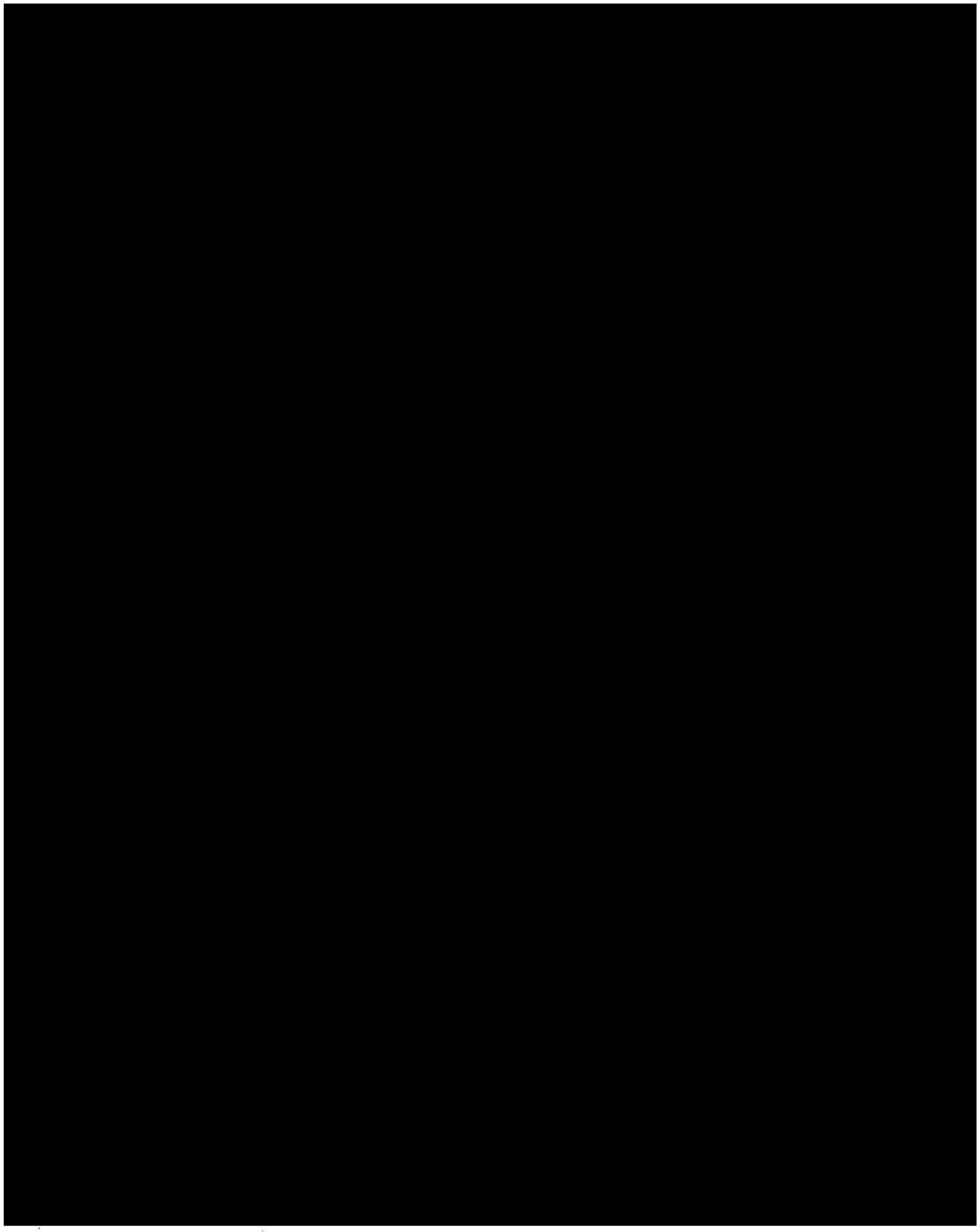

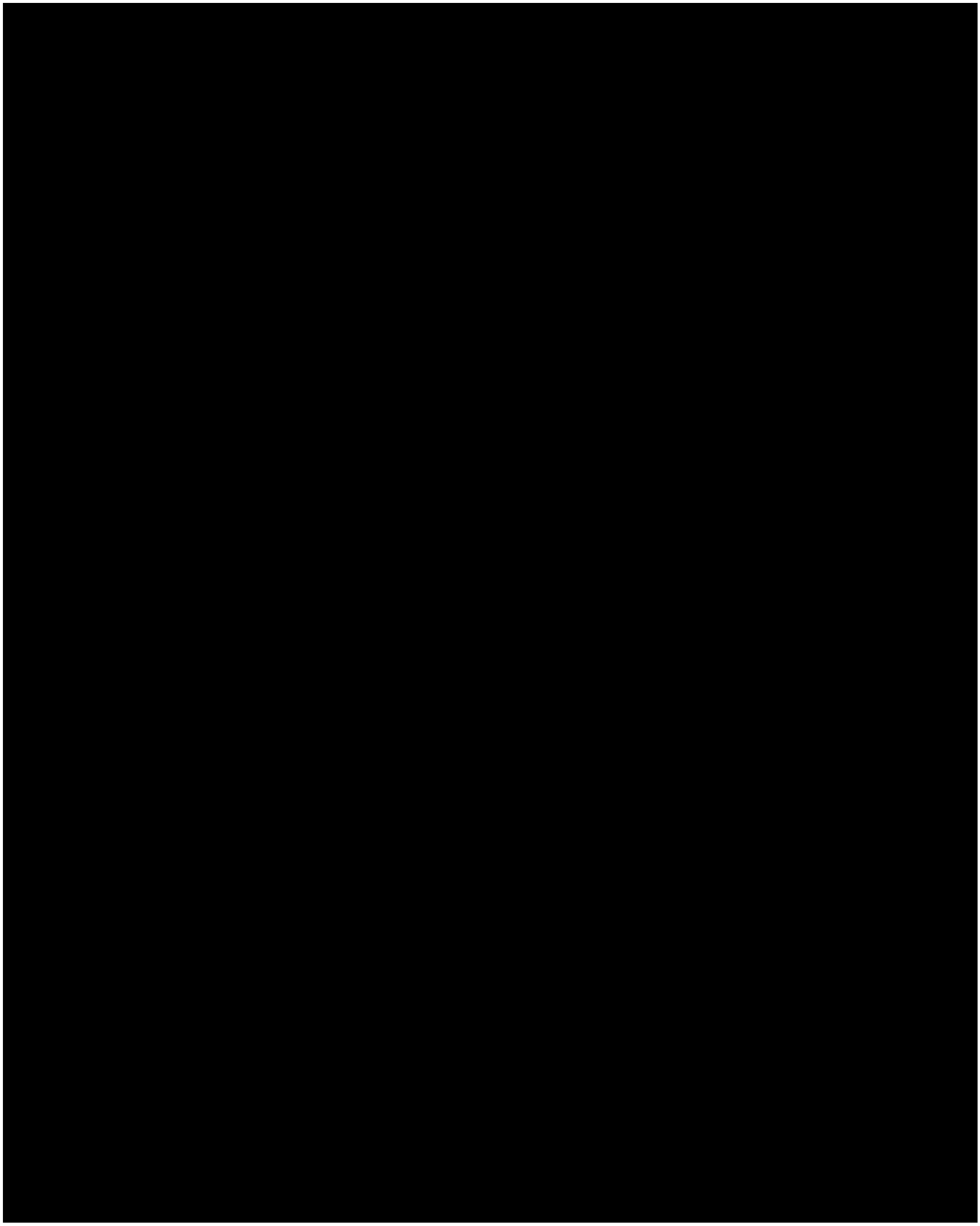

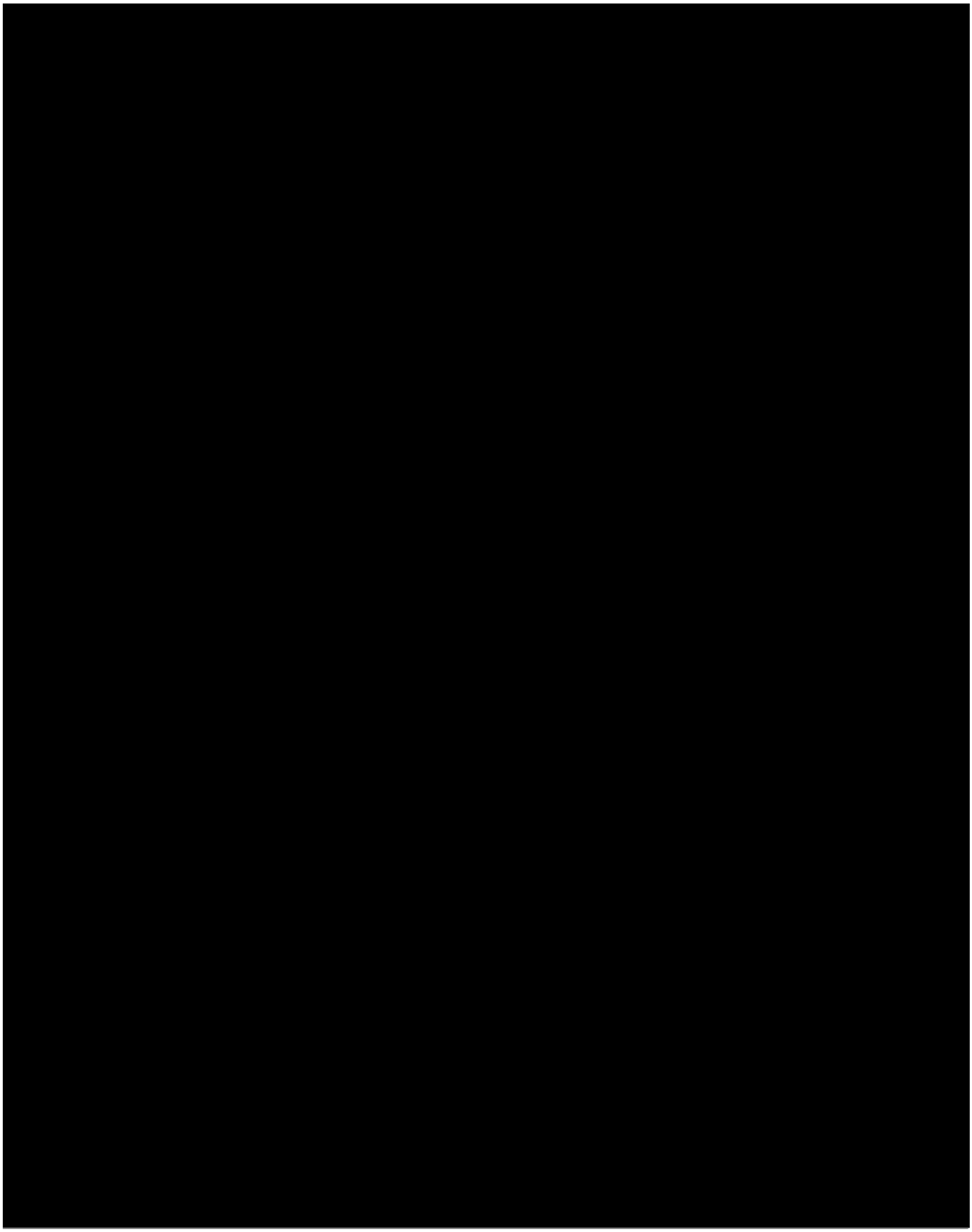

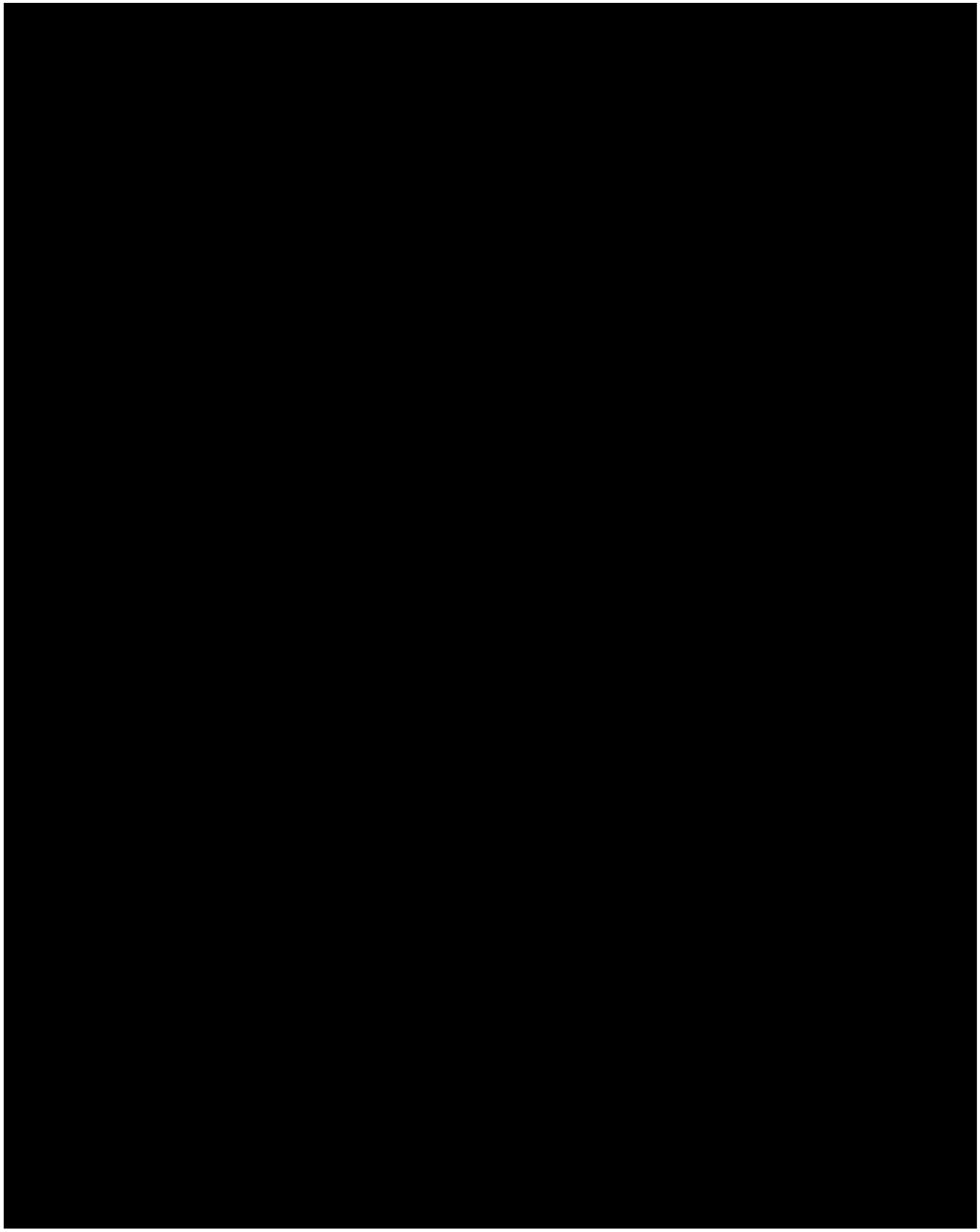

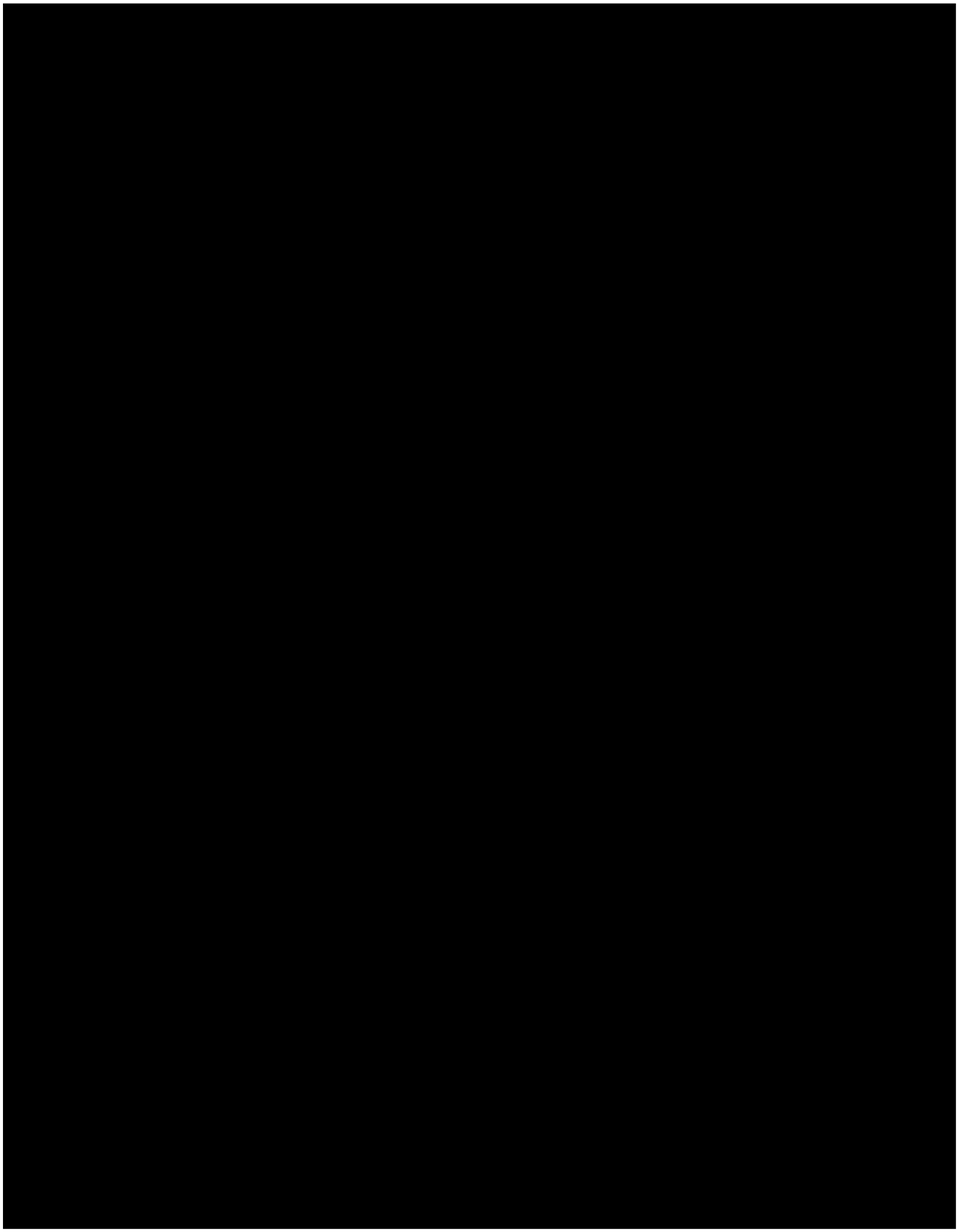

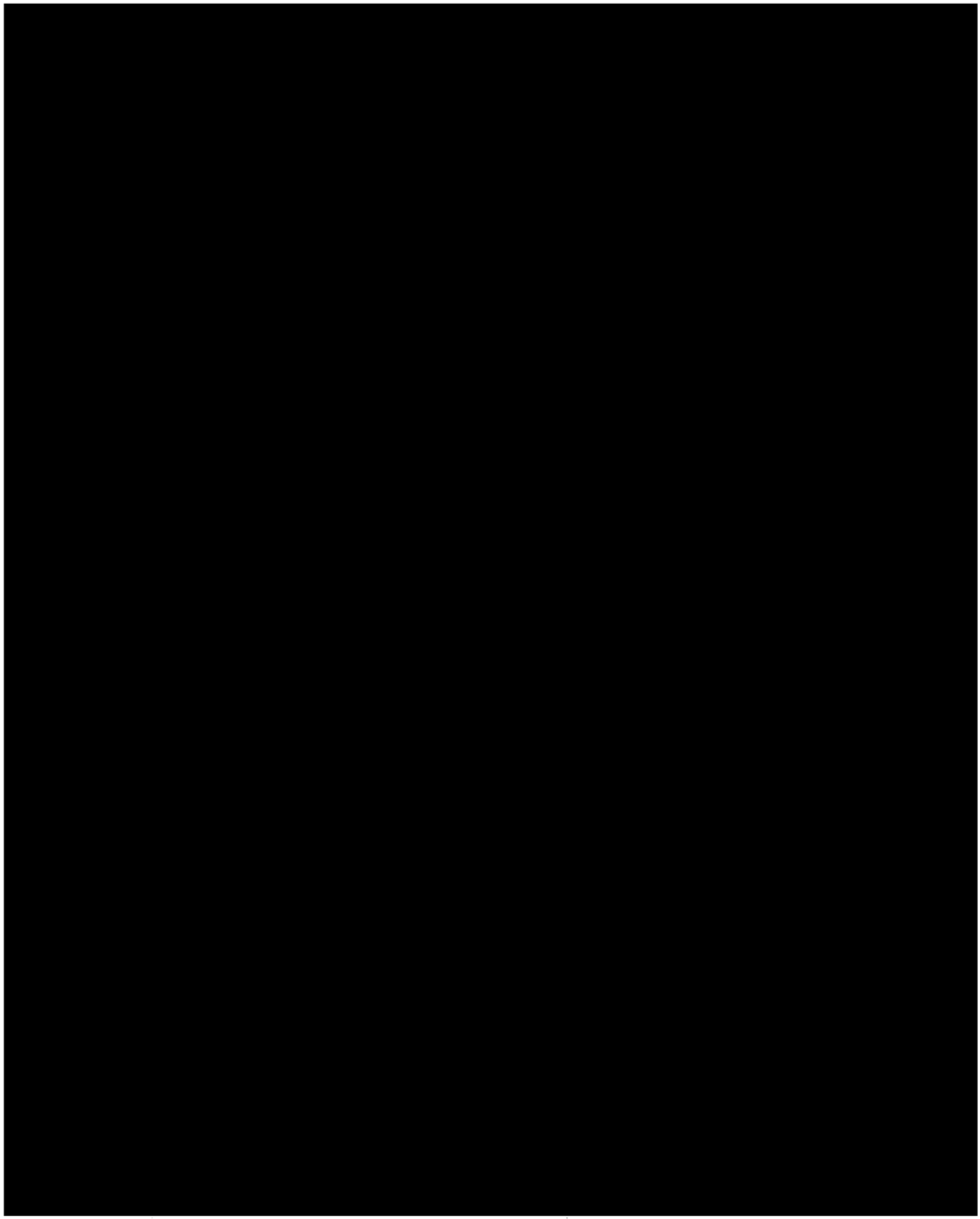

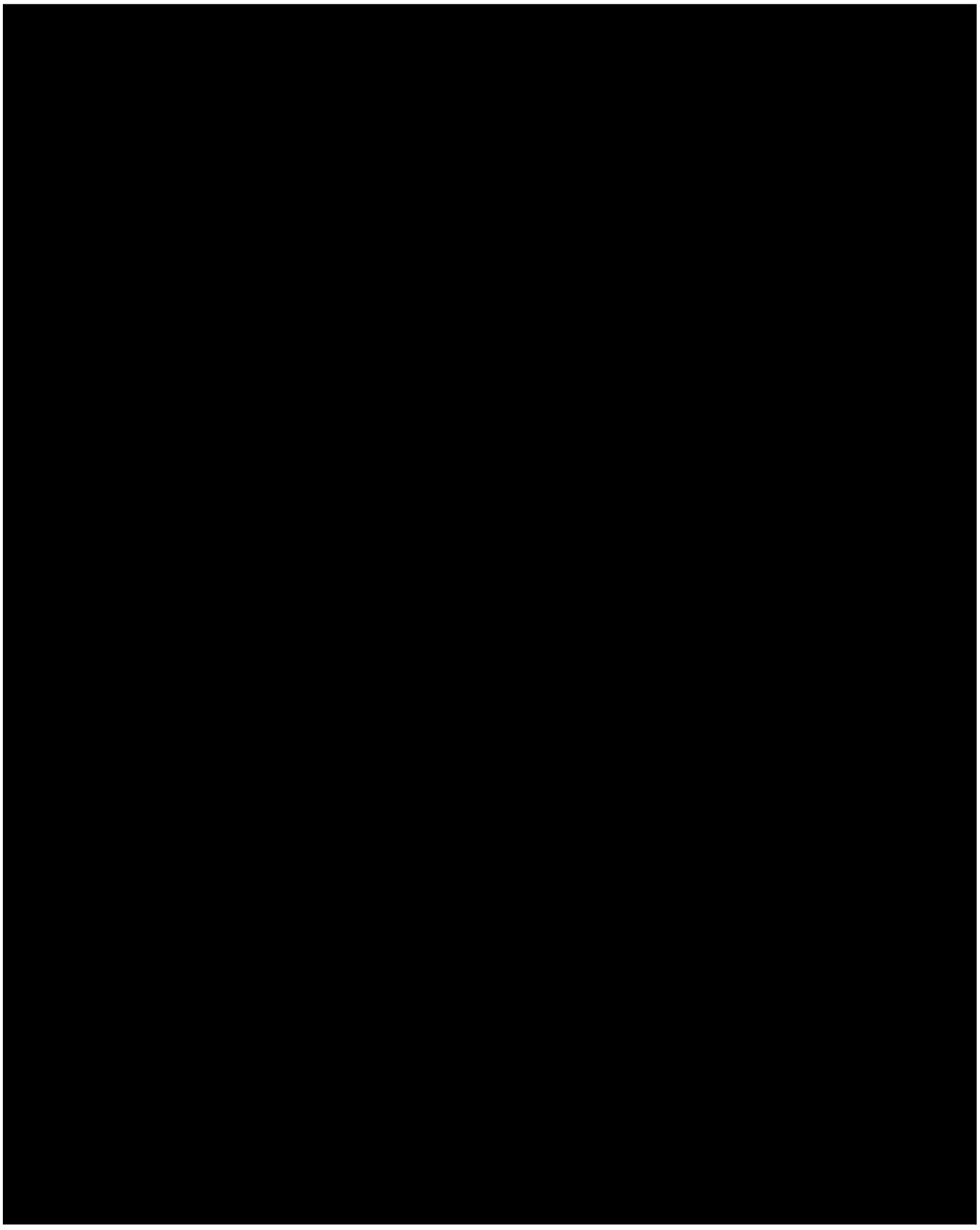

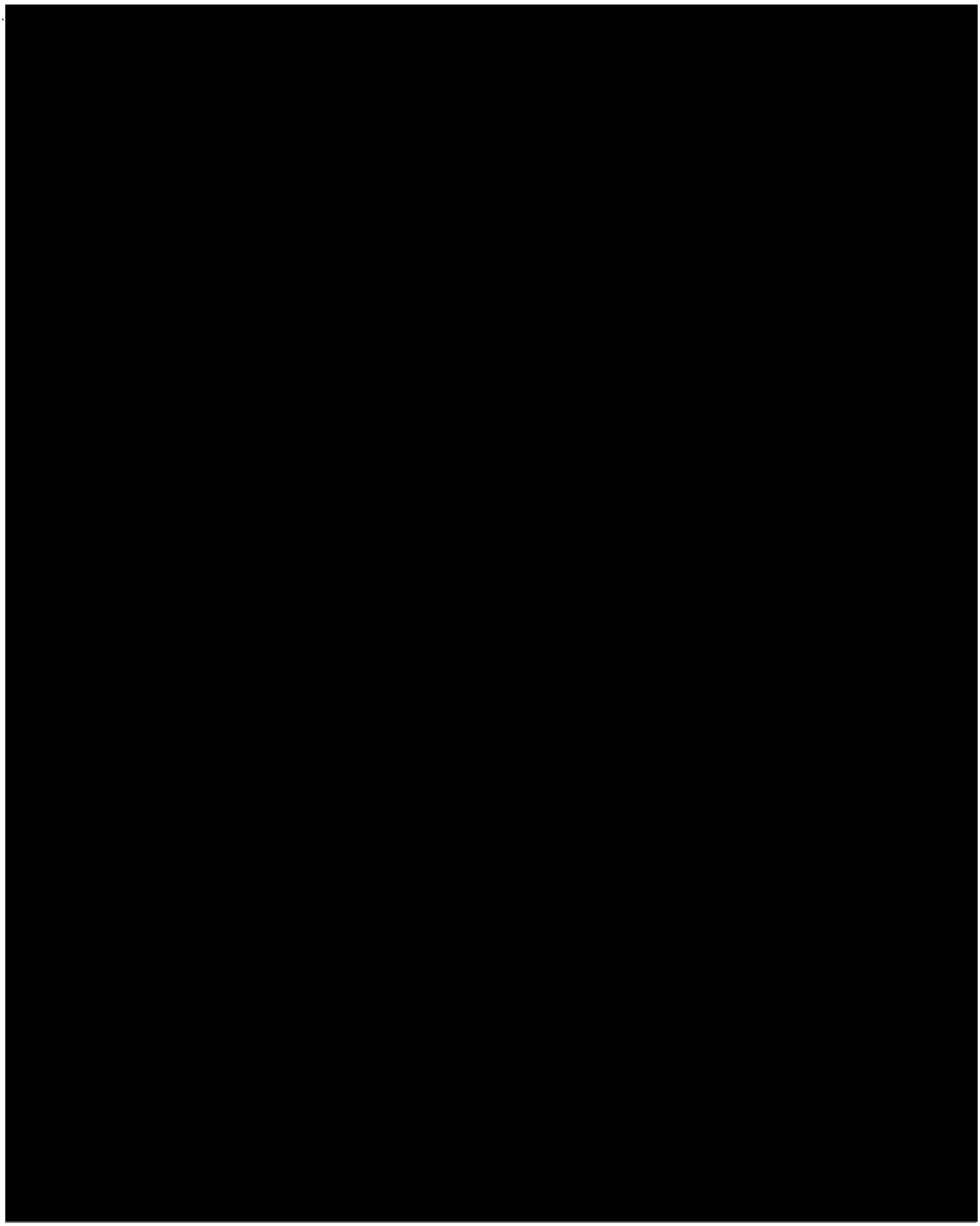

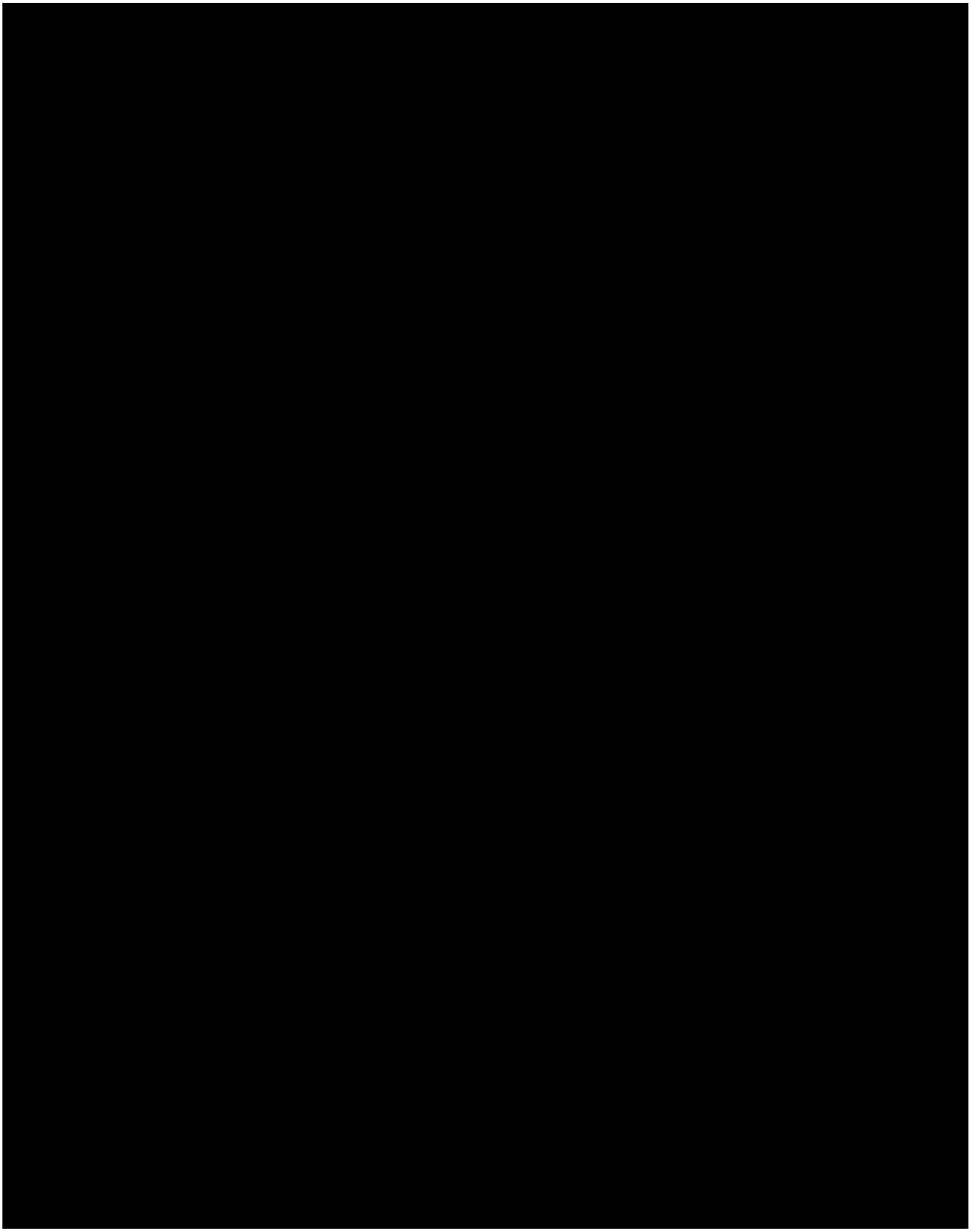

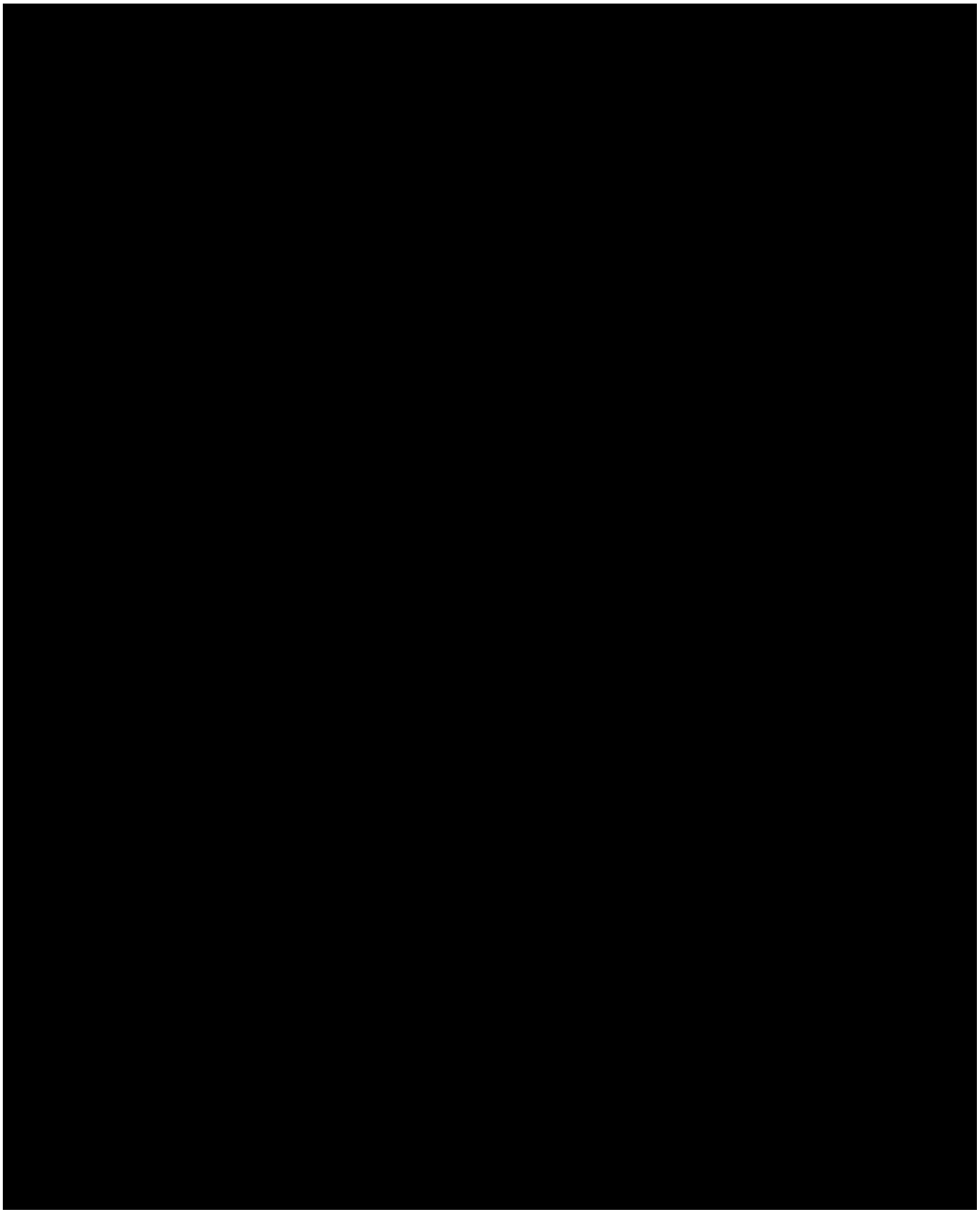

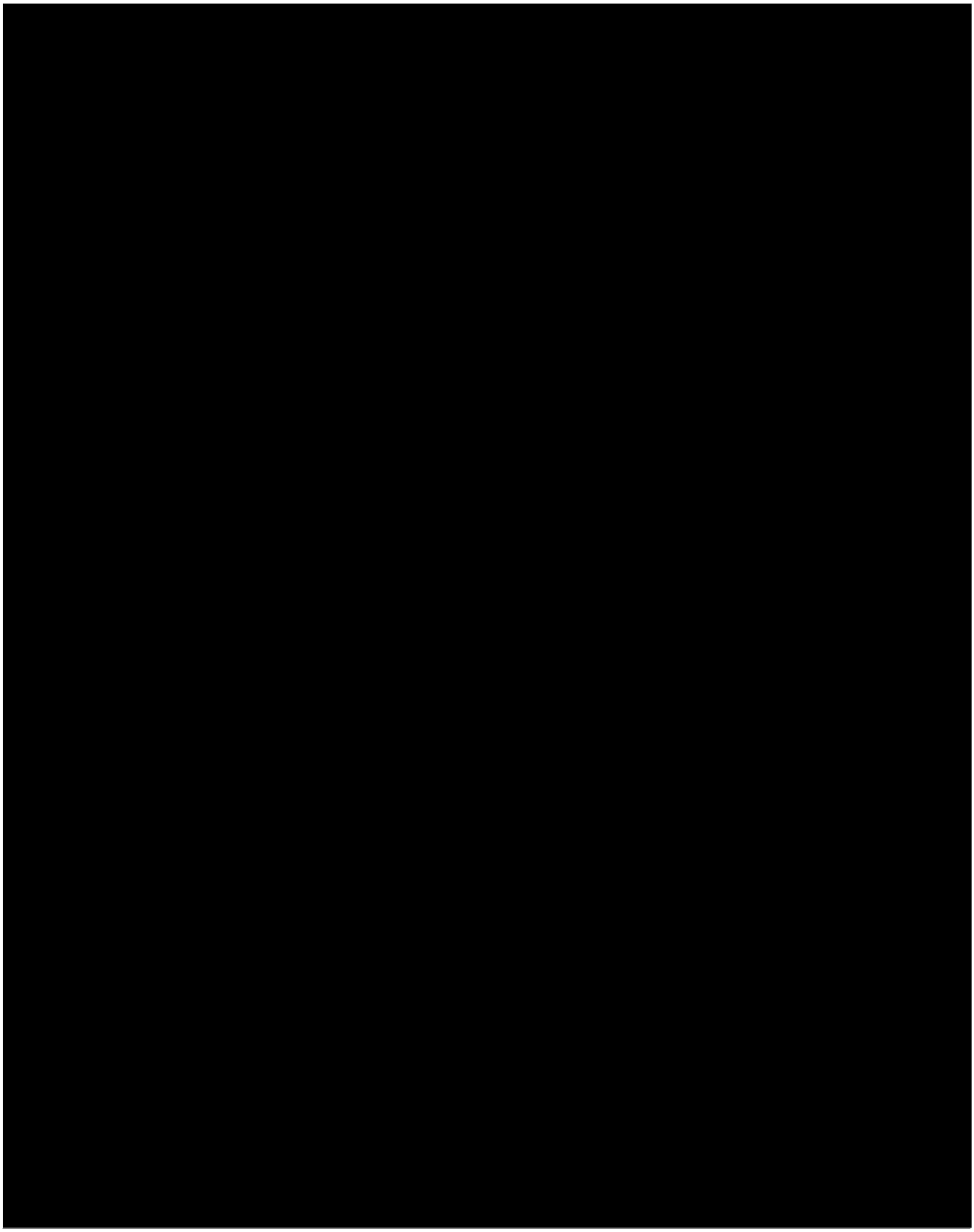

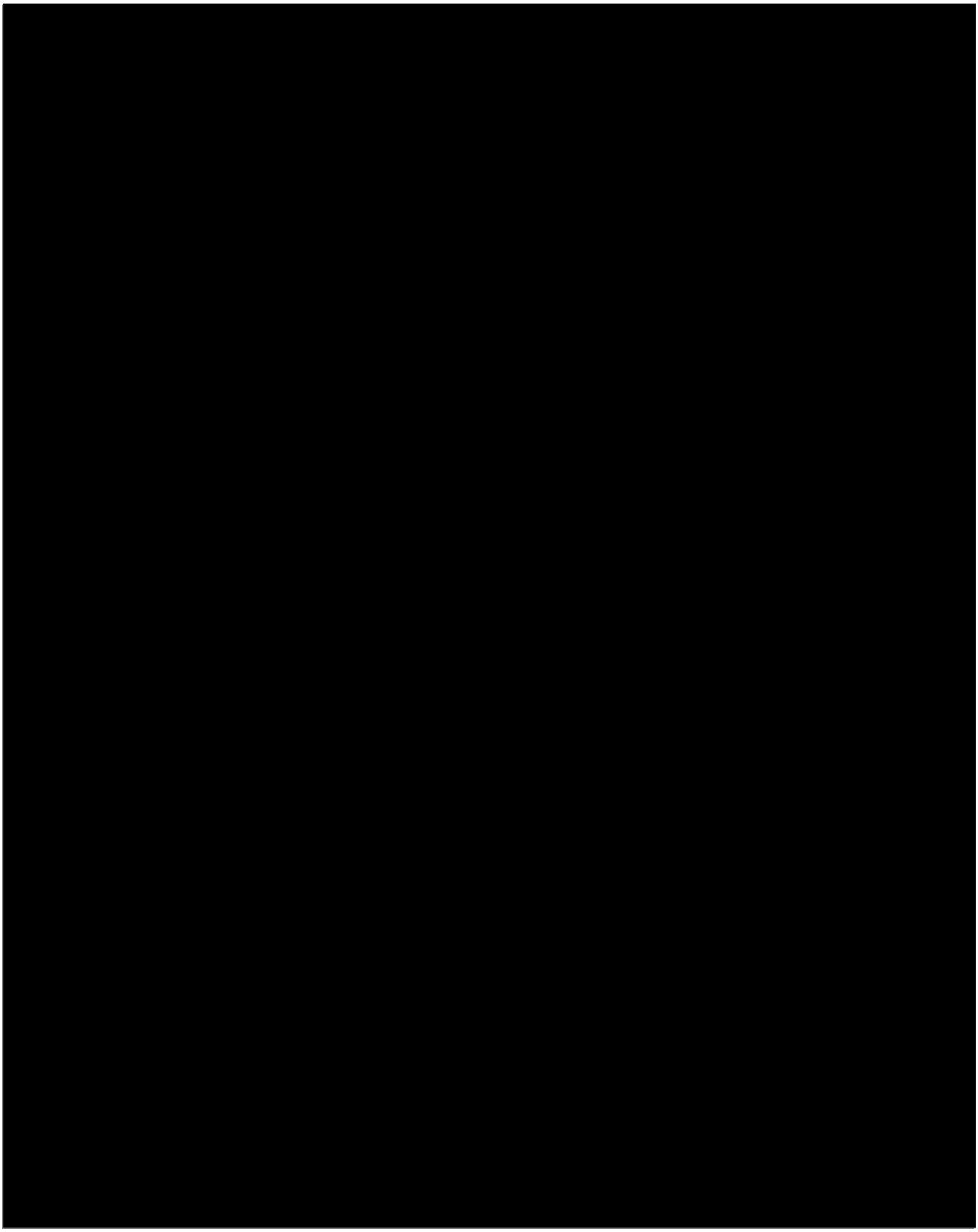

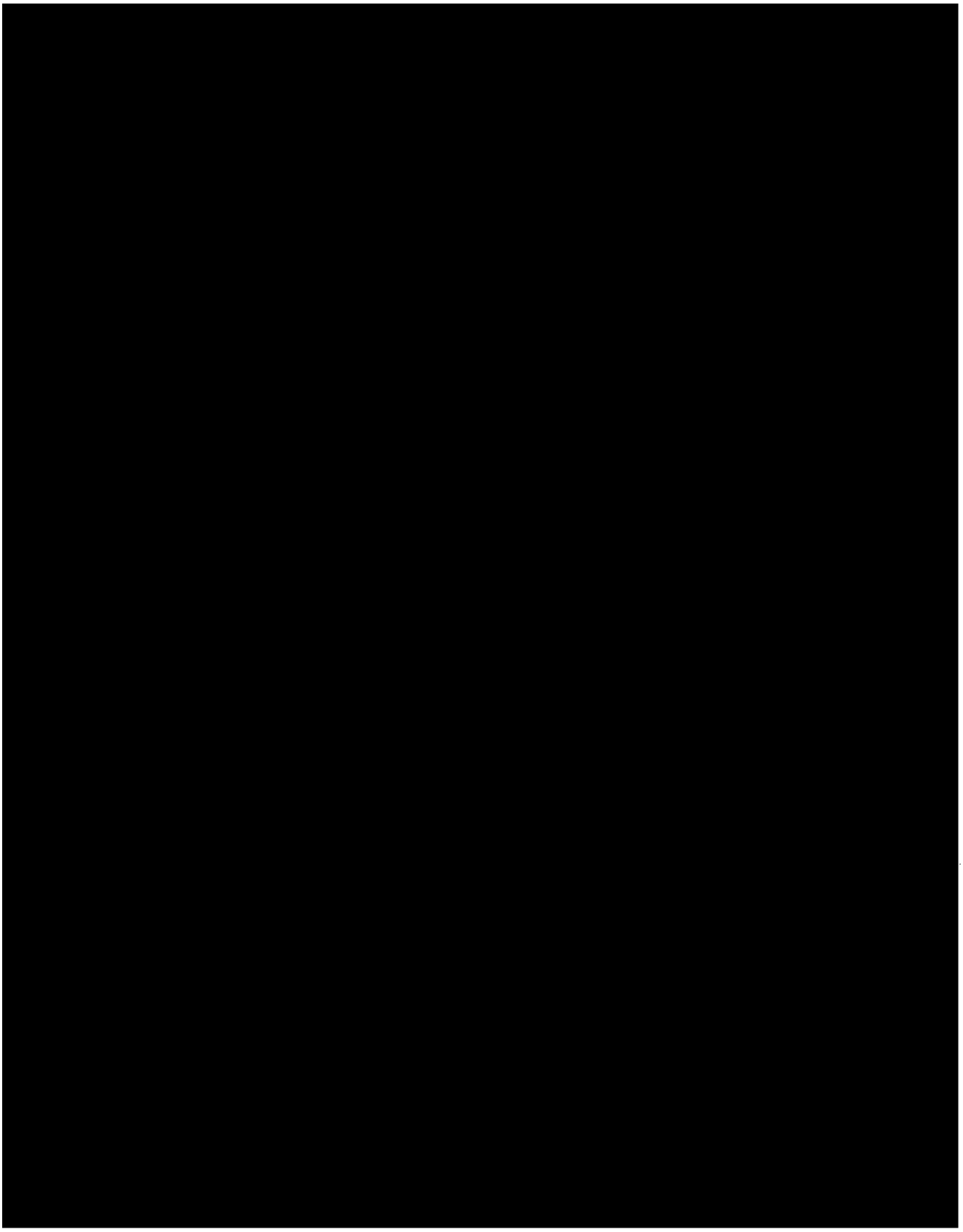

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

IMPURITIES IN NEW DRUG SUBSTANCES
Q3A(R2)

Current *Step 4* version

dated 25 October 2006

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

Q3A(R2)
Document History

First Codification	History	Date	New Codification **November 2005**
Q3	Approval by the Steering Committee under *Step 2* and release for public consultation.	15 March 1994	Q3A
Q3A	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies. Q3 was renamed Q3A.	30 March 1995	Q3A
Q3A(R)	Approval by the Steering Committee of the first Revision under *Step 2* and release for public consultation.	7 October 1999	Q3A(R1)
Q3A(R)	Approval by the Steering Committee of the first Revision under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	6 February 2002	Q3A(R1)

Current *Step 4* version

Q3A(R2)	Approval by the Steering Committee of the revision of the Attachment 2 directly under *Step 4* without further public consultation.	25 October 2006	Q3A(R2)

IMPURITIES IN NEW DRUG SUBSTANCES

ICH Harmonised Tripartite Guideline

Having reached *Step 4* of the ICH Process at the ICH Steering Committee meeting on 7 February 2002, this guideline is recommended for adoption to the three regulatory parties to ICH.

Attachment 2 has been revised on 25 October 2006.

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IMPURITIES IN NEW DRUG SUBSTANCES

1. PREAMBLE

This document is intended to provide guidance for registration applications on the content and qualification of impurities in new drug substances produced by chemical syntheses and not previously registered in a region or member state. It is not intended to apply to new drug substances used during the clinical research stage of development. The following types of drug substances are not covered in this guideline: biological/biotechnological, peptide, oligonucleotide, radiopharmaceutical, fermentation product and semi-synthetic products derived therefrom, herbal products, and crude products of animal or plant origin.

Impurities in new drug substances are addressed from two perspectives:

Chemistry Aspects include classification and identification of impurities, report generation, listing of impurities in specifications, and a brief discussion of analytical procedures; and

Safety Aspects include specific guidance for qualifying those impurities that were not present, or were present at substantially lower levels, in batches of a new drug substance used in safety and clinical studies.

2. CLASSIFICATION OF IMPURITIES

Impurities can be classified into the following categories:

- Organic impurities (process- and drug-related)
- Inorganic impurities
- Residual solvents

Organic impurities can arise during the manufacturing process and/or storage of the new drug substance. They can be identified or unidentified, volatile or non-volatile, and include:

- Starting materials
- By-products
- Intermediates
- Degradation products
- Reagents, ligands and catalysts

Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:

- Reagents, ligands and catalysts
- Heavy metals or other residual metals
- Inorganic salts
- Other materials (e.g., filter aids, charcoal)

Solvents are inorganic or organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of a new drug substance. Since these are

generally of known toxicity, the selection of appropriate controls is easily accomplished (see ICH Guideline Q3C on Residual Solvents).

Excluded from this document are: (1) extraneous contaminants that should not occur in new drug substances and are more appropriately addressed as Good Manufacturing Practice (GMP) issues, (2) polymorphic forms, and (3) enantiomeric impurities.

3. RATIONALE FOR THE REPORTING AND CONTROL OF IMPURITIES

3.1 Organic Impurities

The applicant should summarise the actual and potential impurities most likely to arise during the synthesis, purification, and storage of the new drug substance. This summary should be based on sound scientific appraisal of the chemical reactions involved in the synthesis, impurities associated with raw materials that could contribute to the impurity profile of the new drug substance, and possible degradation products. This discussion can be limited to those impurities that might reasonably be expected based on knowledge of the chemical reactions and conditions involved.

In addition, the applicant should summarise the laboratory studies conducted to detect impurities in the new drug substance. This summary should include test results of batches manufactured during the development process and batches from the proposed commercial process, as well as the results of stress testing (see ICH Guideline Q1A on Stability) used to identify potential impurities arising during storage. The impurity profile of the drug substance batches intended for marketing should be compared with those used in development, and any differences discussed.

The studies conducted to characterise the structure of actual impurities present in the new drug substance at a level greater than (>) the identification threshold given in Attachment 1 (e.g., calculated using the response factor of the drug substance) should be described. Note that any impurity at a level greater than (>) the identification threshold in any batch manufactured by the proposed commercial process should be identified. In addition, any degradation product observed in stability studies at recommended storage conditions at a level greater than (>) the identification threshold should be identified. When identification of an impurity is not feasible, a summary of the laboratory studies demonstrating the unsuccessful effort should be included in the application. Where attempts have been made to identify impurities present at levels of not more than (≤) the identification thresholds, it is useful also to report the results of these studies.

Identification of impurities present at an apparent level of not more than (≤) the identification threshold is generally not considered necessary. However, analytical procedures should be developed for those potential impurities that are expected to be unusually potent, producing toxic or pharmacological effects at a level not more than (≤) the identification threshold. All impurities should be qualified as described later in this guideline.

3.2 Inorganic Impurities

Inorganic impurities are normally detected and quantified using pharmacopoeial or other appropriate procedures. Carry-over of catalysts to the new drug substance should be evaluated during development. The need for inclusion or exclusion of inorganic impurities in the new drug substance specification should be discussed.

Acceptance criteria should be based on pharmacopoeial standards or known safety data.

3.3 Solvents

The control of residues of the solvents used in the manufacturing process for the new drug substance should be discussed and presented according to the ICH Q3C Guideline for Residual Solvents.

4. ANALYTICAL PROCEDURES

The registration application should include documented evidence that the analytical procedures are validated and suitable for the detection and quantification of impurities (see ICH Q2A and Q2B Guidelines for Analytical Validation). Technical factors (e.g., manufacturing capability and control methodology) can be considered as part of the justification for selection of alternative thresholds based on manufacturing experience with the proposed commercial process. The use of two decimal places for thresholds (See Attachment 1) does not necessarily reflect the precision of the analytical procedure used for routine quality control purposes. Thus, the use of lower precision techniques (e.g., thin-layer chromatography) can be acceptable where justified and appropriately validated. Differences in the analytical procedures used during development and those proposed for the commercial product should be discussed in the registration application.

The quantitation limit for the analytical procedure should be not more than (≤) the reporting threshold.

Organic impurity levels can be measured by a variety of techniques, including those that compare an analytical response for an impurity to that of an appropriate reference standard or to the response of the new drug substance itself. Reference standards used in the analytical procedures for control of impurities should be evaluated and characterised according to their intended uses. The drug substance can be used as a standard to estimate the levels of impurities. In cases where the response factors of the drug substance and the relevant impurity are not close, this practice can still be appropriate, provided a correction factor is applied or the impurities are, in fact, being overestimated. Acceptance criteria and analytical procedures used to estimate identified or unidentified impurities can be based on analytical assumptions (e.g., equivalent detector response). These assumptions should be discussed in the registration application.

5. REPORTING IMPURITY CONTENT OF BATCHES

Analytical results should be provided in the application for all batches of the new drug substance used for clinical, safety, and stability testing, as well as for batches representative of the proposed commercial process. Quantitative results should be presented numerically, and not in general terms such as "complies", "meets limit" etc. Any impurity at a level greater than (>) the reporting threshold (see Attachment 1) and total impurities observed in these batches of the new drug substance should be reported with the analytical procedures indicated. Below 1.0%, the results should be reported to two decimal places (e.g., 0.06%, 0.13%); at and above 1.0%, the results should be reported to one decimal place (e.g., 1.3%). Results should be rounded using conventional rules (see Attachment 2). A tabulation (e.g., spreadsheet) of the data is recommended. Impurities should be designated by code number or by an appropriate descriptor, e.g., retention time. If a higher reporting threshold is proposed, it should

be fully justified. All impurities at a level greater than (>) the reporting threshold should be summed and reported as total impurities.

When analytical procedures change during development, reported results should be linked to the procedure used, with appropriate validation information provided. Representative chromatograms should be provided. Chromatograms of representative batches from analytical validation studies showing separation and detectability of impurities (e.g., on spiked samples), along with any other impurity tests routinely performed, can serve as the representative impurity profiles. The applicant should ensure that complete impurity profiles (e.g., chromatograms) of individual batches are available, if requested.

A tabulation should be provided that links the specific new drug substance batch to each safety study and each clinical study in which the new drug substance has been used.

For each batch of the new drug substance, the report should include:

- Batch identity and size
- Date of manufacture
- Site of manufacture
- Manufacturing process
- Impurity content, individual and total
- Use of batches
- Reference to analytical procedure used

6. LISTING OF IMPURITIES IN SPECIFICATIONS

The specification for a new drug substance should include a list of impurities. Stability studies, chemical development studies, and routine batch analyses can be used to predict those impurities likely to occur in the commercial product. The selection of impurities in the new drug substance specification should be based on the impurities found in batches manufactured by the proposed commercial process. Those individual impurities with specific acceptance criteria included in the specification for the new drug substance are referred to as "specified impurities" in this guideline. Specified impurities can be identified or unidentified.

A rationale for the inclusion or exclusion of impurities in the specification should be presented. This rationale should include a discussion of the impurity profiles observed in the safety and clinical development batches, together with a consideration of the impurity profile of batches manufactured by the proposed commercial process. Specified identified impurities should be included along with specified unidentified impurities estimated to be present at a level greater than (>) the identification threshold given in Attachment 1. For impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantitation/detection limit of the analytical procedures should be commensurate with the level at which the impurities should be controlled. For unidentified impurities, the procedure used and assumptions made in establishing the level of the impurity should be clearly stated. Specified, unidentified impurities should be referred to by an appropriate qualitative analytical descriptive label (e.g., "unidentified A", "unidentified with relative retention of 0.9"). A general acceptance criterion of not more than (≤) the identification threshold (Attachment 1) for any unspecified impurity and an acceptance criterion for total impurities should be included.

Acceptance criteria should be set no higher than the level that can be justified by safety data, and should be consistent with the level achievable by the manufacturing process and the analytical capability. Where there is no safety concern, impurity acceptance criteria should be based on data generated on batches of the new drug substance manufactured by the proposed commercial process, allowing sufficient latitude to deal with normal manufacturing and analytical variation and the stability characteristics of the new drug substance. Although normal manufacturing variations are expected, significant variation in batch-to-batch impurity levels can indicate that the manufacturing process of the new drug substance is not adequately controlled and validated (see ICH Q6A Guideline on Specifications, Decision Tree #1, for establishing an acceptance criterion for a specified impurity in a new drug substance). The use of two decimal places for thresholds (See Attachment 1) does not necessarily indicate the precision of the acceptance criteria for specified impurities and total impurities.

In summary, the new drug substance specification should include, where applicable, the following list of impurities:

Organic Impurities

- Each specified identified impurity
- Each specified unidentified impurity
- Any unspecified impurity with an acceptance criterion of not more than (≤) the identification threshold
- Total impurities

Residual Solvents

Inorganic Impurities

7. QUALIFICATION OF IMPURITIES

Qualification is the process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified. The applicant should provide a rationale for establishing impurity acceptance criteria that includes safety considerations. The level of any impurity present in a new drug substance that has been adequately tested in safety and/or clinical studies would be considered qualified. Impurities that are also significant metabolites present in animal and/or human studies are generally considered qualified. A level of a qualified impurity higher than that present in a new drug substance can also be justified based on an analysis of the actual amount of impurity administered in previous relevant safety studies.

If data are unavailable to qualify the proposed acceptance criterion of an impurity, studies to obtain such data can be appropriate when the usual qualification thresholds given in Attachment 1 are exceeded.

Higher or lower thresholds for qualification of impurities can be appropriate for some individual drugs based on scientific rationale and level of concern, including drug class effects and clinical experience. For example, qualification can be especially important when there is evidence that such impurities in certain drugs or therapeutic classes have previously been associated with adverse reactions in patients. In these instances, a lower qualification threshold can be appropriate. Conversely, a higher qualification threshold can be appropriate for individual drugs when the level of concern for safety is less than usual based on similar considerations (e.g., patient

population, drug class effects, clinical considerations). Proposals for alternative thresholds would be considered on a case-by-case basis.

The "Decision Tree for Identification and Qualification" (Attachment 3) describes considerations for the qualification of impurities when thresholds are exceeded. In some cases, decreasing the level of impurity to not more than the threshold can be simpler than providing safety data. Alternatively, adequate data could be available in the scientific literature to qualify an impurity. If neither is the case, additional safety testing should be considered. The studies considered appropriate to qualify an impurity will depend on a number of factors, including the patient population, daily dose, and route and duration of drug administration. Such studies can be conducted on the new drug substance containing the impurities to be controlled, although studies using isolated impurities can sometimes be appropriate.

Although this guideline is not intended to apply during the clinical research stage of development, in the later stages of development the thresholds in this guideline can be useful in evaluating new impurities observed in drug substance batches prepared by the proposed commercial process. Any new impurity observed in later stages of development should be identified if its level is greater than (>) the identification threshold given in Attachment 1 (see the "Decision Tree for Identification and Qualification" in Attachment 3). Similarly, the qualification of the impurity should be considered if its level is greater than (>) the qualification threshold given in Attachment 1. Safety assessment studies to qualify an impurity should compare the new drug substance containing a representative amount of the new impurity with previously qualified material. Safety assessment studies using a sample of the isolated impurity can also be considered.

8. GLOSSARY

Chemical Development Studies: Studies conducted to scale-up, optimise, and validate the manufacturing process for a new drug substance.

Enantiomeric Impurity: A compound with the same molecular formula as the drug substance that differs in the spatial arrangement of atoms within the molecule and is a non-superimposable mirror image.

Extraneous Contaminant: An impurity arising from any source extraneous to the manufacturing process.

Herbal Products: Medicinal products containing, exclusively, plant material and/or vegetable drug preparations as active ingredients. In some traditions, materials of inorganic or animal origin can also be present.

Identified Impurity: An impurity for which a structural characterisation has been achieved.

Identification Threshold: A limit above (>) which an impurity should be identified.

Impurity: Any component of the new drug substance that is not the chemical entity defined as the new drug substance.

Impurity Profile: A description of the identified and unidentified impurities present in a new drug substance.

Intermediate: A material produced during steps of the synthesis of a new drug substance that undergoes further chemical transformation before it becomes a new drug substance.

Ligand: An agent with a strong affinity to a metal ion.

New Drug Substance: The designated therapeutic moiety that has not been previously registered in a region or member state (also referred to as a new molecular entity or new chemical entity). It can be a complex, simple ester, or salt of a previously approved drug substance.

Polymorphic Forms: Different crystalline forms of the same drug substance. These can include solvation or hydration products (also known as pseudo-polymorphs) and amorphous forms.

Potential Impurity: An impurity that theoretically can arise during manufacture or storage. It may or may not actually appear in the new drug substance.

Qualification: The process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified.

Qualification Threshold: A limit above (>) which an impurity should be qualified.

Reagent: A substance other than a starting material, intermediate, or solvent that is used in the manufacture of a new drug substance.

Reporting Threshold: A limit above (>) which an impurity should be reported. Reporting threshold is the same as reporting level in Q2B.

Solvent: An inorganic or an organic liquid used as a vehicle for the preparation of solutions or suspensions in the synthesis of a new drug substance.

Specified Impurity: An impurity that is individually listed and limited with a specific acceptance criterion in the new drug substance specification. A specified impurity can be either identified or unidentified.

Starting Material: A material used in the synthesis of a new drug substance that is incorporated as an element into the structure of an intermediate and/or of the new drug substance. Starting materials are normally commercially available and of defined chemical and physical properties and structure.

Unidentified Impurity: An impurity for which a structural characterisation has not been achieved and that is defined solely by qualitative analytical properties (e.g., chromatographic retention time).

Unspecified impurity: An impurity that is limited by a general acceptance criterion, but not individually listed with its own specific acceptance criterion, in the new drug substance specification.

ATTACHMENT 1
Thresholds

Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

[1] The amount of drug substance administered per day

[2] Higher reporting thresholds should be scientifically justified

[3] Lower thresholds can be appropriate if the impurity is unusually toxic

ATTACHMENT 2

Illustration of Reporting Impurity Results for Identification and Qualification in an Application

The attachment is only illustrative and is not intended to serve as template how results on impurities should be presented in an application file. Normally raw data are not presented.

Example 1: 0.5 g Maximum Daily Dose

Reporting threshold = 0.05%
Identification threshold = 0.10%
Qualification threshold = 0.15%

"Raw" Result (%)	Reported Result (%) Reporting threshold =0.05%	Calculated Total Daily Intake (TDI) (mg) of the impurity (rounded result in mg)	Action	
			Identification (Threshold 0.10% exceeded?)	Qualification (Threshold 0.15% exceeded?)
0.044	Not reported	0.2	None	None
0.0963	0.10	0.5	None	None
0.12	0.12[1)]	0.6	Yes	None[1)]
0.1649	0.16[1)]	0.8	Yes	Yes[1)]

Example 2: 0.8 g Maximum Daily Dose

Reporting threshold = 0.05%
Identification threshold = 0.10%
Qualification threshold = 1.0 mg TDI

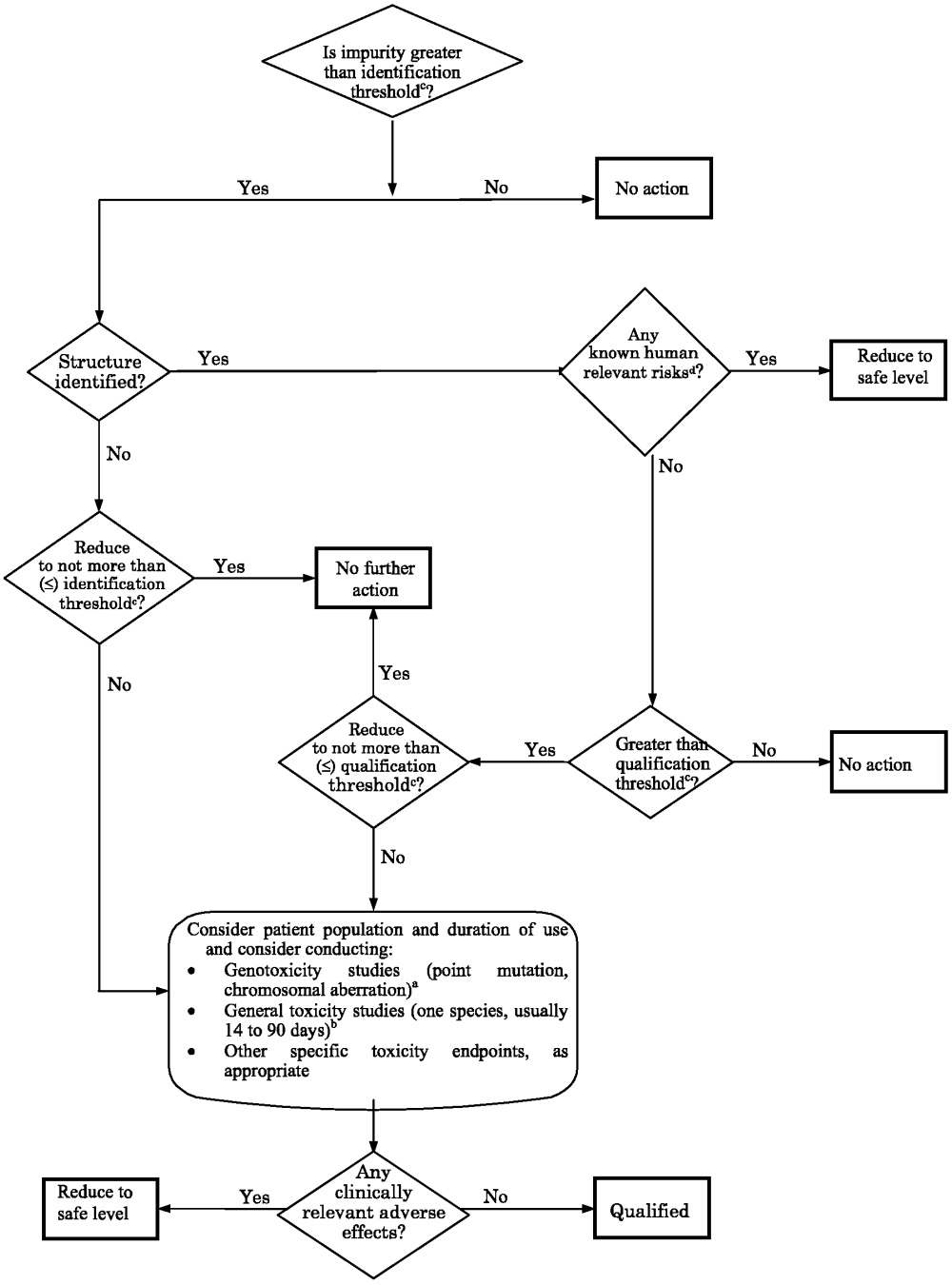
"Raw" Result (%)	Reported Result (%) Reporting threshold =0.05%	Calculated Total Daily Intake (TDI) (mg) of the impurity (rounded result in mg)	Action	
			Identification (Threshold 0.10% exceeded?)	Qualification (Threshold 1.0 mg TDI exceeded?)
0.066	0.07	0.6	None	None
0.124	0.12	1.0	yes	None[1)2)]
0.143	0.14	1.1	yes	Yes[1)]

1) After identification, if the response factor is determined to differ significantly from the original assumptions, it may be appropriate to re-measure the actual amount of the impurity present and re-evaluate against the qualification threshold (see Attachment 1).

2) To verify if a threshold is exceeded, a reported result has to be evaluated against the thresholds as follows: when the threshold is described in %, the reported result rounded to the same decimal place as the threshold should be compared directly to the threshold. When the threshold is described in TDI, the reported result should be converted to TDI, rounded to the same decimal place as the threshold and compared to the threshold. For example the amount of impurity at 0.12% level corresponds to a TDI of 0.96 mg (absolute amount) which is then rounded up to 1.0 mg; so the qualification threshold expressed in TDI (1.0 mg) is not exceeded.

ATTACHMENT 3

Decision Tree for Identification and Qualification



Notes on Attachment 3

a) If considered desirable, a minimum screen (e.g., genotoxic potential), should be conducted.
A study to detect point mutations and one to detect chromosomal aberrations, both in vitro, are considered an appropriate minimum screen.

b) If general toxicity studies are desirable, one or more studies should be designed to allow comparison of unqualified to qualified material. The study duration should be based on available relevant information and performed in the species most likely to maximise the potential to detect the toxicity of an impurity. On a case-by-case basis, single-dose studies can be appropriate, especially for single-dose drugs. In general, a minimum duration of 14 days and a maximum duration of 90 days would be considered appropriate.

c) Lower thresholds can be appropriate if the impurity is unusually toxic.

d) For example, do known safety data for this impurity or its structural class preclude human exposure at the concentration present?

M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

May 2015
ICH

M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

Guidance for Industry

Additional copies are available from:

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Center for Drug Evaluation and Research
Food and Drug Administration
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Email: druginfo@fda.hhs.gov
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or

Office of Communication, Outreach and Development
Center for Biologics Evaluation and Research
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 71, Room 3128
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Phone: 800-835-4709 or 240-402-7800
ocod@fda.hhs.gov
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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

May 2015
ICH

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Contains Nonbinding Recommendations

M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not create any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION (1)[2]

The synthesis of drug substances involves the use of reactive chemicals, reagents, solvents, catalysts, and other processing aids. As a result of chemical synthesis or subsequent degradation, impurities reside in all drug substances and associated drug products. While ICH *Q3A Impurities in New Drug Substances* (Revision 2) (Q3A) and *Q3B(R2) Impurities in New Drug Products* (Q3B) (Refs. 1 and 2)[3] provide guidance for qualification and control for the majority of the impurities, limited guidance is provided for those impurities that are DNA reactive. The purpose of this guidance is to provide a practical framework that is applicable to the identification, categorization, qualification, and control of these mutagenic impurities to limit potential carcinogenic risk. This guidance is intended to complement ICH Q3A, Q3B (Note 1), and *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (Ref. 3).

This guidance emphasizes considerations of both safety and quality risk management in establishing levels of mutagenic impurities that are expected to pose negligible carcinogenic risk. It outlines recommendations for assessment and control of mutagenic impurities that reside or are reasonably expected to reside in final drug substance or product, taking into consideration the intended conditions of human use.

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[1]This guidance was developed within the Expert Working Group (Multidisciplinary) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Steering Committee at Step 4 of the ICH process, June 2014. At Step 4 of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and the United States.

[2] Arabic numbers reflect the organizational breakdown in the document endorsed by the ICH Steering Committee at Step 4 of the ICH process, June 2014.

[3] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance Web page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm or the Vaccines, Blood & Biologics Web page at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. SCOPE OF GUIDANCE (2)

This document is intended to provide guidance for new drug substances and new drug products during their clinical development and subsequent applications for marketing. It also applies to post-approval submissions of marketed products, and to new marketing applications for products with a drug substance that is present in a previously approved product – in both cases, only where:

- Changes to the drug substance synthesis result in new impurities or increased acceptance criteria for existing impurities;
- Changes in the formulation, composition or manufacturing process result in new degradation products or increased acceptance criteria for existing degradation products;
- Changes in indication or dosing regimen are made which significantly affect the acceptable cancer risk level.

Assessment of the mutagenic potential of impurities as described in this guidance is not intended for the following types of drug substances and drug products: biological/biotechnological, peptide, oligonucleotide, radiopharmaceutical, fermentation, herbal, and crude products of animal or plant origin.

This guidance does not apply to drug substances and drug products intended for advanced cancer indications as defined in the scope of ICH S9 (Ref. 4). Additionally, there may be some cases where a drug substance intended for other indications is itself genotoxic at therapeutic concentrations and may be expected to be associated with an increased cancer risk. Exposure to a mutagenic impurity in these cases would not significantly add to the cancer risk of the drug substance. Therefore, impurities could be controlled at acceptable levels for non-mutagenic impurities.

Assessment of the mutagenic potential of impurities as described in this guidance is not intended for excipients used in existing marketed products, flavoring agents, colorants, and perfumes. Application of this guidance to leachables associated with drug product packaging is not intended, but the safety risk assessment principles outlined in this guidance for limiting potential carcinogenic risk can be used if warranted. The safety risk assessment principles of this guidance can be used if warranted for impurities in excipients that are used for the first time in a drug product and are chemically synthesized.

III. GENERAL PRINCIPLES (3)

The focus of this guidance is on DNA reactive substances that have a potential to directly cause DNA damage when present at low levels leading to mutations and therefore, potentially causing cancer. This type of mutagenic carcinogen is usually detected in a bacterial reverse

mutation (mutagenicity) assay. Other types of genotoxicants that are non-mutagenic typically have threshold mechanisms and usually do not pose carcinogenic risk in humans at the level ordinarily present as impurities. Therefore, to limit a possible human cancer risk associated with the exposure to potentially mutagenic impurities, the bacterial mutagenicity assay is used to assess the mutagenic potential and the need for controls. Structure-based assessments are useful for predicting bacterial mutagenicity outcomes based upon the established knowledge. There are a variety of approaches to conduct this evaluation, including a review of the available literature and/or computational toxicology assessment.

A Threshold of Toxicological Concern (TTC) concept was developed to define an acceptable intake for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effects. The methods upon which the TTC is based are generally considered to be very conservative since they involve a simple linear extrapolation from the dose giving a 50% tumor incidence (TD_{50}) to a 1 in 10^6 incidence, using TD_{50} data for the most sensitive species and most sensitive site of tumor induction. For application of a TTC in the assessment of acceptable limits of mutagenic impurities in drug substances and drug products, a value of 1.5 micrograms (μg)/day corresponding to a theoretical 10^{-5} excess lifetime risk of cancer can be justified. Some structural groups were identified to be of such high potency that intakes even below the TTC would theoretically be associated with a potential for a significant carcinogenic risk. This group of high potency mutagenic carcinogens, referred to as the *cohort of concern*, comprises aflatoxin-like-, N-nitroso-, and alkyl-azoxy compounds.

During clinical development, it is expected that control strategies and approaches will be less developed in earlier phases where overall development experience is limited. This guidance bases acceptable intakes for mutagenic impurities on established risk assessment strategies. Acceptable risk during the early development phase is set at a theoretically calculated level of approximately one additional cancer per million. For later stages in development and for marketed products, acceptable increased cancer risk is set at a theoretically calculated level of approximately 1 in 100,000. These risk levels represent a small theoretical increase in risk when compared to human overall lifetime incidence of developing any type of cancer, which is greater than 1 in 3. It is noted that established cancer risk assessments are based on lifetime exposures. Less-Than-Lifetime (LTL) exposures both during development and marketing can have higher acceptable intakes of impurities and still maintain comparable risk levels. The use of a numerical cancer risk value (1 in 100,000) and its translation into risk-based doses (TTC) is a highly hypothetical concept that should not be regarded as a realistic indication of the actual risk. Nevertheless, the TTC concept provides an estimate of safe exposures for any mutagenic compound. However, exceeding the TTC is not necessarily associated with an increased cancer risk given the conservative assumptions employed in the derivation of the TTC value. The most likely increase in cancer incidence is actually much less than 1 in 100,000. In addition, in cases where a mutagenic compound is a non-carcinogen in a rodent bioassay, there would be no predicted increase in cancer risk. Based on all the above considerations, any exposure to an impurity that is later identified as a mutagen is not necessarily associated with an increased cancer risk for patients already exposed to the impurity. A risk assessment would determine whether any further actions would be taken.

Where a potential risk has been identified for an impurity, an appropriate control strategy leveraging process understanding and/or analytical controls should be developed to ensure that the mutagenic impurity is at or below the acceptable cancer risk level.

There may be cases when an impurity is also a metabolite of the drug substance. In such cases, the risk assessment that addresses mutagenicity of the metabolite can qualify the impurity.

IV. CONSIDERATIONS FOR MARKETED PRODUCTS (4)

This guidance is not intended to be applied retrospectively (i.e., to products marketed prior to adoption of this guidance). However, some types of post-approval changes warrant a reassessment of safety relative to mutagenic impurities. This section applies to these post-approval changes for products marketed prior to, or after, the adoption of this guidance. Section VIII.E (8.5) (Lifecycle Management) contains additional recommendations for products marketed after adoption of this guidance.

A. Post-Approval Changes to the Drug Substance Chemistry, Manufacturing, and Controls (4.1)

Post-approval submissions involving the drug substance chemistry, manufacturing, and controls should include an evaluation of the potential risk impact associated with mutagenic impurities from changes to the route of synthesis, reagents, solvents, or process conditions after the starting material. Specifically, changes should be evaluated to determine whether the changes result in any new mutagenic impurities or higher acceptance criteria for existing mutagenic impurities. Reevaluation of impurities not impacted by changes is not recommended. For example, when only a portion of the manufacturing process is changed, the assessment of risk from mutagenic impurities should be limited to whether any new mutagenic impurities result from the change, whether any mutagenic impurities formed during the affected step are increased, and whether any known mutagenic impurities from up-stream steps are increased. Regulatory submissions associated with such changes should describe the assessment as outlined in Section IX.B (9.2). Changing the site of manufacture of drug substance, intermediates, or starting materials or changing raw materials supplier will not require a reassessment of mutagenic impurity risk.

When a new drug substance supplier is proposed, evidence that the drug substance produced by this supplier using the same route of synthesis as an existing drug product marketed in the assessor's region is considered to be sufficient evidence of acceptable risk/benefit regarding mutagenic impurities and an assessment per this guidance is not required. If this is not the case, then an assessment per this guidance is strongly recommended.

B. Post-Approval Changes to the Drug Product Chemistry, Manufacturing, and Controls (4.2)

Post-approval submissions involving the drug product (e.g., change in composition, manufacturing process, dosage form) should include an evaluation of the potential risk associated with any new mutagenic degradation products or higher acceptance criteria for existing mutagenic degradation products. If appropriate, the regulatory submission should include an updated control strategy. Reevaluation of the drug substance associated with drug products is not recommended or expected provided there are no changes to the drug substance. Changing the site of manufacture of drug product will not require a reassessment of mutagenic impurity risk.

C. Changes to the Clinical Use of Marketed Products (4.3)

Changes to the clinical use of marketed products that can warrant a reevaluation of the mutagenic impurity limits include a significant increase in clinical dose, an increase in duration of use (in particular when a mutagenic impurity was controlled above the lifetime acceptable intake for a previous indication that may no longer be appropriate for the longer treatment duration associated with the new indication), or a change in indication from a serious or life-threatening condition where higher acceptable intakes were justified (Section VII.E (7.5)) to an indication for a less serious condition where the existing impurity acceptable intakes may no longer be appropriate. Changes to the clinical use of marketed products associated with new routes of administration or expansion into patient populations that include pregnant women and/or pediatrics will not warrant a reevaluation, assuming no increases in daily dose or duration of treatment.

D. Other Considerations for Marketed Products (4.4)

Application of this guidance to marketed products may be warranted if there is specific cause for concern. The existence of impurity structural alerts alone is considered insufficient to trigger follow-up measures, unless it is a structure in the cohort of concern (Section III (3)). However a specific cause for concern would be new relevant impurity hazard data (classified as Class 1 or 2, Section 6) generated after the overall control strategy and specifications for market authorization were established. This new relevant impurity hazard data should be derived from high-quality scientific studies consistent with relevant regulatory testing guidelines, with data records or reports readily available. Similarly, a newly discovered impurity that is a known Class 1 or Class 2 mutagen that is present in a marketed product could also be a cause for concern. In both of these cases when the applicant becomes aware of this new information, an evaluation per this guidance should be conducted.

V. DRUG SUBSTANCE AND DRUG PRODUCT IMPURITY ASSESSMENT (5)

Actual and potential impurities that are likely to arise during the synthesis and storage of a new drug substance, and during manufacturing and storage of a new drug product, should be assessed.

The impurity assessment is a two-stage process:

- Actual impurities that have been identified should be considered for their mutagenic potential.
- An assessment of potential impurities likely to be present in the final drug substance is carried out to determine whether further evaluation of their mutagenic potential is warranted.

The steps as applied to synthetic impurities and degradation products are described in Sections V.A (5.1) and V.B (5.2), respectively.

A. Synthetic Impurities (5.1)

Actual impurities include those observed in the drug substance above the ICH Q3A reporting thresholds. Identification of actual impurities is expected when the levels exceed the

identification thresholds outlined by ICH Q3A. It is acknowledged that some impurities below the identification threshold may also have been identified.

Potential impurities in the drug substance can include starting materials, reagents, and intermediates in the route of synthesis from the starting material to the drug substance.

The risk of carryover into the drug substance should be assessed for identified impurities that are present in starting materials and intermediates, and impurities that are reasonably expected by-products in the route of synthesis from the starting material to the drug substance. As the risk of carryover may be negligible for some impurities (e.g., those impurities in early synthetic steps of long routes of synthesis), a risk-based justification could be provided for the point in the synthesis after which these types of impurities should be evaluated for mutagenic potential.

For starting materials that are introduced late in the synthesis of the drug substance (and where the synthetic route of the starting material is known), the final steps of the starting material synthesis should be evaluated for potential mutagenic impurities.

Actual impurities where the structures are known and potential impurities as defined above should be evaluated for mutagenic potential as described in Section VI (6).

B. Degradation Products (5.2)

Actual drug substance degradation products include those observed above the ICH Q3A reporting threshold during storage of the drug substance in the proposed long-term storage conditions and primary and secondary packaging. Actual degradation products in the drug product include those observed above the ICH Q3B reporting threshold during storage of the drug product in the proposed long-term storage conditions and primary and secondary packaging, and also include those impurities that arise during the manufacture of the drug product. Identification of actual degradation products is expected when the levels exceed the identification thresholds outlined by ICH Q3A/Q3B. It is acknowledged that some degradation products below the identification threshold may also have been identified.

Potential degradation products in the drug substance and drug product are those that may be reasonably expected to form during long-term storage conditions. Potential degradation products include those that form above the ICH Q3A/Q3B identification threshold during accelerated stability studies (e.g., 40°C/75% relative humidity for 6 months) and confirmatory photostability studies as described in ICH Q1B (Ref. 5), but are yet to be confirmed in the drug substance or drug product under long-term storage conditions in the primary packaging.

Knowledge of relevant degradation pathways can be used to help guide decisions on the selection of potential degradation products to be evaluated for mutagenicity, e.g., from degradation chemistry principles, relevant stress testing studies, and development stability studies.

Actual and potential degradation products likely to be present in the final drug substance or drug product and where the structure is known should be evaluated for mutagenic potential as described in Section VI (6).

C. Considerations for Clinical Development (5.3)

It is expected that the impurity assessment described in Sections V.A (5.1) and V.B (5.2) applies to products in clinical development. However, it is acknowledged that the available information is limited. For example, information from long-term stability studies and photostability studies may not be available during clinical development and thus information on potential degradation products may be limited. Additionally, the thresholds outlined in ICH Q3A/Q3B do *not* apply to products in clinical development and, consequently, fewer impurities will be identified.

VI. HAZARD ASSESSMENT ELEMENTS (6)

Hazard assessment involves an initial analysis of actual and potential impurities by conducting database and literature searches for carcinogenicity and bacterial mutagenicity data in order to classify them as Class 1, 2, or 5 according to Table 1. If data for such a classification are not available, an assessment of Structure-Activity Relationships (SAR) that focuses on bacterial mutagenicity predictions should be performed. This could lead to a classification into Class 3, 4, or 5.

Table 1: Impurities Classification With Respect to Mutagenic and Carcinogenic Potential and Resulting Control Actions

Class	Definition	Proposed action for control (details in Section VII (7) and VIII (8))
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive,* no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5 If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

*Or other relevant positive mutagenicity data indicative of DNA-reactivity-related induction of gene mutations (e.g., positive findings in in vivo gene mutation studies)

A computational toxicology assessment should be performed using Quantitative Structure-Activity Relationship ((Q)SAR) methodologies that predict the outcome of a bacterial mutagenicity assay (Ref. 6). Two (Q)SAR prediction methodologies that complement each other should be applied. One methodology should be expert rule-based, and the second methodology should be statistical-based. (Q)SAR models utilizing these prediction methodologies should follow the general validation principles set forth by the Organisation for Economic Co-operation and Development (OECD).

The absence of structural alerts from two complementary (Q)SAR methodologies (expert rule-based and statistical) is sufficient to conclude that the impurity is of no mutagenic concern, and no further testing is recommended (Class 5 in Table 1).

If warranted, the outcome of any computer system-based analysis can be reviewed with the use of expert knowledge in order to provide additional supportive evidence on relevance of any positive, negative, conflicting, or inconclusive prediction and to provide a rationale to support the final conclusion.

To follow up on a relevant structural alert (Class 3 in Table 1), either adequate control measures could be applied or a bacterial mutagenicity assay with the impurity alone can be conducted. An appropriately conducted negative bacterial mutagenicity assay (Note 2) would overrule any structure-based concern, and no further genotoxicity assessments would be recommended (Note 1). These impurities should be considered non-mutagenic (Class 5 in Table 1). A positive bacterial mutagenicity result would warrant further hazard assessment and/or control measures (Class 2 in Table 1). For instance, when levels of the impurity cannot be controlled at an appropriate acceptable limit, it is recommended that the impurity be tested in an in vivo gene mutation assay in order to understand the relevance of the bacterial mutagenicity assay result under in vivo conditions. The selection of other in vivo genotoxicity assays should be scientifically justified based on knowledge of the mechanism of action of the impurity and expected target tissue exposure (Note 3). In vivo studies should be designed taking into consideration existing ICH genotoxicity guidances. Results in the appropriate in vivo assay may support setting compound specific impurity limits.

An impurity with a structural alert that is shared (e.g., same structural alert in the same position and chemical environment) with the drug substance or related compounds can be considered as non-mutagenic (Class 4 in Table 1) if the testing of such material in the bacterial mutagenicity assay was negative.

VII. RISK CHARACTERIZATION (7)

As a result of hazard assessment described in Section VI (6), each impurity will be assigned to one of the five classes in Table 1. For impurities belonging in Classes 1, 2, and 3, the principles of risk characterization used to derive acceptable intakes are described in this section.

A. TTC-Based Acceptable Intakes (7.1)

A TTC-based acceptable intake of a mutagenic impurity of 1.5 µg per person per day is considered to be associated with a negligible risk (theoretical excess cancer risk of <1 in 100,000 over a lifetime of exposure) and can, in general, be used for most pharmaceuticals as a default to derive an acceptable limit for control. This approach would usually be used for

mutagenic impurities present in pharmaceuticals for long-term treatment (> 10 years) and where no carcinogenicity data are available (Classes 2 and 3).

B. Acceptable Intakes Based on Compound-Specific Risk Assessments (7.2)

1. Mutagenic Impurities With Positive Carcinogenicity Data (Class 1 in Table 1) (7.2.1)

Compound-specific risk assessments to derive acceptable intakes should be applied instead of the TTC-based acceptable intakes where sufficient carcinogenicity data exist. For a known mutagenic carcinogen, a compound-specific acceptable intake can be calculated based on carcinogenic potency and linear extrapolation as a default approach. Alternatively, other established risk assessment practices such as those used by international regulatory bodies may be applied either to calculate acceptable intakes or to use already existing values published by regulatory authorities (Note 4).

Compound-specific calculations for acceptable intakes can be applied case-by-case for impurities which are chemically similar to a known carcinogen compound class (class-specific acceptable intakes) provided that a rationale for chemical similarity and supporting data can be demonstrated (Note 5).

2. Mutagenic Impurities With Evidence for a Practical Threshold (7.2.2)

The existence of mechanisms leading to a dose response that is non-linear or has a practical threshold is increasingly recognized, not only for compounds that interact with non-DNA targets but also for DNA-reactive compounds, whose effects may be modulated by, for example, rapid detoxification before coming into contact with DNA, or by effective repair of induced damage. The regulatory approach to such compounds can be based on the identification of a No-Observed Effect Level (NOEL) and use of uncertainty factors (see ICH Q3C(R5), Ref. 7) to calculate a permissible daily exposure (PDE) when data are available.

The acceptable intakes derived from compound-specific risk assessments (Section VII.B (7.2)) can be adjusted for shorter duration of use in the same proportions as defined in the following sections (Section VII.C.1 (7.3.1) and VII.C.2 (7.3.2)) or should be limited to not more than 0.5%, whichever is lower. For example, if the compound-specific acceptable intake is 15 µg/day for lifetime exposure, the less than lifetime limits (Table 2) can be increased to a daily intake of 100 µg (> 1-10 years treatment duration), 200 µg (> 1-12 months) or 1200 µg (< 1 month). However, for a drug with a maximum daily dose of, for instance, 100 milligrams (mg), the acceptable daily intake for the < 1-month duration, would be limited to 0.5% (500 µg) rather than 1200 µg.

C. Acceptable Intakes in Relation to Less-Than-Lifetime (LTL) Exposure (7.3)

Standard risk assessments of known carcinogens assume that cancer risk increases as a function of cumulative dose. Thus, cancer risk of a continuous low dose over a lifetime would be equivalent to the cancer risk associated with an identical cumulative exposure averaged over a shorter duration.

The TTC-based acceptable intake of 1.5 µg/day is considered to be protective for a lifetime of daily exposure. To address LTL exposures to mutagenic impurities in pharmaceuticals, an approach is applied in which the acceptable cumulative lifetime dose (1.5 µg/day x 25,550 days = 38.3 mg) is uniformly distributed over the total number of exposure days during LTL exposure. This would allow higher daily intake of mutagenic impurities than would be the case for lifetime exposure and still maintain comparable risk levels for daily and non-daily treatment regimens. Table 2 is derived from the above concepts and illustrates the acceptable intakes for LTL to lifetime exposures for clinical development and marketing. In the case of intermittent dosing, the acceptable daily intake should be based on the total number of dosing days instead of the time interval over which the doses were administered and that number of dosing days should be related to the relevant duration category in Table 2. For example, a drug administered once per week for 2 years (i.e., 104 dosing days) would have an acceptable intake per dose of 20 µg.

Table 2: Acceptable Intakes for an Individual Impurity

Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

1. Clinical Development (7.3.1)

Using this LTL concept, acceptable intakes of mutagenic impurities are recommended for limited treatment periods during clinical development of up to 1 month, 1 to 12 months and more than 1 year up to completion of Phase 3 clinical trials (Table 2). These adjusted acceptable intake values maintain a 10^{-6} risk level in early clinical development when benefit has not yet been established and a 10^{-5} risk level for later stages in development (Note 6).

An alternative approach to the strict use of an adjusted acceptable intake for any mutagenic impurity could be applied for Phase 1 clinical trials for dosing up to 14 days. For this approach, only impurities that are known mutagenic carcinogens (Class 1) and known mutagens of unknown carcinogenic potential (Class 2), as well as impurities in the cohort of concern chemical class, should be controlled (see Section VIII (8)) to acceptable limits as described in Section VII (7). All other impurities would be treated as non-mutagenic impurities. This includes impurities which contain structural alerts (Class 3), which alone would not trigger action for an assessment for this limited Phase 1 duration.

2. Marketed Products (7.3.2)

The treatment duration categories with acceptable intakes in Table 2 for marketed products are intended to be applied to anticipated exposure durations for the great majority of patients. The proposed intakes, along with various scenarios for applying those intakes, are described in Note 7, Table 4. In some cases, a subset of the population of patients may extend treatment beyond the marketed drug's categorical upper limit (e.g., treatment exceeding 10 years for an acceptable intake of 10 µg/day, perhaps receiving 15 years of treatment). This would result in a negligible increase (in the example given, a fractional increase to 1.5/100,000) compared to the overall calculated risk for the majority of patients treated for 10 years.

D. Acceptable Intakes for Multiple Mutagenic Impurities (7.4)

The TTC-based acceptable intakes should be applied to each individual impurity. When there are two Class 2 or Class 3 impurities, individual limits apply. When there are three or more Class 2 or Class 3 impurities specified on the drug substance specification, total mutagenic impurities should be limited as described in Table 3 for clinical development and marketed products.

For combination products, each active ingredient should be regulated separately.

Table 3: Acceptable Total Daily Intakes for Multiple Impurities

Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Total Daily intake [µg/day]	120	60	30	5

Only specified Class 2 and 3 impurities on the drug substance specification should be included in the calculation of the total limit. However, impurities with compound-specific or class-related acceptable intake limits (Class 1) should not be included in the total limits of Class 2 and Class 3 impurities. Also, degradation products that form in the drug product would be controlled individually and a total limit would not be applied.

E. Exceptions and Flexibility in Approaches (7.5)

- Higher acceptable intakes may be justified when human exposure to the impurity will be much greater from other sources, e.g., food, or endogenous metabolism (e.g., formaldehyde).
- Case-by-case exceptions to the use of the appropriate acceptable intake can be justified in cases of severe disease, reduced life expectancy, late onset but chronic disease, or limited therapeutic alternatives.
- Compounds from some structural classes of mutagens can display extremely high carcinogenic potency (cohort of concern), i.e., aflatoxin-like-, N-nitroso-, and alkyl-azoxy structures. If these compounds are found as impurities in pharmaceuticals, acceptable intakes for these high-potency carcinogens would likely be significantly lower than the acceptable intakes defined in this guidance. Although the principles of this guidance can be used, a case-by-case approach, using, e.g., carcinogenicity data from closely related structures, if available, should usually be developed to justify acceptable intakes for pharmaceutical development and marketed products.

The above risk approaches described in Section VII (7) are applicable to all routes of administration, and no corrections to acceptable intakes are generally warranted. Exceptions to consider may include situations where data justify route-specific concerns that should be evaluated case by case. These approaches are also applicable to all patient populations based upon the conservative nature of the risk approaches being applied.

VIII. CONTROL

A control strategy is a planned set of controls derived from current product and process understanding that assures process performance and product quality (ICH Q10, Ref. 8). A control strategy can include, but is not limited to, the following:

- Controls on material attributes (including raw materials, starting materials, intermediates, reagents, solvents, primary packaging materials);

- Facility and equipment operating conditions;
- Controls implicit in the design of the manufacturing process;
- In-process controls (including in-process tests and process parameters);
- Controls on drug substance and drug product (e.g., release testing).

When an impurity has been characterized as Class 1, 2, or 3 in Table 1, it is important to develop a control strategy that assures that the level of this impurity in the drug substance and drug product is below the acceptable limit. A thorough knowledge of the chemistry associated with the drug substance manufacturing process, and of the drug product manufacturing process, along with an understanding of the overall stability of the drug substance and drug product is fundamental to developing the appropriate controls. Developing a strategy to control mutagenic impurities in the drug product is consistent with risk management processes identified in ICH Q9 (Ref. 9). A control strategy that is based on product and process understanding and utilization of risk management principles will lead to a combination of process design and control and appropriate analytical testing, which can also provide an opportunity to shift controls upstream and minimize the need for end-product testing.

A. Control of Process Related Impurities (8.1)

There are 4 potential approaches to development of a control strategy for drug substance:

Option 1

Include a test for the impurity in the drug substance specification with an acceptance criterion at or below the acceptable limit using an appropriate analytical procedure.

For an Option 1 control approach, it is possible to apply periodic verification testing per ICH Q6A (Ref 10). Periodic verification testing is justified when it can be shown that levels of the mutagenic impurity in the drug substance are less than 30% of the acceptable limit for at least 6 consecutive pilot scale or 3 consecutive production scale batches. If this condition is not fulfilled, a routine test in the drug substance specification is recommended. See Section VIII.C (8.3) for additional considerations.

Option 2

Include a test for the impurity in the specification for a raw material, starting material or intermediate, or as an in-process control, with an acceptance criterion at or below the acceptable limit using an appropriate analytical procedure.

Option 3

Include a test for the impurity in the specification for a raw material, starting material or intermediate, or as an in-process control, with an acceptance criterion above the acceptable limit of the impurity in the drug substance, using an appropriate analytical procedure coupled with demonstrated understanding of fate and purge and associated process controls that assure the level in the drug substance is below the acceptable limit without the need for any additional testing later in the process.

This option can be justified when the level of the impurity in the drug substance will be less than 30% of the acceptable limit by review of data from laboratory scale experiments (spiking experiments are encouraged) and, where warranted, is supported by data from pilot scale or commercial scale batches. See Appendix 2, Case Examples 1 and 2. Alternative approaches can be used to justify Option 3.

Option 4

Understand process parameters and impact on residual impurity levels (including fate and purge knowledge) with sufficient confidence that the level of the impurity in the drug substance will be below the acceptable limit such that no analytical testing is recommended for this impurity (i.e., the impurity does not need to be listed on any specification).

A control strategy that relies on process controls in lieu of analytical testing can be appropriate if the process chemistry and process parameters that have an impact on the levels of mutagenic impurities are understood and the risk of an impurity residing in the final drug substance above the acceptable limit is determined to be negligible. In many cases, justification of this control approach based on scientific principles alone is sufficient. Elements of a scientific risk assessment can be used to justify an option 4 approach. The risk assessment can be based on physicochemical properties and process factors that influence the fate and purge of an impurity, including chemical reactivity, solubility, volatility, ionizability, and any physical process steps designed to remove impurities. The result of this risk assessment might be shown as an estimated purge factor for clearance of the impurity by the process (Ref. 11).

Option 4 is especially useful for those impurities that are inherently unstable (e.g., thionyl chloride that reacts rapidly and completely with water) or for those impurities that are introduced early in the synthesis and are effectively purged.

In some cases an Option 4 approach can be appropriate when the impurity is known to form, or is introduced late in the synthesis; however, process-specific data should then be provided to justify this approach.

B. Considerations for Control Approaches (8.2)

For Option 4 approaches where justification based on scientific principles alone is not considered sufficient, as well as for Option 3 approaches, analytical data to support the control approach is strongly recommended. This could include, as appropriate, information on the structural changes to the impurity caused by downstream chemistry (*fate*); analytical data on pilot scale batches; and, in some cases, laboratory scale studies with intentional addition of the impurity (*spiking studies*). In these cases, it is important to demonstrate that the fate/purge argument for the impurity is robust and will consistently assure a negligible probability of an impurity residing in the final drug substance above the acceptable limit. Where the purge factor is based on developmental data, it is important to address the expected scale-dependence or independence. In the case that the small scale model used in the development stage is considered to not represent the commercial scale, confirmation of suitable control in pilot scale and/or initial commercial batches is generally appropriate. The need for data from pilot/commercial batches is influenced by the magnitude of the purge factor calculated from laboratory or pilot scale data, point of entry of the impurity, and knowledge of downstream process purge points.

If Options 3 and 4 cannot be justified, then a test for the impurity on the specification for a raw material, starting material or intermediate, or as an in-process control (Option 2) or drug substance (Option 1) at the acceptable limit should be included. For impurities introduced in the last synthetic step, an Option 1 control approach would normally be expected unless otherwise justified.

The application of *As Low As Reasonably Practicable* (ALARP) is not necessary if the level of the mutagenic impurity is below acceptable limits. Similarly, it is not necessary to demonstrate that alternate routes of synthesis have been explored.

In cases where control efforts cannot reduce the level of the mutagenic impurity to below the acceptable limit and levels are as low as reasonably practical, a higher limit may be justified based on a risk/benefit analysis.

C. Considerations for Periodic Testing (8.3)

The above options include situations where a test is recommended to be included in the specification, but where routine measurement for release of every batch may not be necessary. This approach, referred to as *periodic or skip testing* in ICH Q6A could also be called *Periodic Verification Testing*. This approach may be appropriate when it can be demonstrated that processing subsequent to impurity formation/introduction clears the impurity. It should be noted that allowance of Periodic Verification Testing is contingent upon use of a process that is under a state of control (i.e., produces a quality product that consistently meets specifications and conforms to an appropriately established facility, equipment, processing, and operational control regimen). If upon testing, the level of the mutagenic impurity fails to meet the acceptance criteria established for the periodic test, the drug producer should immediately commence full testing (i.e., testing of every batch for the attribute specified) until the cause of the failure has been conclusively determined, corrective action has been implemented, and the process is again documented to be in a state of control. As noted in ICH Q6A, regulatory authorities should be notified of a periodic verification test failure to evaluate the risk/benefit of previously released batches that were not tested.

D. Control of Degradation Products (8.4)

For a potential degradation product that has been characterized as mutagenic, it is important to understand if the degradation pathway is relevant to the drug substance and drug product manufacturing processes and/or their proposed packaging and storage conditions. A well-designed accelerated stability study (e.g., 40°C/75% relative humidity, 6 months) using the proposed packaging, with appropriate analytical procedures, is recommended to determine the relevance of the potential degradation product. Alternatively, well-designed kinetically equivalent shorter-term stability studies at higher temperatures using the proposed commercial packaging may be used to determine the relevance of the degradation pathway prior to initiating longer-term stability studies. This type of study would be especially useful to understand the relevance of those potential degradation products that are based on knowledge of potential degradation pathways but not yet observed in the product.

Based on the result of these accelerated studies, if it is anticipated that the degradation product will form at levels approaching the acceptable limit under the proposed packaging and storage conditions, then efforts to control formation of the degradation product is

recommended. In these cases, monitoring for the drug substance or drug product degradation in long-term primary stability studies at the proposed storage conditions (in the proposed commercial pack) is expected unless otherwise justified. Whether or not a specification limit for the mutagenic degradation product is appropriate will generally depend on the results from these stability studies.

If it is anticipated that formulation development and packaging design options are unable to control mutagenic degradation product levels to less than the acceptable limit and levels are as low as reasonably practicable, a higher limit can be justified based on a risk/benefit analysis.

E. Lifecycle Management (8.5)

This section is intended to apply to those products approved after the issuance of this guidance.

The quality system elements and management responsibilities described in ICH Q10 are intended to encourage the use of science-based and risk-based approaches at each lifecycle stage, thereby promoting continual improvement across the entire product lifecycle. Product and process knowledge should be managed from development through the commercial life of the product up to and including product discontinuation.

The development and improvement of a drug substance or drug product manufacturing process usually continues over its lifecycle. Manufacturing process performance, including the effectiveness of the control strategy, should be periodically evaluated. Knowledge gained from commercial manufacturing can be used to further improve process understanding and process performance and to adjust the control strategy.

Any proposed change to the manufacturing process should be evaluated for the impact on the quality of drug substance and drug product. This evaluation should be based on understanding of the manufacturing process and should determine whether appropriate testing to analyze the impact of the proposed changes is warranted. Additionally, improvements in analytical procedures may lead to structural identification of an impurity. In those cases, the new structure would be assessed for mutagenicity as described in this guidance.

Throughout the lifecycle of the product, it will be important to reassess if testing is recommended when intended or unintended changes occur in the process. This applies when there is no routine monitoring at the acceptable limit (Option 3 or Option 4 control approaches), or when applying periodic rather than batch-by-batch testing. This testing should be performed at an appropriate point in the manufacturing process.

In some cases, the use of statistical process control and trending of process measurements can be useful for continued suitability and capability of processes to provide adequate control on the impurity. Statistical process control can be based on process parameters that influence impurity formation or clearance, even when that impurity is not routinely monitored (e.g., Option 4).

All changes should be subject to internal change management processes as part of the quality system (ICH Q10). Changes to information filed and approved in a dossier should be reported to regulatory authorities in accordance with regional regulations and guidelines.

F. Considerations for Clinical Development (8.6)

It is recognized that product and process knowledge increases over the course of development and therefore it is expected that data to support control strategies in the clinical development trial phases will be less than at the marketing registration phase. A risk-based approach based on process chemistry fundamentals is encouraged to prioritize analytical efforts on those impurities with the highest likelihood of being present in the drug substance or drug product. Analytical data may not be expected to support early clinical development when the likelihood of an impurity being present is low, but in a similar situation analytical data may be appropriate to support the control approach for the marketing application. It is also recognized that commercial formulation design occurs later in clinical development and therefore efforts associated with drug product degradation products will be limited in the earlier phases.

IX. DOCUMENTATION

Information relevant to the application of this guidance should be provided at the following stages:

A. Clinical Trial Applications (9.1)

- It is expected that the number of structures assessed for mutagenicity and the collection of analytical data will both increase throughout the clinical development period.
- For Phase 1 studies of 14 days or less, a description of efforts to mitigate risks of mutagenic impurities focused on Class 1 and Class 2 impurities and those in the cohort of concern as outlined in Section VII (7) should be included. For Phase 1 clinical trials of more than 14 days and for Phase 2a clinical trials as well, Class 3 impurities that have analytical controls should also be included.
- For Phase 2b and Phase 3 clinical development trials, a list of the impurities assessed by (Q)SAR should be included, and any Class 1, 2, or 3 actual and potential impurities should be described along with plans for control. The *in silico* (Q)SAR systems used to perform the assessments should be described. The results of bacterial mutagenicity tests of actual impurities should be reported.
- Chemistry arguments may be appropriate instead of analytical data for potential impurities that present a low likelihood of being present as described in Section VIII.F (8.6).

B. Common Technical Document (Marketing Application) (9.2)

- For actual and potential process related impurities and degradation products where assessments according to this guidance are conducted, the mutagenic impurity classification and rationale for this classification should be provided:
  - This would include the results and description of *in silico* (Q)SAR systems used and, as appropriate, supporting information to arrive at the overall conclusion for Class 4 and 5 impurities.

  - When bacterial mutagenicity assays were performed on impurities, study reports should be provided for bacterial mutagenicity assays on impurities.

- Justification for the proposed specification and the approach to control should be provided (e.g., ICH Q11 example 5b, Ref. 12). For example, this information could include the acceptable intake, the location, and sensitivity of relevant routine monitoring. For Option 3 and Option 4 control approaches, a summary of knowledge of the purge factor, and identification of factors providing control (e.g., process steps, solubility in wash solutions), is important.

NOTES

Note 1 The ICH M7 Guidance recommendations provide a state-of-the-art approach for assessing the potential of impurities to induce point mutations and ensure that such impurities are controlled to safe levels so that below or above the ICH Q3A/Q3B qualification threshold, no further qualification for mutagenic potential is required. This includes the initial use of (Q)SAR tools to predict bacterial mutagenicity. In cases where the amount of the impurity exceeds 1 mg daily dose for chronic administration, evaluation of genotoxic potential as recommended in ICH Q3A/Q3B could be considered. In cases where the amount of the impurity is less than 1 mg, no further genotoxicity testing is required regardless of other qualification thresholds.

Note 2 To assess the mutagenic potential of impurities, a single bacterial mutagenicity assay can be carried out with a fully adequate protocol according to ICH S2(R1) and OECD 471 guidelines (Refs. 13 and 14). The assays should be performed in compliance with Good Laboratory Practices (GLP) regulations; however, lack of full GLP compliance does not necessarily mean that the data cannot be used to support clinical trials and marketing authorizations. Such deviations should be described in the study report. For example, the test article may not be prepared or analyzed in compliance with GLP regulations. In some cases, the selection of bacterial tester strains may be limited to those proven to be sensitive to the identified alert. For impurities that are not feasible to isolate or synthesize or when compound quantity is limited, it may not be possible to achieve the highest test concentrations recommended for an ICH-compliant bacterial mutagenicity assay according to the current testing guidelines. In this case, bacterial mutagenicity testing could be carried out using a miniaturized assay format with proven high concordance to the ICH-compliant assay to enable testing at higher concentrations with justification.

Note 3 Tests to Investigate the in vivo Relevance of in vitro Mutagens (Positive Bacterial Mutagenicity)

In vivo test	Factors to justify choice of test as fit-for-purpose
Transgenic mutation assays	• For any bacterial mutagenicity positive. Justify selection of assay tissue/organ
Pig-a assay (blood)	• For directly acting mutagens (bacterial mutagenicity positive without S9)*
Micronucleus test (blood or bone marrow)	• For directly acting mutagens (bacterial mutagenicity positive without S9) and compounds known to be clastogenic*
Rat liver Unscheduled DNA Synthesis (UDS) test	• In particular for bacterial mutagenicity positive with S9 only • Responsible liver metabolite known o to be generated in test species used o to induce bulky adducts
Comet assay	• Justification should be provided (chemical class specific mode of action to form alkaline labile sites or single-strand breaks as preceding DNA damage that can

	potentially lead to mutations • Justify selection of assay tissue/organ
Others	• With convincing justification

*For indirect acting mutagens (requiring metabolic activation), adequate exposure to metabolite(s) should be demonstrated.

Note 4 Example of linear extrapolation from the TD_{50}

It is possible to calculate a compound-specific acceptable intake based on rodent carcinogenicity potency data such as TD_{50} values (doses giving a 50% tumor incidence equivalent to a cancer risk probability level of 1:2). Linear extrapolation to a probability of 1 in 100,000 (i.e., the accepted lifetime risk level used) is achieved by simply dividing the TD_{50} by 50,000. This procedure is similar to that employed for derivation of the TTC.

Calculation example: Ethylene oxide

TD_{50} values for ethylene oxide according to the Carcinogenic Potency Database are 21.3 mg/kg body weight/day (rat) and 63.7 mg/kg body weight/day (mouse). For the calculation of an acceptable intake, the lower (i.e., more conservative) value of the rat is used.

To derive a dose to cause tumors in 1 in 100,000 animals, divide by 50,000:

21.3 mg/kilograms (kg) ÷ 50,000 = 0.42 µg/kg

To derive a total human daily dose:

0.42 µg/kg/day x 50 kg body weight = 21.3 µg/person/day

Hence, a daily life-long intake of 21.3 µg ethylene oxide would correspond to a theoretical cancer risk of 10^{-5} and therefore be an acceptable intake when present as an impurity in a drug substance.

Alternative methods and published regulatory limits for cancer risk assessment

As an alternative of using the most conservative TD_{50} value from rodent carcinogenicity studies irrespective of its relevance to humans, an in-depth toxicological expert assessment of the available carcinogenicity data can be done in order to initially identify the findings (e.g., species, organ) with highest relevance to human risk assessment as a basis for deriving a reference point for linear extrapolation. Also, in order to better take into account directly the shape of the dose-response curve, a benchmark dose such as a benchmark dose lower confidence limit 10% (BMDL10, an estimate of the lowest dose which is 95% certain to cause no more than a 10% cancer incidence in rodents) may be used instead of TD_{50} values as a numerical index for carcinogenic potency. Linear extrapolation to a probability of 1 in 100,000 (i.e., the accepted lifetime risk level used) is then achieved by simply dividing the BMDL10 by 10,000.

Compound-specific acceptable intakes can also be derived from published recommended values from internationally recognized bodies such as World Health Organization (WHO, International Program on Chemical Safety [IPCS] Cancer Risk Assessment Programme) and others using the appropriate 10^{-5} lifetime risk level. In general, a regulatory limit that is applied should be based on the most current and scientifically supported data and/or methodology.

Note 5 A compound-specific calculation of acceptable intakes for mutagenic impurities may be applied for mutagenic impurities (without carcinogenicity data) which are structurally similar to a chemically defined class of known carcinogen. For example, factors that are associated with the carcinogenic potency of monofunctional alkyl chlorides have been identified (Ref. 15) and can be used to modify the safe acceptable intake of monofunctional alkyl chlorides, a group of alkyl chlorides commonly used in drug synthesis. Compared to multifunctional alkyl chlorides, the monofunctional compounds are much less potent carcinogens with TD_{50} values ranging from 36 to 1810 mg/kg/day (n=15; epichlorohydrin with two distinctly different functional groups is excluded). A TD_{50} value of 36 mg/kg/day can thus be used as a still very conservative class-specific potency reference point for calculation of acceptable intakes for monofunctional alkyl chlorides. This potency level is at least 10-fold lower than the TD_{50} of 1.25 mg/kg/day corresponding to the default lifetime TTC (1.5 µg/day) and therefore justifies lifetime and less-than-lifetime daily intakes for monofunctional alkyl chlorides 10 times the default ones.

Note 6 Establishing less-than-lifetime acceptable intakes for mutagenic impurities in pharmaceuticals has precedence in the establishment of the staged TTC limits for clinical development (Ref. 16). The calculation of less-than-lifetime Acceptable Intakes (AI) is predicated on the principle of Haber's rule, a fundamental concept in toxicology where concentration (C) x time (T) = a constant (k). Therefore, the carcinogenic effect is based on both dose and duration of exposure.

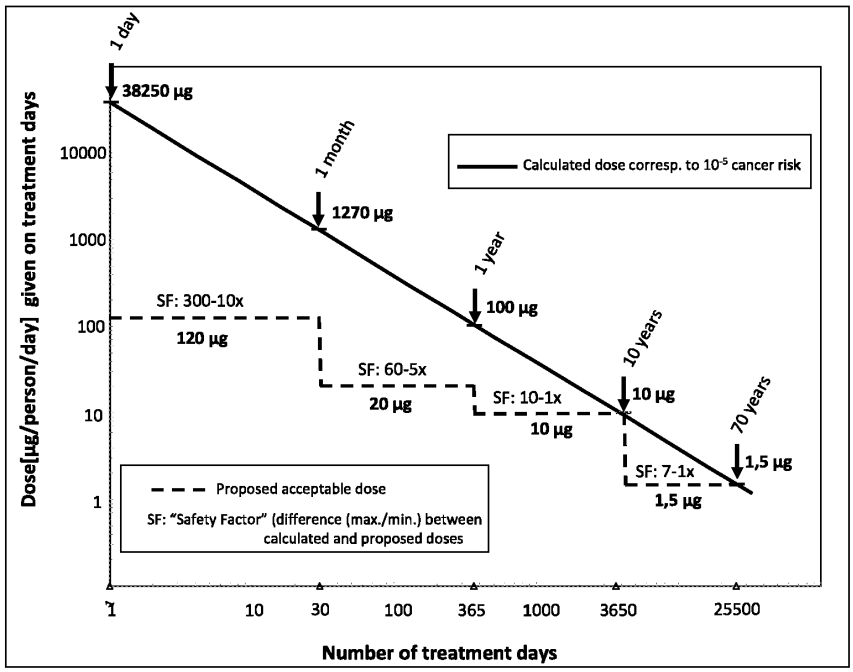

Figure 1: Illustration of calculated daily dose of a mutagenic impurity corresponding to a theoretical 1:100,000 cancer risk as a function of duration of treatment in comparison to the acceptable intake levels as recommended in Section VII.C (7.3).

The solid line in Figure 1 represents the linear relationship between the amount of daily intake of a mutagenic impurity corresponding to a 10^{-5} cancer risk and the number of treatment days. The calculation is based on the TTC level as applied in this guidance for life-long treatment, i.e., 1.5 µg per person per day using the formula:

$$\text{Less-than-lifetime AI} = \frac{1.5\ \mu g \times (365\ \text{days} \times 70\ \text{years lifetime} = 25{,}550)}{\text{Total number of treatment days}}$$

The calculated daily intake levels would thus be 1.5 µg for treatment duration of 70 years, 10 µg for 10 years, 100 µg for 1 year, 1270 µg for 1 month and approximately 38.3 mg as a single dose, all resulting in the same cumulative intake and therefore theoretically in the same cancer risk (1 in 100,000).

The dashed step-shaped curve represents the actual daily intake levels adjusted to less-than-lifetime exposure as recommended in Section VII (7) of this guidance for products in clinical development and marketed products. These proposed levels, are, in general, significantly lower than the calculated values thus providing safety factors that increase with shorter treatment durations.

The proposed accepted daily intakes are also in compliance with a 10^{-6} cancer risk level if treatment durations are not longer than 6 months and are therefore applicable in early clinical trials with volunteers/patients where benefit has not yet

been established. In this case, the safety factors as shown in the upper graph would be reduced by a factor of 10.

Note 7

Table 4: Examples of clinical use scenarios with different treatment durations for applying acceptable intakes

Scenario[1]	**Acceptable Intake (µg/day)**
Treatment duration of ≤ 1 month: e.g., drugs used in emergency procedures (antidotes, anesthesia, acute ischemic stroke), actinic keratosis, treatment of lice	120
Treatment duration of > 1-12 months: e.g., anti-infective therapy with maximum up to 12 months treatment (Hepatitis C Virus), parenteral nutrients, prophylactic flu drugs (~ 5 months), peptic ulcer, Assisted Reproductive Technology (ART), pre-term labor, preeclampsia, pre-surgical (hysterectomy) treatment, fracture healing (these are acute use but with long half-lives)	20
Treatment duration of >1-10 years: e.g., stage of disease with short life expectancy (severe Alzheimer's), non-genotoxic anticancer treatment being used in a patient population with longer-term survival (breast cancer, Chronic Myelogenous Leukemia), drugs specifically labeled for less than 10 years of use, drugs administered intermittently to treat acute recurring symptoms[2] (chronic Herpes, gout attacks, substance dependence such as smoking cessation), macular degeneration, Human Immunodeficiency Virus (HIV)[3]	10
Treatment duration of >10 years to lifetime: e.g., chronic use indications with high likelihood for lifetime use across broader age range (hypertension, dyslipidemia, asthma, Alzheimer's Disease (AD) (except severe AD), hormone therapy (e.g., Growth Hormone, Thyroid Hormone, Para Thyroid Hormone), lipodystrophy, schizophrenia, depression, psoriasis, atopic dermatitis, Chronic Obstructive Pulmonary Disease (COPD), cystic fibrosis, seasonal and perennial allergic rhinitis	1.5

[1] This table shows general examples; each example should be examined on a case-by-case basis. For example, 10 µg/day may be acceptable in cases where the life expectancy of the patient may be limited, e.g., severe Alzheimer's disease, even though the drug use could exceed 10-year duration.

[2] Intermittent use over a period >10 yrs., but based on calculated cumulative dose, it falls under the >1-10 yr. category.

[3] HIV is considered a chronic indication, but resistance develops to the drugs after 5-10 years and the therapy is changed to other HIV drugs.

GLOSSARY

Acceptable intake:
In the context of this guidance, an intake level that poses negligible cancer risk, or for serious/life-threatening indications where risk and benefit are appropriately balanced.

Acceptable limit:
Maximum acceptable concentration of an impurity in a drug substance or drug product derived from the acceptable intake and the daily dose of the drug.

Acceptance criterion:
Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures.

Control strategy:
A planned set of controls, derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.

Cumulative intake:
The total intake of a substance that a person is exposed to over time.

Degradation Product:
A molecule resulting from a chemical change in the drug molecule brought about over time and/or by the action of light, temperature, pH, water, or by reaction with an excipient and/or the immediate container/closure system.

DNA-reactive:
The potential to induce direct DNA damage through chemical reaction with DNA.

Expert knowledge:
In the context of this guidance, expert knowledge can be defined as a review of pre-existing data and the use of any other relevant information to evaluate the accuracy of an *in silico* model prediction for mutagenicity.

Genotoxicity:
A broad term that refers to any deleterious change in the genetic material regardless of the mechanism by which the change is induced.

Impurity:
Any component of the drug substance or drug product that is not the drug substance or an excipient.

Mutagenic impurity:
An impurity that has been demonstrated to be mutagenic in an appropriate mutagenicity test model, e.g., bacterial mutagenicity assay.

Periodic verification testing:
Also known as *periodic or skip testing* in ICH Q6A.

(Q)SAR and SAR:
In the context of this guidance, refers to the relationship between the molecular (sub) structure of a compound and its mutagenic activity using (Quantitative) Structure-Activity Relationships derived from experimental data.

Purge factor:
Purge reflects the ability of a process to reduce the level of an impurity, and the purge factor is defined as the level of an impurity at an upstream point in a process divided by the level of an impurity at a downstream point in a process. Purge factors may be measured or predicted.

Structural alert:
In the context of this guidance, a chemical grouping or molecular (sub) structure which is associated with mutagenicity.

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APPENDICES

Appendix 1: Scope Scenarios for Application of the ICH M7 Guidance

Scenario	Applies to Drug Substance	Applies to Drug Product	Comments
Registration of new drug substances and associated drug product	Yes	Yes	Primary intent of the M7 Guidance
Clinical trial applications for new drug substances and associated drug product	Yes	Yes	Primary intent of the M7 Guidance
Clinical trial applications for new drug substances for an anti-cancer drug per ICH S9	No	No	Out of scope of M7 Guidance
Clinical trial applications for new drug substances for an orphan drug	Yes	Yes	There may be exceptions on a case-by-case basis for higher impurity limits
Clinical trial application for a new drug product using an existing drug substance where there are no changes to the drug substance manufacturing process	No	Yes	Retrospective application of the M7 Guidance is not intended for marketed products unless there are changes made to the synthesis. Since no changes are made to the drug substance synthesis, the drug substance would not require reevaluation. Since the drug product is new, application of this guidance is expected.
A new formulation of an approved drug substance is filed	No	Yes	See Section IV.B (4.2)
A product that is previously approved in a member region is filed for the first time in a different member region. The product is unchanged.	Yes	Yes	As there is no mutual recognition, an existing product in one member region filed for the first time in another member region would be considered a new product.
A new supplier or new site of the drug substance is registered. There are no changes to the manufacturing process used in this registered application.	No	No	As long as the synthesis of the drug substance is consistent with previously approved methods, then reevaluation of mutagenic impurity risk is not necessary. The applicant would need to demonstrate that no changes have been made to a previously approved process/product. Refer to Section IV.A (4.1).

An existing product (approved after the issuance of ICH M7 with higher limits based on ICH S9) associated with an advanced cancer indication is now registered for use in a non-life threatening indication	Yes	Yes	Since the patient population and acceptable cancer risk have changed, the previously approved impurity control strategy and limits will require reevaluation. See Section IV.C (4.3).
New combination product is filed that contains one new drug substance and an existing drug substance	Yes (new drug substance) No (existing drug substance)	Yes	M7 would apply to the new drug substance. For the existing drug substance, retrospective application of M7 to existing products is not intended. For the drug product, this would classify as a new drug product so the guidance would apply to any new or higher levels of degradation products.

Appendix 2: Case Examples to Illustrate Potential Control Approaches

Case 1: Example of an Option 3 Control Strategy

An intermediate X is formed two steps away from the drug substance and impurity A is routinely detected in intermediate X. The impurity A is a stable compound and carries over to the drug substance. A spike study of the impurity A at different concentration levels in intermediate X was performed at laboratory scale. As a result of these studies, impurity A was consistently removed to less than 30% of the TTC-based limit in the drug substance even when impurity A was present at 1% in intermediate X. Since this intermediate X is formed only two steps away from the drug substance and the impurity A level in the intermediate X is relatively high, the purging ability of the process has also been confirmed by determination of impurity A in the drug substance in multiple pilot-scale batches and results were below 30% of the TTC-based limit. Therefore, control of the impurity A in the intermediate X with an acceptance limit of 1.0% is justified and no test is warranted for this impurity in the drug substance specification.

Case 2: Example of an Option 3 Control Strategy: Based on Predicted Purge From a Spiking Study Using Standard Analytical Methods

A starting material Y is introduced in step 3 of a 5-step synthesis, and an impurity B is routinely detected in the starting material Y at less than 0.1% using standard analytical methods. In order to determine whether the 0.1% specification in the starting material is acceptable, a purge study was conducted at laboratory scale where impurity B was spiked into starting material Y with different concentration levels up to 10% and a purge factor of > 500 fold was determined across the final three processing steps. This purge factor applied to a 0.1% specification in starting material Y would result in a predicted level of impurity B in the drug substance of less than 2 parts per million (ppm). As this is below the TTC-based limit of 50 ppm for this impurity in the drug substance, the 0.1% specification of impurity B

in starting material Y is justified without the need for providing drug substance batch data on pilot scale or commercial scale batches.

Case 3: Example of an Option 2 and 4 Control Strategy: Control of Structurally Similar Mutagenic Impurities

The Step 1 intermediate of a 5-step synthesis is a nitroaromatic compound that may contain low levels of impurity C, a positional isomer of the step 1 intermediate and also a nitroaromatic compound. The amount of impurity C in the step 1 intermediate has not been detected by ordinary analytical methods, but it may be present at lower levels. The step 1 intermediate is positive in the bacterial mutagenicity assay. The step 2 hydrogenation reaction results in a 99% conversion of the step 1 intermediate to the corresponding aromatic amine. This is confirmed via in-process testing. An assessment of purge of the remaining step 1 nitroaromatic intermediate was conducted, and a high purge factor was predicted based on purge points in the subsequent step 3 and 4 processing steps. Purge across the step 5 processing step is not expected and a specification for the step 1 intermediate at the TTC-based limit was established at the step 4 intermediate (Option 2 control approach). The positional isomer impurity C would be expected to purge via the same purge points as the step 1 intermediate and therefore will always be much lower than the step 1 intermediate itself; therefore, no testing is required and an Option 4 control strategy for impurity C can be supported without the need for any additional laboratory or pilot scale data.

Case 4: Example of an Option 4 Control Strategy: Highly Reactive Impurity

Thionyl chloride is a highly reactive compound that is mutagenic. This reagent is introduced in step 1 of a 5-step synthesis. At multiple points in the synthesis, significant amounts of water are used. Since thionyl chloride reacts instantaneously with water, there is no chance of any residual thionyl chloride to be present in the drug substance. An Option 4 control approach is suitable without the need for any laboratory or pilot scale data.

Implementation of Guidance:

Implementation of M7 is encouraged after publication; however, because of the complexity of the guidance, application of M7 is not expected prior to 18 months after ICH publication. The following exceptions to the 18-month timeline apply.

1. Ames tests should be conducted according to M7 upon ICH publication. However, Ames tests conducted prior to publication of M7 need not be repeated.

2. When development programs have started Phase 2b and Phase 3 clinical trials prior to publication of M7, these programs can be completed up to and including marketing application submission and approval, with the following exceptions to M7:
   - No need for two (Q)SAR assessments as outlined in Section VI (6).
   - No need to comply with the scope of product impurity assessment as outlined in Section V (5).
   - No need to comply with the documentation recommendations as outlined in Section IX (9).

3. Given the similar challenges for development of a commercial manufacturing process, application of the aspects of M7 listed above to new marketing applications that do not include Phase 2b and Phase 3 clinical trials would not be expected until 36 months after ICH publication of M7.

What's New with Impurities in Pharmaceuticals?

Southern California Pharmaceutical Discussion Group

January 15, 2015

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ICH Q3 Impurities

Q3A Drug Substances– 1995 (step 4), R1 (2002), R2 (2006)

Q3B Drug Products– 1996 (step 4), R1 (2003), R2 (2006)

Q3C Residual Solvents– 1997, R1-5 (2002, 2005, 2009, 2011)

- Most ICH guidelines on impurities in drug substances and drug products are >15 years old
- What else is there to say?

Filling the Gaps

- M7 – Genotoxic Impurities – Step 4 (June 2014)
  - changes from EMA and FDA guidance
- Q3D Elemental Impurities – Step 4 (Dec. 2014)
  - USP <232>, <233>
- Other gaps?
- Revisions needed?

ICH M7 – Genotoxic Impurities

Filling the ICH Q3 A/B gap for “impurities that are expected to be unusually potent, producing toxic or pharmacological effects at a level not more than (≤) the identification threshold.”

- Identification of "unusually potent" impurities not described
- No threshold of concern given

EMA* guideline and FDA** draft guidance:

Threshold of Toxicological Concern (TTC), 1.5 μg/day

*http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002903.pdf

**http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079235.pdf

Assessing Impurities – ICH M7

All impurities (actual and potential), where the structures are known, should be evaluated for mutagenic potential.

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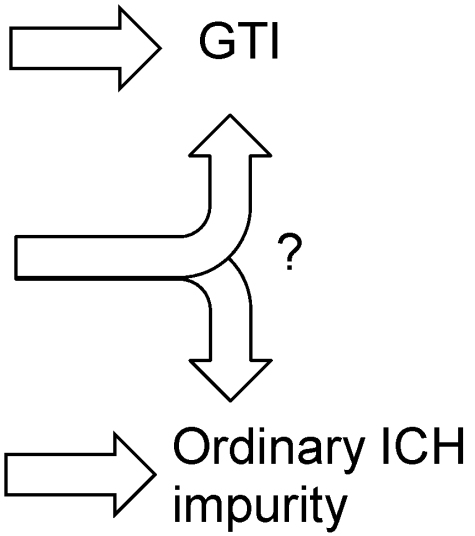
Classifying impurities – PhRMA paper recommendation*, adopted in M7

All **identified** or **predicted** impurities should be classified into one of five classes:

Class 1. Known to be genotoxic and carcinogenic
Class 2. Known to be genotoxic but with unknown carcinogenic potential

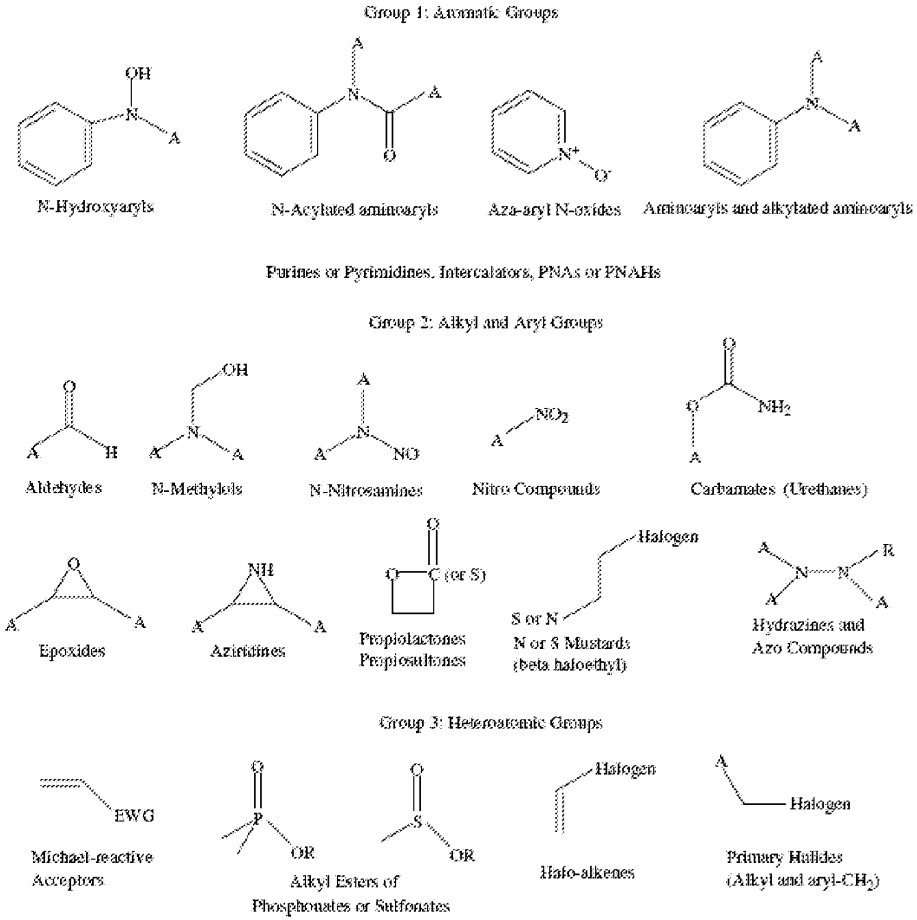
Class 3. With a unique alerting structure and of unknown genotoxic potential

Class 4. With an alerting structure related to the parent API
Class 5. With no structural alert



*Müller et al., *Reg. Tox. Pharmacol.* 44, 198-211 (2006);

Alerting Structures – examples



Müller et al.

Assessing Impurities – ICH M7, cont.

Is an impurity potentially genotoxic?

Search databases and literature for carcinogenicity and bacterial mutagenicity data in order to classify impurity as Class 1, 2, or 5

When data are not available:

Use Structure-Activity Relationships (SAR) that focus on bacterial mutagenicity predictions. This could lead to a classification into Class 3, 4, or 5.

(Q)SAR/*in silico* assessments

Two (Q)SAR prediction methodologies that complement each other should be applied.

- Expert rule-based
- Statistical-based
- follow Organisation for Economic Co-operation and Development (OECD) validation principles
- review with expert knowledge

Absence of structural alerts from 2 Q(SAR) predictions = normal impurity

Toxicity Prediction Software

Freely available software

Caesar models
Lazar
OncoLogic
PASS
EPI Suite
OECD QSAR Application Toolbox
Toxtree
T.E.S.T

Commercially available software

ACD/Tox Suite
BioEpisteme
HazardExpert
MDL QSAR
MultiCASE
TOPKAT
q-Tox
ADMET Predictor
DEREK
Leadscope
Molcode Toolbox
OASIS TIMES
ToxAlert
CSGenoTox

Review of Software Tools for Toxicity Prediction, M. F. Gatnik and A. Worth, European Commission Joint Research Centre, Institute for Health and Consumer Protection
https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/doc/EUR_24489_EN.pdf

Industry Survey: Dobo et al., Reg. Tox. Pharmacol. 62 (2012) 449–455

Ames testing

- Negative result classifies compound as normal ICH impurity and overrides a positive *in silico* prediction for genotoxicity
- Test on the isolated impurity preferred vs. impurity in drug substance; ≥250 µg/plate needed for compound of interest
- GLP studies expected but test article characterization may not comply fully; exceptions also allowed for compounds difficult to prepare or isolate

ICH M7 - TTC-based acceptable limits

Acceptable Daily Intakes* for an Individual Impurity, μg/day Clinical trials or marketed product								
	Single Dose	< 14 days	≤ 1 mo.	≤ 3 mo.	≤ 6 mo.	≤ 12 mo.	>1 – 10 years	>10 years to lifetime
M7	**	**	120	20	20	20	10	1.5
EMA	120	60	60	30	10	5	1.5 (marketed)	1.5

*Compound-specific risk assessments to derive acceptable intakes should be applied instead of the TTC-based acceptable intakes where sufficient carcinogenicity data exist.

**Clinical trials of up to 14 days – class 3 impurities can be treated as normal impurities

Mitigating factors in application of TTC

- Indication – life-saving therapy
- Exposure from other sources, e.g., foods or endogenous metabolism (e.g., formaldehyde)
- Reduced life expectancy
- Late onset but chronic disease
- Limited therapeutic alternatives

Strategies to Address GTIs

- Modify synthesis to remove compounds of concern or move them as early in the synthesis as possible
- Purification – provide rationale and/or data to demonstrate that GTI has negligible risk of being in drug substance
- Specification – commit to analytical testing and acceptance limit at intermediate (higher levels?) or drug substance (staged TTC levels)
- Degradation product GTI – packaging and storage to prevent formation, implement specification through shelf-life

ICH M7 Control Options for Process Impurities

Option 1
Specification for impurity in drug substance with acceptable limit.

Option 2
Specification at precursor with drug substance acceptable limit.

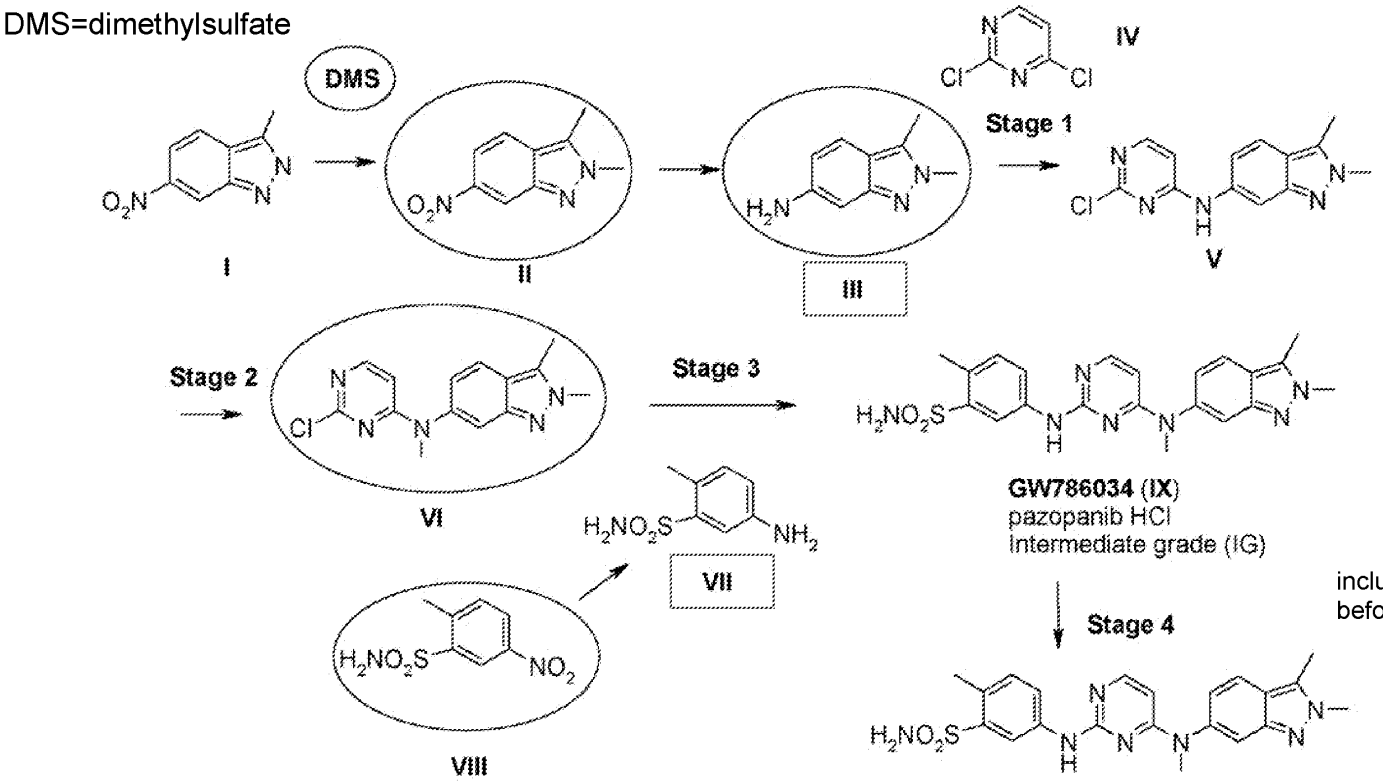
Option 3
Specification at precursor with higher limit. Fate and purge data and associated process controls needed that assure the level in the drug substance is below the acceptable limit.

Option 4
No routine testing. Compelling fate and purge knowledge with sufficient confidence that the level of the impurity in the drug substance will be below the acceptable limit.

Probability of regulatory acceptance

Example – GSK, pazopanib HCl

DMS=dimethylsulfate



III and VII designated as API starting materials

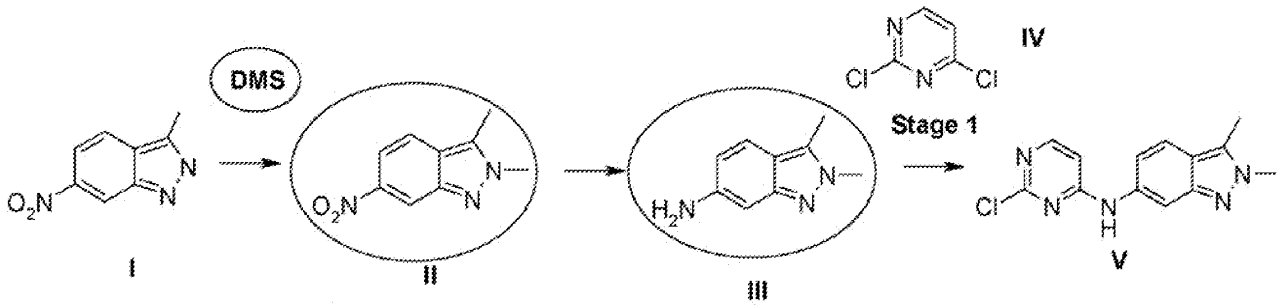
D.Q. Liu, T.K. Chen, M.A. McGuire, A.S. Kord, J. Pharm. Biomed. Anal. 50 (2009) 144-150

Strategy

- Develop TTC-level LC-MS method for GTIs
- Analyze materials throughout process to show lack of carry-through (up to 79 batches of API)
- Perform impurity rejection studies to show process capability
- Establish tests for GTIs at levels higher than TTC using LC methods at starting materials or intermediates

Note: Pazopanib HCl (Votrient) is a tyrosine kinase inhibitor approved for the treatment of renal cell carcinoma

Impurity rejection efficiency - DMS



DMS introduced 2 steps back from starting material III
20 batches of III showed <1.7 ppm DMS
79 batches of API showed <1.7 ppm DMS

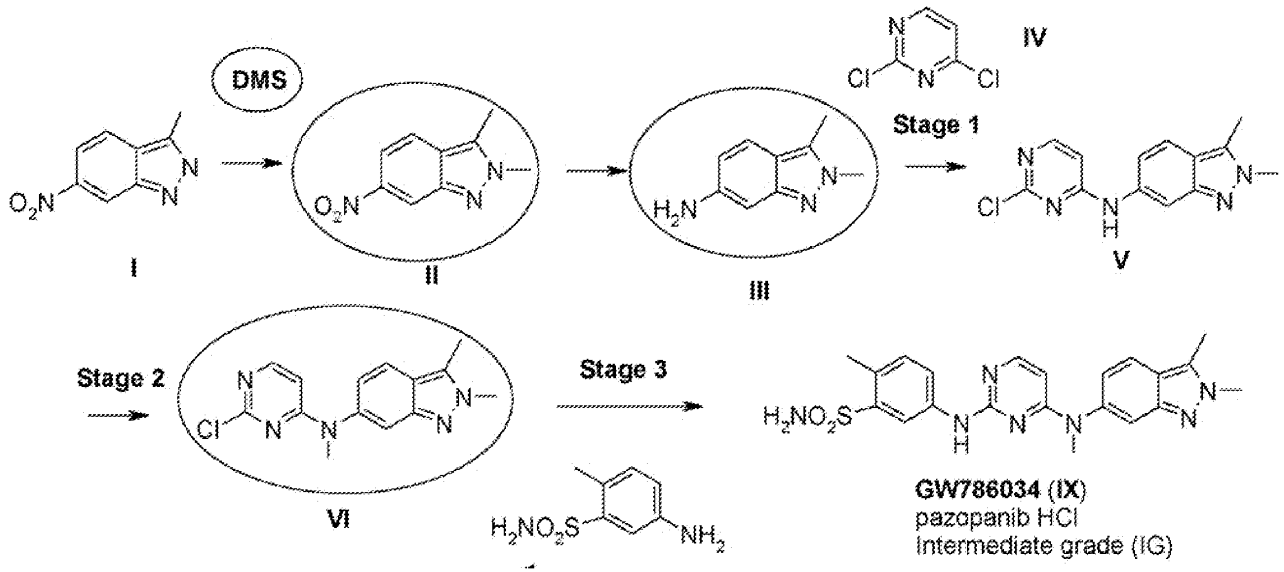
50,000 ppm DMS spiked in III ⟶ <1.7 ppm in V

>29,000 fold rejection in step 1 followed by 4 subsequent steps

0.1% acceptance criterion set for DMS in starting material III

IS ROUTINE TESTING FOR DMS NEEDED?

Impurity rejection efficiency – compound II

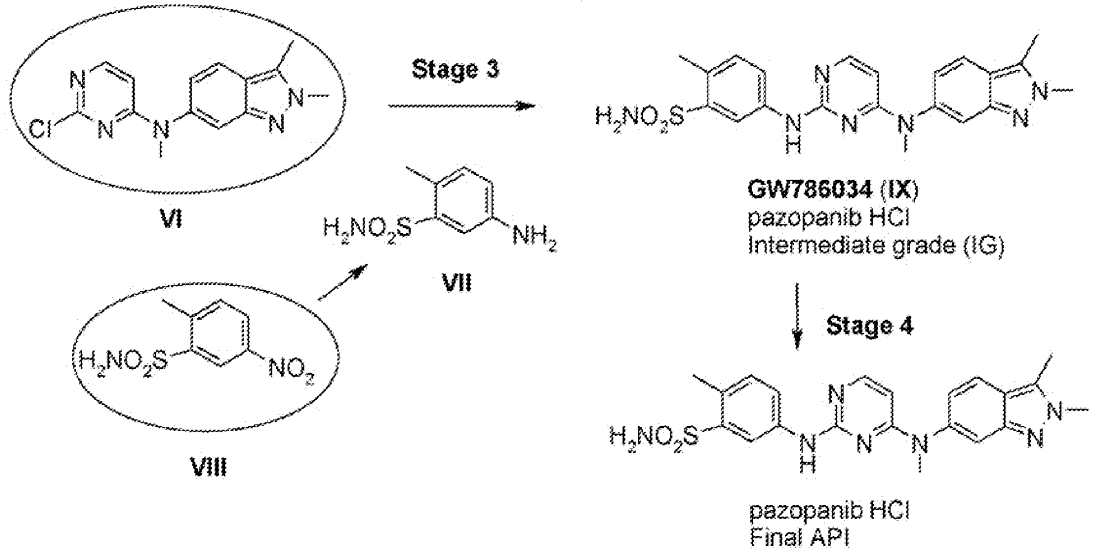


79 batches of API showed <1.7 ppm II
16 batches of III showed <24 ppm II

50,000 ppm in III	⟶	670 ppm in V	75 fold rejection
670 ppm in V	⟶	23 ppm in VI	29 fold rejection
23 ppm in VI	⟶	<1.7 ppm in IX	>13 fold rejection

0.1% limit set for II in III

Higher Upstream limits for VI and VIII



limit of NMT 0.1% set for VIII in VII

limit of NMT 0.6% set for VI in IX

Genotoxic degradation products

- Identify potential degradation product GTIs during predictive stress testing
- Does degradation product GTI form with time under normal storage conditions?
- Can degradation product GTI formation be prevented through formulation design, packaging, or storage conditions?
- Establish specification if necessary

Risk of Producing an Alerting Structure from Drug Degradation*

- Analysis of over 1100 known degradation products from more than 350 drugs suggests that degradation of drugs may lead to unique structure alerting functional groups in about 5-8% of the degradation products.
- Roughly 50% or less of these alerting structures can be expected to be Ames positive
- An average of 8-9 major deg products are observed in stress testing for a typical drug, so most drugs will have zero or one deg product for follow-up as a potential GTI

*S.W. Baertschi et al., Stress Testing and Degradation-Derived Genotoxic Impurities: Scientific, Practical and Regulatory Considerations, Conference on Small Molecule Science, August 2, 2011, Chapel Hill, NC

ICH M7- Control for degradants

Is degradant relevant?

- check accelerated stability study data (e.g., 40°C/75% relative humidity, 6 months)
- kinetically equivalent shorter term stability studies at higher temperatures in the proposed commercial package may be used to determine the relevance of the degradation pathway prior to initiating longer term stability studies.

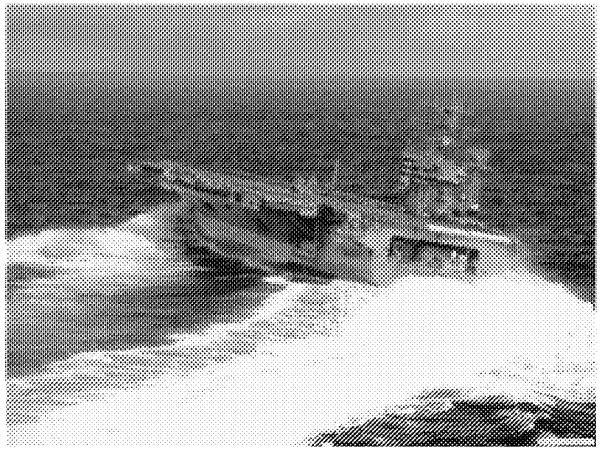
S.W. Baertschi et al., "Stress testing as a predictive tool for the assessment of potential genotoxic degradants", in Pharmaceutical Stress Testing, 2nd Ed., S.W. Baertschi, K.M. Alsante, R. A. Reed, 2011, Informa, London.

ICH M7 – Other Considerations

- Not applied to products for advanced cancer indications (see ICH S9)
- Not applied to drug substances that are themselves genotoxic
- Will be applied to changes in existing authorizations if new or greater levels of previous impurities are present
- Assess potentially genotoxic impurities which may be present at levels below the Q3 A/B ID thresholds (same as current guidelines)
- Previous data from similar compounds may be used with justification to discharge risk

Elemental Impurities

USP <232> Limits
USP <233> Procedures
replacing <231> Heavy Metals



ICH Q3D

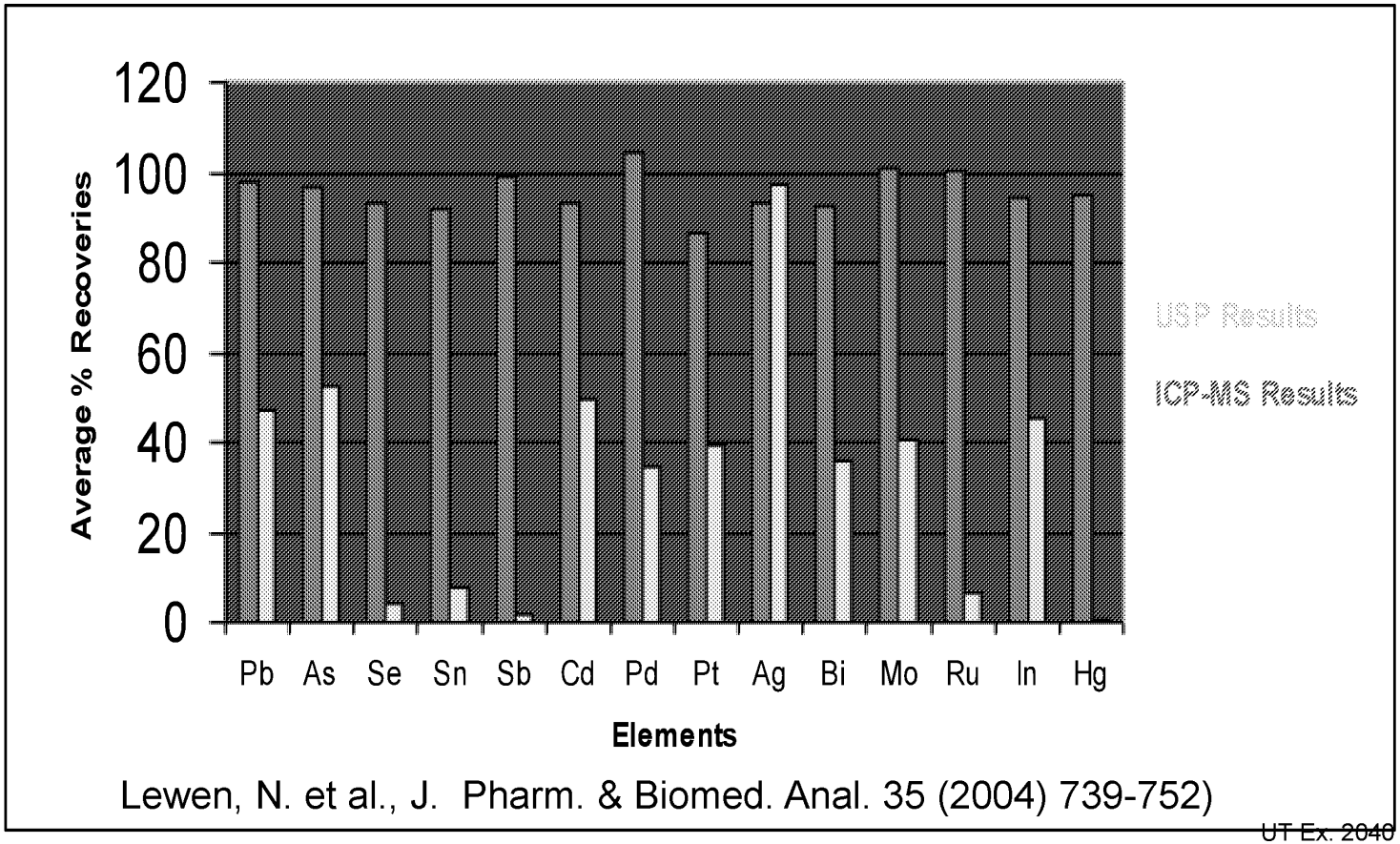
Disclaimer

Information in this presentation related to USP Elemental Impurities is from publically-available sources.

The speaker does not represent official USP positions or policy on Elemental Impurities or any other topic.

USP <231> Heavy Metals

<231> has been in use for many years. What's the problem?



Lewen, N. et al., J. Pharm. & Biomed. Anal. 35 (2004) 739-752)

USP key issue – elemental impurities

<232> Elemental Impurities – Limits

<233> Elemental Impurities – Procedures

Revisions proposed in PF 40(2)

http://www.usp.org/usp-nf/key-issues/elemental-impurities

Implementation date when chapters apply to drug product monographs: ~~December 1, 2015~~

BUT, what about ICH Q3D?

- Elemental Impurities Key Issues Page Updated (14–Jan–2015)

January 14, 2015: USP is announcing plans to establish *January 1, 2018* as the new date of applicability of General Chapters <232> Elemental Impurities—Limits and <2232> Elemental Contaminants in Dietary Supplements.

Heavy metals limits – USP proposal

Table 1. Elemental Impurities for Drug Products

Element	Oral Daily Dose PDE[a] (µg/day)	Parenteral Daily Dose PDE (µg/day)	Inhalational Daily Dose PDE (µg/day)	LVP Component Limit (µg/g)
Cadmium	~~25~~ 5.0 1S (*USP38*)	2.5	~~4.5~~ 3.4 1S (*USP38*)	0.25
Lead	~~5~~ 5.0 1S (*USP38*)	~~5~~ 5.0 1S (*USP38*)	~~5~~ 5.0 1S (*USP38*)	0.5
Inorganic arsenic[b]	~~4.5~~ 15 1S (*USP38*)	~~4.5~~ 15 1S (*USP38*)	~~4.5~~ 1.9 1S (*USP38*)	~~0.15~~ 1.5 1S (*USP38*)
Inorganic mercury[b]	15	1.5	~~4.5~~ 1.2 1S (*USP38*)	0.15
Iridium	100	10	1.5	1.0
Osmium	100	10	1.5	1.0
Palladium	100	10	~~4.5~~ 1.0 1S (*USP38*)	1.0
Platinum	100	10	1.5	1.0
Rhodium	100	10	1.5	1.0
Ruthenium	100	10	1.5	1.0

PDE = permitted daily exposure

Limits, cont.

Chromium	—[c]	—[c]	~~25~~ 2.9 1S (*USP38*)	—[c]
Molybdenum	~~100~~ 180 1S (*USP38*)	~~10~~ 90 1S (*USP38*)	~~10~~ 7.6 1S (*USP38*)	~~1.0~~ 9.0 1S (*USP38*)
Nickel	~~500~~ 600 1S (*USP38*)	~~50~~ 60 1S (*USP38*)	~~1.5~~ 6.0 1S (*USP38*)	~~5.0~~ 6.0 1S (*USP38*)
Vanadium	~~100~~ 120 1S (*USP38*)	~~10~~ 12 1S (*USP38*)	~~20~~ 1.2 1S (*USP38*)	~~1.0~~ 1.2 1S (*USP38*)
Copper	~~1000~~ 1300 1S (*USP38*)	~~100~~ 130 1S (*USP38*)	~~100~~ 13 1S (*USP38*)	~~10~~ 13 1S (*USP38*)

Options for compliance

Drug product analysis (Q3D option 3)

Daily Dose PDE ≥ measured value (µg/g) × maximum daily dose (g/day)

Summation option (add metals present in each component, Q3D option 2b)

Daily Dose PDE $\geq [\Sigma^{M}_{1}(CM \times WM)] \times DD$

M = each ingredient used to manufacture a dosage unit
CM = element concentration in component (drug substance or excipient) (µg/g)
WM = weight of component in a dosage unit (g/dosage unit)
DD = number of units in the maximum daily dose (unit/day)

Individual component option (Large volume parenterals only)

API and excipients meet limits given in Table 1 for LVP components

Testing

If, by validated processes and supply-chain control, manufacturers can demonstrate the absence of impurities, then further testing is not needed.

USP Updates – stay tuned

October 2014 expert panel recommendation: ***Limits be revised to align with the ICH Q3D Step 4 document to the extent possible.***

Separately, ***USP is considering potential adjustments to the elemental impurities implementation timeline*** as specified in General Notices 5.60.30 (Dec.1, 2015) based on developments related to the anticipated ICH Q3D Step 4 document.

http://www.usp.org/usp-nf/key-issues/elemental-impurities

ICH Q3D – Guideline for Elemental Impurities

- Focus is on risk assessment for occurrence of and limits for elemental impurities
- Step 4 guideline published December 16, 2014

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_Step_4.pdf

ICH Q3D: Risk-based assessment of need for control of metal impurities

Table 5.1: Elements to be Considered in the Risk Assessment

Element	Class	If intentionally added (all routes)	If not intentionally added		
			Oral	Parenteral	Inhalation
Cd	1	yes	yes	yes	yes
Pb	1	yes	yes	yes	yes
As	1	yes	yes	yes	yes
Hg	1	yes	yes	yes	yes
Co	2A	yes	yes	yes	yes
V	2A	yes	yes	yes	yes
Ni	2A	yes	yes	yes	yes
Tl	2B	yes	no	no	no
Au	2B	yes	no	no	no
Pd	2B	yes	no	no	no
Ir	2B	yes	no	no	no
Os	2B	yes	no	no	no
Rh	2B	yes	no	no	no
Ru	2B	yes	no	no	no
Se	2B	yes	no	no	no
Ag	2B	yes	no	no	no
Pt	2B	yes	no	no	no
Li	3	yes	no	yes	yes
Sb	3	yes	no	yes	yes
Ba	3	yes	no	no	yes
Mo	3	yes	no	no	yes
Cu	3	yes	no	yes	yes
Sn	3	yes	no	no	yes
Cr	3	yes	no	no	yes

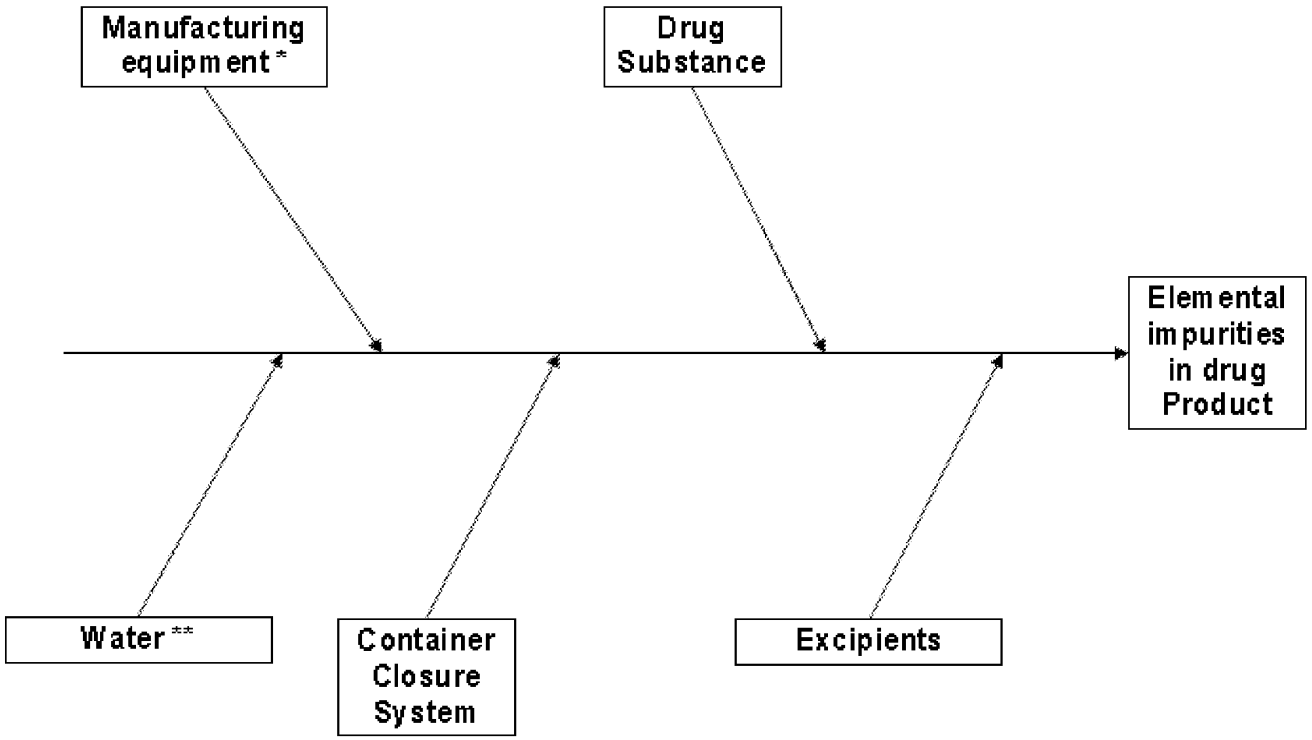
ICH Q3D – Risk assessment

Identify: Identify known and potential sources of elemental impurities that may find their way into the drug product.

Evaluate: Evaluate the presence of a particular elemental impurity in the drug product by determining the observed or predicted level of the impurity and comparing with the established PDE.

Control: Summarize and document the risk assessment. Identify if controls built into the process are sufficient or identify additional controls to be considered to limit elemental impurities in the drug product.

Sources of Elemental Impurities



ICH Q3D: Limits for Elemental Impurities

Table A.2.1: Permitted Daily Exposures for Elemental Impurities[1]

Element	Class[2]	Oral PDE µg/day		Parenteral PDE, µg/day		Inhalation PDE, µg/day	
Cd	1	5	5.0	2	2.5	2	3.4
Pb	1	5	5.0	5	5.0	5	5.0
As	1	15	15	15	15	2	1.9
Hg	1	30	15	3	1.5	1	1.2
Co	2A	50		5		3	
V	2A	100	120	10	12	1	1.2
Ni	2A	200	600	20	60	5	6.0
Tl	2B	8		8		8	
Au	2B	100		100		1	
Pd	2B	100	100	10	10	1	1.0
Ir	2B	100	100	10	10	1	1.5
Os	2B	100	100	10	10	1	1.5
Rh	2B	100	100	10	10	1	1.5
Ru	2B	100	100	10	10	1	1.5
Se	2B	150		80		130	
Ag	2B	150		10		7	
Pt	2B	100	100	10	10	1	1.5
Li	3	550		250		25	
Sb	3	1200		90		20	
Ba	3	1400		700		300	
Mo	3	3000	180	1500	90	10	7.6
Cu	3	3000	1300	300	130	30	13
Sn	3	6000		600		60	
Cr	3	11000		1100		3	2.9

Red = USP <232> PDEs

Option 1: Assume 10 g/day dose. If all components meet PDE concentration, they may be used in any proportion

Option 2a: Use the actual dose to calculate PDE concentration. If all components meet the PDE, they may be used in any proportion.

Option 2b: Use the amounts of each component present and data on metals present to set limits for individual components.

Option 3: Drug product analysis with limits based on daily dose

Q3D – Other Limit Considerations

- When PDEs are necessary for other routes of administration, the concepts described in the guideline may be used to derive PDEs.
  - Consider local effects, bioavailability, quality considerations

- Higher PDEs may be permitted for:

  - Intermittent dosing;
  - Short term dosing (i.e., 30 days or less);
  - Specific indications (e.g., life-threatening, unmet medical needs, rare diseases).

ICH Q3D Implementation

- Application of Q3D to existing products is not expected prior to 36 months after publication of the guideline by ICH. December 2017?
- Will USP implementation timing be revised to December 2017? – January 1, 2018
- Q3D implementation plan 21 Oct 2014
  - Training materials
  - FAQ document

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_IWG_Final_Concept_Paper_October_21_2014.pdf

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Q3D_IWG_Final_Business_Plan_October_21_2014.pdf

USP <233> Method Proposal

Procedure 1 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP–AES or ICP–OES).

Procedure 2 can be used for elemental impurities generally amenable to detection by inductively coupled plasma–mass spectrometry (ICP–MS).

Performance-based method

Analysts are free to select a method/procedure that works for their samples

The method selected may include plasma spectrochemistry, atomic absorption spectroscopy, OR ANY OTHER METHOD that displays adequate accuracy, sensitivity and specificity.

Other ICH Impurity Questions

- Other gaps?
- Thresholds and limits
- Impurity control in
  - multisource products
  - starting materials
  - reagents
- Depth of impurity investigations

Products not covered by ICH

Excluded product types:

- biological/biotechnological (ICH Q6B)
- peptide (PhEur general monograph 2034)
- oligonucleotide
- radiopharmaceutical
- fermentation product and semi-synthetic products (EMA guideline for antibiotics)
- herbal products
- crude products of animal or plant origin

Extractable and Leachable Impurities

Multitude of guidelines, but lack of harmonized expectations, focus has been on inhaled and nasal products

- Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products; FDA Draft Guidance for Industry
- Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products – Chemistry, Manufacturing, and Controls Documentation; FDA Guidance for Industry
- CHMP Guideline on the Pharmaceutical Quality of Inhalation and Nasal Products
- PQRI: Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products

Threshold limit disconnects

Drug substance process impurity:

- 0.2% in a drug substance with 0.5 mg maximum daily dose requires identification and qualification
- Patient exposure - Total daily intake (TDI) of impurity: 1 μg
- TDI is less than 1.5 μg/day limit for a potentially genotoxic impurity

Residual solvent:

- A drug substance with benzene at 2 ppm meets Q3C(R5) limit, but for a dose of 2 g/day, TDI is 4 μg
- A drug substance with benzene present at 5 ppm exceeds limit, but for a dose of 2 mg/day, TDI is 0.01 μg

D.J. Snodin, S.D. McCrossen, "Guidelines and pharmacopoeial standards for pharmaceutical impurities: Overview and critical assessment", Reg. Tox. Pharmacol. 63 (2012) 298–312

Degradation impurities threshold concerns

Degradation impurity in drug substance

Dose:	1 mg	10 mg	100 mg
ID threshold (TDI)	1 μg	10 μg	100 μg
Qual threshold (TDI)	1.5 μg	15 μg	150 μg

Degradation impurity in drug product

Dose:	1 mg	10 mg	100 mg
ID threshold (TDI)	5 μg	20 μg	200 μg
Qual threshold (TDI)	10 μg	50 μg	200 μg

Should thresholds for degradation impurities in a drug substance be consistent with those for the drug product?

Other Threshold Considerations

Should more latitude in application of Q3 thresholds be allowed based on:

-Chronic vs. limited-duration therapy
-Indication
-Population

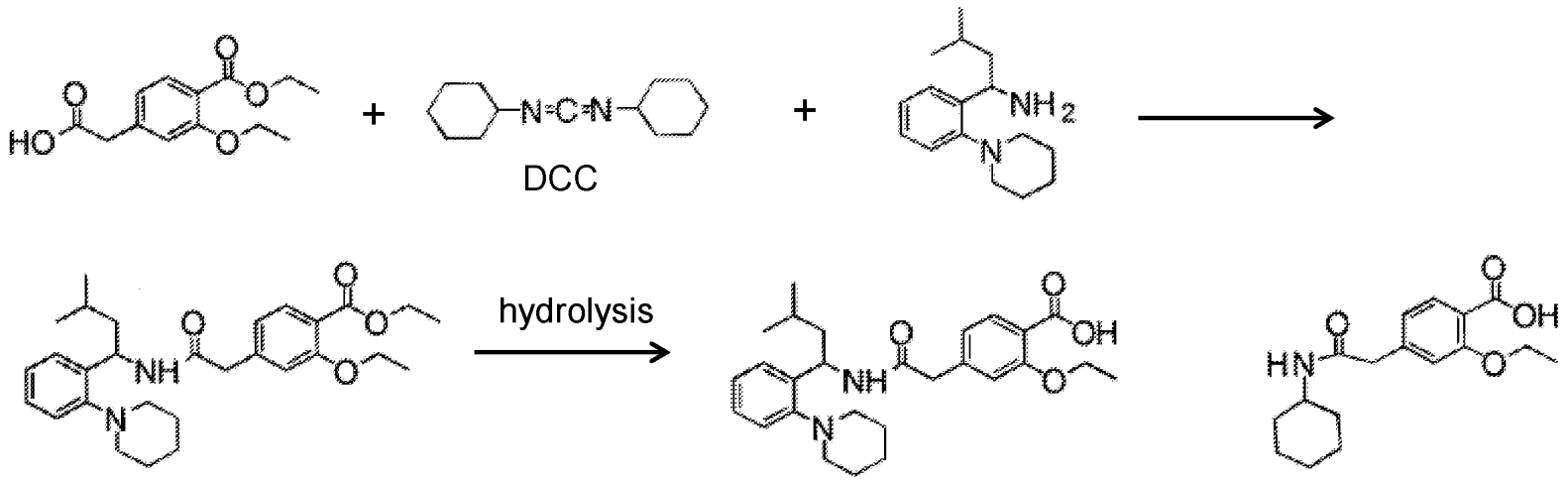
Should *in silico*, literature, or other structure-based rationale be acceptable for impurity qualification in lieu of additional animal studies?

Risk assessment for impurities in multi-sourced materials (starting materials, reagents)

- What method(s) are used to prepare the material?
- What impurities could be introduced with the material?
- What is the likelihood QC methods will detect new impurities?
- Does supplier's change control for manufacturing changes evaluate the potential for new impurities?
- Does buyer's change control evaluate potential for new impurities from a different supplier?

Example: Impurity from reagent

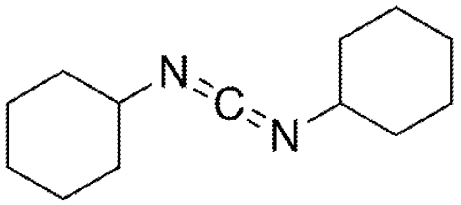
Repaglinide synthesis



Impurity derived from cyclohexylamine in DCC

K.V.S.R. Krishna Reddy et al., J. Pharm. Biomed.Anal. 32 (2003) 461-467

DCC Quality?



74 suppliers (52 from China)

Quality range: 98.0-99.5%
Unknown control of cyclohexylamine impurity

Switching suppliers without knowledge of the impact of cyclohexylamine as an impurity could generate a new impurity in the drug substance

Do suppliers control cyclohexylamine levels?
What levels are acceptable for regalinide process?
Would the QC impurities test for repaglinide detect the cyclohexylamine-derived impurity?

Ambiguity in Impurity Investigation
How much is enough?

Q3A(R2)

…summarise the *actual and potential impurities most likely to arise* during the synthesis, purification, and storage of the new drug substance.

This discussion can be limited to those impurities that *might reasonably be expected* based on knowledge of the chemical reactions and conditions involved.

Potential Impurity: An impurity that *theoretically* can arise during manufacture or storage. It may or may not actually appear in the new drug substance.

??????

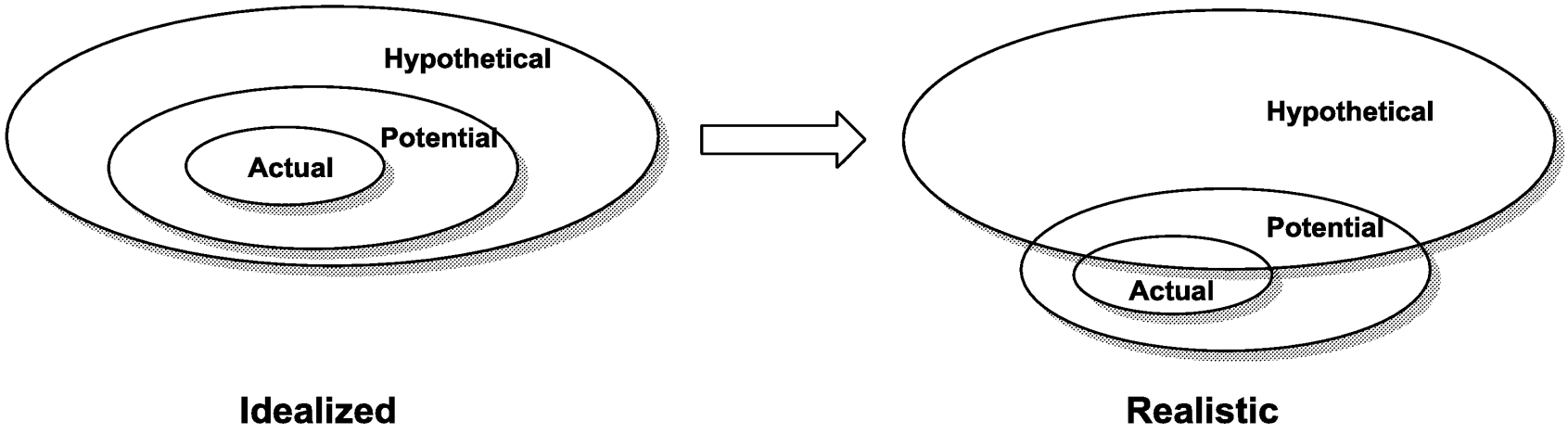
How are “most likely to arise” and “might reasonably be expected” interpreted?

“Theoretical” impurities can encompass an unreasonably large number of compounds, especially if genotoxicity thresholds are considered.

In Practice…

Focus investigation on potential, not hypothetical/theoretical impurities

- Theoretical/hypothetical: based on *in cerebro* or *in silico* predictions of synthetic by-products or degradation chemistry
- Potential: used in process, found during process development ,or formed as major degradation products during stress testing
- Actual or relevant: impurities present in drug or with a high likelihood of being present



*S. Baertschi et al., Conference on Small Molecule Science UNC-Chapel Hill, NC, August 2, 2011

Summary

Despite ongoing questions, ICH impurity guidelines have provided harmonized expectations for drug development.

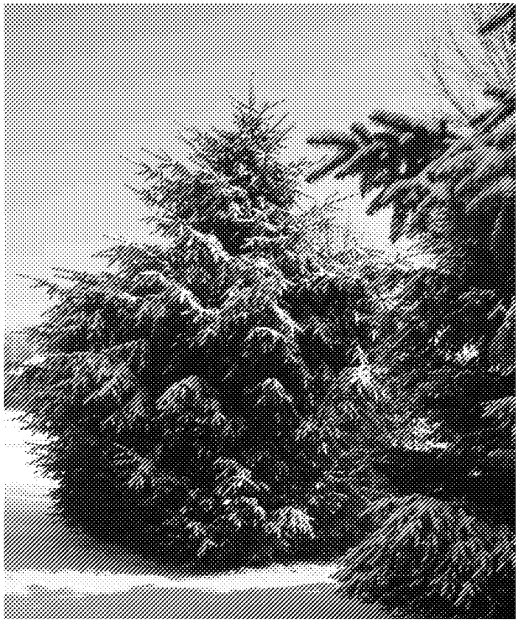
Changes being effected:

- Genotoxic impurities
- Elemental impurities

Opportunities for improvement:

- guidance for other product types
- greater flexibility when justified
- reexamination of threshold rationale and inconsistency

Thank You!



Biotech

Janet Woodcock - FDA

by *Damian Garde* |

The gatekeeper of biopharma's biggest market

Name: Janet Woodcock
Title: Director of the FDA's Center for Drug Evaluation and Research

FDA meetings have made for intriguing theater over the past year. Advocates chastise the agency for its perceived gender bias. Pharma execs squabble about safety data that happen to defend their multi-billion-dollar products. Parents of children with rare diseases read tearful entreaties for the agency to approve new drugs with debatable supporting evidence.

Each, wittingly or otherwise, is trying to get through to Janet Woodcock, a 20-plus-year FDA veteran who runs the agency's drug-approval arm and has the power to alter the course of the industry.

Woodcock is director of the FDA's Center for Drug Evaluation and Research, a division tasked with vetting new drug applications. Under her leadership, the agency is approving more and more new drugs each year--45 last year and 41 in 2014--all the while facing mounting criticism from critics who say the FDA is too close to the business it regulates, industry insiders who claim the process is still too slow, and patient advocates who argue the agency needs to rethink its approach to rare diseases.

Case in point: Duchenne muscular dystrophy.

Two companies, BioMarin Pharmaceuticals ($BMRN) and Sarepta Therapeutics ($SRPT), are petitioning the agency to approve treatments that could help about 13% of boys with the deadly, muscle-wasting disease. Each is armed with data from small studies in which their candidate drugs charted only intermittent efficacy, below the standard usually required to win FDA approval. There are no approved treatments for the disease, and parents of DMD patients are clamoring for anything that might improve and extend the lives of their children, even if it's a long shot.

The question facing Woodcock becomes: Should the FDA bend its standards in response to unmet need, or would relenting open up a loophole that biopharma companies could later exploit to the detriment of patients?

Woodcock always stays on-message in her public comments, sticking to the point that the agency makes its decisions based solely on safety and efficacy data. But the FDA, under her watch, has been increasingly flexible in the approval process.

CDER's cancer division, led by Richard Pazdur, has repeatedly approved new cancer medications with shallow efficacy records, clearing them to treat only the most desperate patients until companies come back with enough data to justify broader use. And the agency seemed to cave to public pressure last year when it approved Addyi, a twice-rejected female libido treatment whose scant effectiveness didn't outweigh its side effects in the eyes of many critics.

Each case boils down to the same fundamental issue: What makes a drug approvable? Woodcock, as gatekeeper of the world's biggest drug market, plays a sizable role answering that question, giving her the power to shift the dynamics of biopharma.

-- Damian Garde (email | Twitter)

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The Political Economy Of FDA Drug Review: Processing, Politics, And Lessons For Policy

Patients, more than pharmaceutical firms, shape the political costs to the FDA of delaying drug approval.

by Daniel P. Carpenter

ABSTRACT: U.S. Food and Drug Administration (FDA) drug review bears a structural similarity to many decisions made by other regulatory agencies: high uncertainty, low reversibility, avoidance of observable error, and high political stakes that induce lobbying by interested parties. This paper explores the policy lessons to be learned from viewing FDA drug review as a politically shaped exercise in information processing. I argue that the incentives facing regulators induce limits on the degree to which drug review can be accelerated, that the same incentives could render privatization initiatives problematic, and that political pressures could play a useful role in identifying priority drugs.

CONSIDER TWO HYPOTHETICAL CONSUMERS, one a pharmaceutical consumer ("patient") who wishes to try a new drug for some ailment, the other a vitamin consumer who wishes to take zinc supplements to ward off a cold or flu. There are few institutional restrictions upon the consumption decisions of the vitamin consumer, at least in the United States. She is free to purchase vitamin products over the counter, and the vitamin manufacturer is free to sell them without prior authorization or licensing.

Not so with pharmaceuticals. The marketplace for pharmaceuticals is one of the most highly regulated industries in the U.S. economy.[1] To use any new pharmaceutical product, the patient must secure the approval of two agents: a licensed physician and the U.S. Food and Drug Administration (FDA).[2]

No discussion of the past, present, or future of the pharmaceutical industry can ignore the critical role played by the FDA in its evolution. The agency's drug review decisions are essentially final (contesting them is extremely difficult and costly) and immensely consequential (regulators in other nations frequently cue off of the FDA's decisions). If the FDA so chooses, it can materially impede the flow of new products to the pharmaceutical marketplace, or it can help accelerate that

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"FDA officials want good press but also to preserve a reputation for scientific rigor among academics and medical professionals."

flow. Just as important, major changes are now occurring at the FDA—including the merger of its drugs and biologics review divisions—that will shape the future of the marketplace.

In this essay I use some lessons from political science to illuminate the way the FDA operates. My central claim is that FDA drug review is an exercise in learning shaped by organized interests. Both the learning and the politics have some peculiar features. The learning—more specifically, "optimal stopping" (deciding not just if but when to terminate drug review and approve an application)—is driven by the FDA's desire to safeguard its reputation for protecting the public's health.[3] The politics involves the mobilization of drug-specific lobbies—mainly the firm submitting the drug and the patients for whom the drug is intended.

The learning incentive and the politics incentive combine to form a trade-off. The FDA will highly prize new information on a drug and will often delay approval to obtain that information. In most cases, however, there is a political cost to delay, and in recent years that cost has been shaped even more by patients and their lobbies than by pharmaceutical firms.

FDA officials seem keenly aware of these trade-offs and of the political difficulties that rejection of a new drug application (NDA) can cause, especially when few or no existing therapies exist for a given disease. In 1991 Paul Leber (then director of the FDA's Division of Neuropharmacological Drug Products) read the initial drug application for Burroughs-Wellcome's Lamictal (lamotrigine) for partial epileptic seizures. He found the NDA to be "disorganized" and poorly keyed to protocols for clinical trials. He considered a "refuse-to-file" (RTF) action, a major embarrassment for the pharmaceutical firm in which the FDA returns the NDA without reviewing it. Yet Leber decided against an RTF, reasoning that since "no new anti-epileptic product had been marketed over the previous 12 years...a refuse to file action, although justified, could have untoward political consequences."[4]

In this essay I consider the lessons and policy implications of this trade-off. My argument and evidence here are both taken from a larger project on the evolution of pharmaceutical regulation at the FDA over the past half-century. This project entails a massive empirical enterprise, including collection of data on more than 17,000 pharmaceutical products (and more than 2,000 new chemical entities, or NCEs) developed over the past fifty years; more than 250 companies that have submitted NDAs; the epidemiology of more than 250 medical conditions ("primary indications") for which NDAs have been submitted; more than 3,000 disease and patient advocacy groups representing medical conditions for which NDAs have been submitted; and more than 4,000 personnel who have served in the FDA from 1980 to the present.

Protecting Reputation

I posit that the FDA behaves in ways that enhance its reputation for protecting consumer safety and public health. As gauged by public opinion polls, the FDA remains one of the most popular agencies in government, regularly securing 70 percent or greater "approval" of its performance among sampled respondents.[5] Buttressing this popularity are powerful symbolic lessons of history: The FDA is widely credited with saving thousands of American lives in its response to the sulfanilamide tragedy of 1937 and the thalidomide scandal of 1959–1961.[6] Congress dramatically strengthened pharmaceutical regulations after each of these events. In short, whether or not the agency deserves it, the FDA clearly possesses a reputation for protecting public health and consumer safety.

This reputation did not arise by accident but is the result of refined bureaucratic strategies. FDA officials have labored for years to craft and guard their organizational legitimacy. They have done so through frequent contact with their various constituents: attending professional meetings; giving lectures to ensure that the FDA's perspective on a given issue is heard; and cultivating the advice of academic and medical scientists, particularly on FDA advisory committees.[7]

Bureaucratic reputations often have multiple audiences. The FDA's reputation is no exception; it has scientific, political, and popular facets. FDA officials want good press (or no bad press) in the news media but also want to preserve a reputation for scientific rigor among academics and medical professionals. As Alison Lawton, a long-time FDA observer, noted recently, "The FDA is very responsive to what I would call 'opinion leaders' in the scientific and medical communities. It cares very much about what these people think as to how the agency is doing."[8]

Another critical audience lies in Congress, particularly in the committees that oversee the FDA. For much of the FDA's history, these bodies have launched criticisms at the agency for approving drugs too frequently and too quickly, and the FDA appears to sense these criticisms acutely. Commissioner Alexander Schmidt offered some testimony to this effect in 1974:

> In all of the FDA's history, I am unable to find a single instance where a Congressional committee investigated the failure of FDA to approve a new drug. But, the times when hearings have been held to criticize our approval of new drugs have been so frequent that we aren't able to count them... The message to FDA staff could not be clearer. Whenever a controversy over a new drug is resolved by its approval, the Agency and the individuals involved likely will be investigated. Whenever such a drug is disapproved, no inquiry will be made.[9]

Contrary to the claims of some analysts, there is nothing inherently "selfish" or "inefficient" about reputation protection.[10] Reputation is simply a currency of bureaucratic politics. Agencies with strong reputations can more easily attract desired personnel; fend off budget cuts; and lobby for the programs, funds, and other things they desire. There are other things that bureaucracies protect and "maximize," but for many agencies such as the FDA, reputation protection serves as the simplest and most powerful dynamic governing their behavior.[11]

Drug Review As A Stopping Problem

In reviewing NDAs, the FDA must choose not only whether to approve, but when to approve. Every time the FDA reviews a new drug, it "invests" (takes a chance with) its reputation. There are three critical aspects of this decision.

■ **Inherent uncertainty.** FDA officials know that even the most successful clinical trials cannot eliminate the possibility that a drug will turn out to be unsafe or inefficacious. Consider, for example, the 1996 review of SmithKline Beecham's Requip (ropinerole) for idiopathic Parkinson's disease. In his summary memorandum, FDA official Paul Leber discussed Requip's safety data and added an important cautionary note:

> Because no pharmacologically active drug substance is entirely free of risk, the conclusion that a drug has been shown to be "safe for use," is actually no more than an opinion... Accordingly, risk to benefit assessments are inherently arguable, all the more so because each turns not only on personal sentiments about the nature of risks and benefits of a drug, but upon incomplete and imperfect information concerning the drug's risks.[12]

Similarly, in 1997 FDA official Rudolph Widmark summarized safety data from Wyeth's Duract (bromfenac sodium) for postoperative pain relief and cautioned:

> In our safety review of NDA study we usually do not get definitive answers based on unequivocal data but are forced to interpret "flagging" events. We think that in the case of bromfenac, we have seen a "liver flag" that can be only fully explored through responsible marketing of the drug.[13]

Some uncertainty will always remain in drug review, and the marginal benefit of more trials and more delay tends to decline as the drug review gets longer.

■ **Asymmetric observability of error.** In the language of decision theory, a "Type I error" occurs when a decisionmaker accepts as true a hypothesis that is in fact false. A "Type II error" occurs when a decisionmaker rejects a hypothesis that is in fact true. The FDA, then, may be said to commit a Type I error when it approves a "bad" drug and a Type II error when it fails to approve a drug that should have been approved. For most of the FDA's history, Type I errors have been more visible than Type II errors.[14] As the remarks from former Commissioner Schmidt illustrate, the FDA has often been excoriated for approving a bad drug (or approving it too quickly) and only recently has been criticized for approving drugs too slowly.

■ **Low (reputational) reversibility.** Finally, the damage of a faulty approval decision is difficult to undo. Of course, the FDA can always secure a recall of a faulty product or compel the manufacturer to attach a "black box warning." Yet these steps will only publicize the error that the agency has made. Even though drug approvals are procedurally reversible, the FDA views drug approval as irreversible from the standpoint of reputation.

My model of drug review, then, is one in which agency reviewers are conducting a "cost-benefit" analysis of the drug at each moment in the review process. The costs of approval can be thought of as the publicly observable hazards of the drug (the FDA rarely gets criticized for approving a safe but inefficacious drug, again because safety errors are much more visible than efficacy errors). The benefit of

approval is to reduce the political pressure that patients, medical professionals, and pharmaceutical firms can apply directly or indirectly upon the FDA (witness the AIDS lobby of the late 1980s and early 1990s).

There is a hitch, however, and the problem is not as simple as it might seem. When the FDA sees approval as irreversible, probability decision theory predicts that the FDA will approve a drug only when the benefits of approval exceed both the costs of approval and the benefits of waiting further.[15] We can think of the benefits of waiting as the amount of information gleaned from another look at the file, or perhaps another test conducted by the drug company. As it turns out, this "value of waiting" is highest during the earliest stages of review, when the least is known about the drug.[16]

The Politics Of Review: New Factors In The Policy Arena

If neither doctors, patients, nor drug firms could apply public pressure for FDA approvals, the agency would find it much easier to delay drugs indefinitely. The costs of delay are not simply scientific; they are political. That is, they are subject to how well those who demand drugs can press their case before the agency, Congress, the media, and other public fora. Before the 1980s it was rare for anyone outside of clinical or academic circles to criticize the FDA for delay. Put differently, few in the media or in Congress were complaining of the agency's Type II errors.

Today the situation is much different, and the political power of patients matters as much as or more than the political power of firms. The best evidence for this proposition comes in two strategies that are now widely adopted by pharmaceutical firms: (1) Firms themselves have in the past six to eight years created, fostered, and subsidized a number of patient advocacy groups; and (2) firms regularly seek alliances with patient advocates in pressing the case for priority status, accelerated approval, or simply approval before the FDA. The second of these is a much more common, and much more successful, strategy. Put differently, politically strategic pharmaceutical firms know that industry lobbying is less successful than patient advocacy, and their regulatory behavior adapts to this fact.

FDA drug approval has in recent years been powerfully shaped by two related factors: the increase of patient advocacy groups and the increasing visibility of Type II errors.

■ **Patient advocacy groups.** The past two decades have witnessed an explosion of interest groups and in no field more dramatically than in health.[17] Several studies have shown that the increase is largely attributable to nonprofit and citizens' groups. As part of the FDA project, my research team and I have tracked the evolution of disease-advocacy groups over the past half-century. As of 2000 we aggregated more than 3,100 disease-specific advocacy groups with at least some involvement in political issues. We were able to find founding dates for more than half of these groups. We found evidence of an explosion of high-specificity health groups in the 1970s and especially the 1980s.[18]

Type II errors. The rise in patient advocacy has led to a balancing of the visibility of Type II versus Type I errors. Before the 1980s it was rare for the public's attention to be drawn to a drug that the FDA had not approved or was reviewing slowly. The AIDS epidemic changed this, less because AIDS protestors changed the FDA than because FDA officials foresaw the extraordinary politics of AIDS and rushed HIV treatments to approval.[19] Yet AIDS was only the beginning of a much larger story of disease-based political mobilization in the United States. To a degree never before witnessed, disease-specific lobbies now press Congress for medical research funding, insurers and state governments for favorable coverage rulings, and the FDA for quick approvals (Exhibit 1).

Asthma versus arthritis. Although people affected by a variety of diseases generally have begun to mobilize and lobby, not all are organized equally. Consider two examples. By just about any measure of public health, asthma is a more severe problem in the United States than arthritis (including rheumatoid arthritis). Exhibit 1 provides some raw figures. The annual death rate from asthma and related illnesses averaged nine times that for arthritis in the 1990s. Or consider that in 1997 hospitalization data from the federal government's Healthcare Cost and Utilization Project (HCUP), there are twice as many hospitalizations for asthma than for osteoarthritis. In addition, the average age of those hospitalized for arthritis was 69, but for asthma, 23.4; hence, when quality-adjusted life years (QALYs) are accounted for, asthma poses an even more costly public health burden.[20]

Yet arthritis receives far more media coverage than asthma does. If we examine coverage in the *Washington Post* for a given year in the 1990s, there were 105 (nonobituary) stories mentioning asthma that year, but almost twice as many (204) mentioning arthritis. Similarly, during 1967–1997 there were 117 arthritis-related stories on the nightly newscasts of the three major networks (ABC, NBC,

EXHIBIT 1
Epidemiology, Media Coverage, And FDA Drug Approval Times

	Death rate per 10,000	Total hospitalizations (1997)	Average days per hospitalization	*Washington Post* stories (1998)	TV news stories (1967–1998)	Median FDA approval time (1983–2000)
Arthritis versus asthma						
Arthritis	0.0027	356,405	0.00[a]	204	117	15.6
Asthma	0.0203	599,591	3.08	105	78	32.6
Three common cancers						
Breast	0.19	120,834	1.95	387	523	11.1
Lung	0.39	133,734	6.80	271	253	14.3
Prostate	0.14	134,882	3.04	165	111	19.3

SOURCES: Hospitalization data from the Healthcare Cost and Utilization Project (HCUP), 1997; and author-collected data on news coverage and Food and Drug Administration (FDA) approval times.

[a] Negligible number.

and CBS), but only 78 asthma-related stories.[21] Perhaps this is not surprising, since the early 1980s arthritis drugs have consistently been approved with much greater speed (an average of twenty months) than have drugs for asthma (an average of thirty-two months, or a full year more).

Certain cancers. Consider also the most common and deadly forms of cancer—breast, lung, and prostate. Prostate cancer is the most prevalent of these conditions, and lung cancer is by far the deadliest, but breast cancer has far more organizations, research dollars, media coverage, and quick drug approvals (Exhibit 1).

Advocacy groups' slow rise. One puzzle is why it took so long for patient and disease advocacy groups to become better organized, given that some have been around a long time. I can only hazard two educated guesses. First, political scientists have characterized the period before the 1970s as one dominated by industry, labor, and trade associations. In the 1980s and 1990s citizens' groups came to the fore. Disease and patient advocacy groups thus might be part of a larger trend. Second, the rise of disease advocacy groups could present an example of organizational learning. Many unorganized disease communities, witnessing the political and economic successes of the AIDS and breast cancer advocacy coalitions, have been motivated to form their own groups and to enter the political arena.[22]

Examples of patients' power. As illustrative evidence of the power of patient advocates in publicizing possible Type II errors, consider the recent approval of AstraZeneca's Iressa (gefitinib) for Stage III non-small-cell lung cancer patients. Despite strong skepticism from the Center for Drug Evaluation and Research (CDER) statistical reviewer and from industry watchers, the FDA approved Iressa in May 2003, in part for two reasons. First, the *Wall Street Journal* ran several editorials urging the FDA to approve it (the most vocal on 24 September 2002). So strong and visible was this pressure that FDA officials are reported to have complained to AstraZeneca about the editorials, worried of a link between the company and the editorial page. Second and more important, lung cancer patient advocates strongly supported approval, and several representatives of the groups offered robust and emotional testimony for the drug at a critical FDA advisory committee meeting in September 2002. As one journalist wrote following the meeting, "These patients—all of whom took Iressa through a compassionate use program—seemed to be the wild card that really helped AstraZeneca in the end. The company has allowed more than 18,000 patients access to Iressa outside its clinical trials, creating a very vocal and persuasive lobbying voice in the drug's favor."[23]

■ **Resources.** Perhaps the most pervasive influence upon FDA drug approval times has been the presence or absence of plentiful FDA staff to review new applications. One important reason that FDA drug approval times slowed in the 1970s, engendering complaints of a "drug lag," is that the 1962 Amendments to the 1938 Food, Drug, and Cosmetic Act piled many new responsibilities onto the FDA without a proportionate increase in personnel. The median FDA review time for new molecular entities (NMEs) submitted in 1978 was 30.8 months, and 30.0 months in 1983. In

other words, the average drug was taking two and a half years to get approved; many others took considerably longer.

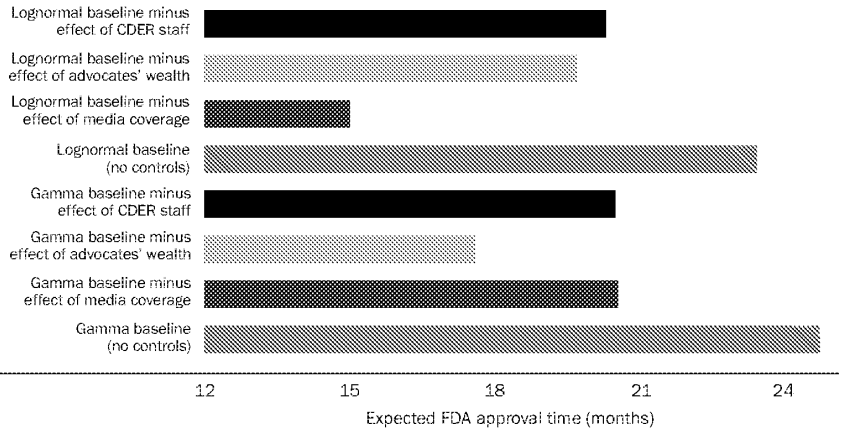
In recent years this situation has begun to change, particularly with the enactment of the Prescription Drug User Fee Act (PDUFA) of 1992 and subsequent renewals of this legislation. PDUFA is a more complicated law than I can assay here, but its most important provisions create (1) a system of per application "user fees" that fund increases in the reviewer staff at CDER, and (2) an incentive structure whereby the legislation is renewed only if the FDA meets specified performance goals.[24] In part because of PDUFA and in part because CDER staff totals began to rise five years before PDUFA, the average review time for NMEs has greatly declined, to thirteen months in 2002. The key here is that FDA staff began to increase appreciably five years before PDUFA was enacted and that the long-run effect of resources on review times is negative and large (with an elasticity of −1.6 to −2.2, meaning that a 10 percent increase in CDER personnel yields a 16–22 percent decrease in expected drug approval time).

■ **Some statistical evidence.** Iressa's approval points to two factors: organized patients and media coverage of the disease. One way of assessing the influence of media and patient groups is to conduct a duration analysis of drug review times in which measures for these constructs are included as explanatory variables. Using data on 540 NCEs reviewed by the FDA over the past twenty-five years, I conducted a set of maximum likelihood duration analyses, regressing the approval time for a drug upon (1) a measure of the wealth of advocacy groups that existed for the primary indication disease of the drug; (2) a measure of the amount of news coverage the primary indication disease received in the three years before the NDA was submitted; and (3) a set of controls, including epidemiological variables, fixed effects for the submitting firms, a set of "shared frailties" (akin to random effects) for the primary indications of the drugs, and proxies for the number of previously existing therapies for the drug's primary indication and the staff resources of the FDA.[25]

Exhibit 2 shows the reduction in expected FDA approval time from a one-standard-deviation increase in the variable in question. Begin with the "baseline" predictions of the two models estimated, lognormal and gamma. These are 23.7 and 24.1 months, respectively. If all other covariates of FDA review times are held at their means, the marginal effect of a one-standard-deviation increase in *Washington Post* stories ranges from 4.2 to 8.4 months' reduction in expected approval time from the baseline. Similarly, marginal effects of a standardized increase in group wealth range from 3.7 to 7.1 months' reduction in expected review time. Finally, a one-standard-deviation increase in CDER staff (200 full-time equivalents, or FTEs) yields a reduction of three to four months in approval time for all drugs. Note that since the models include disease-specific frailty parameters, these observed associations cannot be attributed to unmeasured disease-level heterogeneity in drugs.

In short, there is considerable evidence—from anecdote, from factual inspec-

EXHIBIT 2
Marginal Effects Of FDA Staff, Advocates' Wealth, And Media Coverage On New Molecular Entity (NME) Approval Times



SOURCE: Author's maximum likelihood duration analyses of Food and Drug Administration (FDA) approval times.
NOTES: Estimates from lognormal and gamma duration models. Effect of one-standard-deviation increase in each item depicted. See text for details. CDER is Center for Drug Evaluation and Research, FDA.

tion of the FDA's behavior, and from statistical analyses of drug review times—that the political organization and newsworthiness of patients is negatively associated with drug review times (that is, it causes these review times to get shorter). This should come as little surprise to readers aware of the FDA's response to the AIDS epidemic. The statistical analyses reported here suggest that the FDA's responsiveness to disease politics is by no means an artifact of AIDS, but extends to other diseases as well.

What Does The Future Hold?

■ **Further cuts in approval times?** While approval times for NMEs (especially priority drugs) have become shorter during the past fifteen years, one implication of the perspective presented here is that further reductions (if they can be generated at all) are likely to come at a much higher marginal cost. Trimming two months from the median approval time when yearly averages were thirty to thirty-six months was much easier; now that CDER reviewers commonly crank through priority drugs in six months or less, further reductions will likely come only through dramatic procedural change. As CDER official Kenneth Edmunds recently cautioned, "My fear is that there is a law of diminishing returns setting in. It may be expensive to wring that last 5 percent of improvement out of the FDA without some things giving way. All the easy water has been drained out of the system. All the fat is near the bone now."[26]

■ **Third-party review and privatization?** There have been several proposals to rely on non-FDA ("third party") reviewers or to privatize the FDA's review processes. Among these are actual provisions for occasional third-party review in recent legislation (for medical devices in the 1997 FDA Modernization Act, or FDAMA) and proposals from the Hoover Institution to make the FDA a certifier of private reviewers.[27] In both cases, reformers wish to exploit interorganizational competition among reviewers to reduce approval times and increase the likelihood of approval.

The form and likely effects of privatization are far too complex a subject to be tackled here. Still, it is difficult to believe that the incentives facing FDA drug reviewers would not materialize under privatization. In the simplest case, third-party reviewers would rationally decide to compete not just over quickness in review but also over minimization of visible errors. In the presence of any tort liability, moreover, the procedural conservatism of third-party reviewers might be greater than that under the FDA status quo.[28] Whether privatization would still be preferable to the status quo is another issue, but the limits posed by the incentives of drug review ought to be kept in mind.

■ **Disease politics as usual?** Patient advocacy groups and media attention can accelerate drug review, particularly for diseases that have well-organized advocates and are newsworthy. The problem, as often in politics, is one of inequality: Better-organized and more publicly salient groups get a disproportionate share of the "benefits" (quicker approvals). From a policy standpoint, there are at least two ways of looking at this pattern. One steady conclusion is that it represents an unfortunate (perhaps indefensible) result of distributive politics. Another interpretation would see these political patterns as more benign. With scarce resources, the FDA cannot avoid privileging one disease over another in its drug approval decisions. Moreover, more highly organized and "newsworthy" medical conditions are likely to be (but might not always be) more prevalent, deadlier ones. In short, the FDA must make tough (moral) choices, and disease politics could provide crucial information and guidance in doing so.

PHARMACEUTICAL REGULATION is an immensely complex process, and no combination of theoretical modeling and empirical analysis can ever do it full explanatory justice. There is also much that the FDA does in drug regulation that I have not covered here, including labeling and advertising regulation, the promotion of good manufacturing practices, and the regulation of clinical trials. Nonetheless, viewing FDA drug review as a learning exercise shaped by organized interests sheds illumination upon the process and its policy implications. The FDA protects its reputation, views its approval decisions as irreversible, and responds dramatically to patient advocacy groups and their coverage in the media because they make the consequences of delay and rejection more visible.

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NOTES

1. The stringency of regulation is arguably higher in other nations, because the United States does not (at this writing) directly constrain pharmaceutical prices.
2. This is a very simplified representation of the pharmaceutical marketplace. For more, see the FDA's Web site, www.fda.gov/cder.
3. For a general theory and empirical investigation of how agencies engage in "reputation-maximization," see D.P. Carpenter, *The Forging of Bureaucratic Autonomy: Reputations, Networks, and Policy Innovation in Executive Agencies, 1862–1928* (Princeton, N.J.: Princeton University Press, 2001).
4. "Lamictal Efficacy Comparable to Carbamazepine in First-Line Epilepsy, Glasgow Study; Lamotrigine in Phase III for Monotherapy, Pediatrics," *Pharmaceutical Approvals Monthly*, F-D-C Reports (January 1996): 29.
5. See Pew Research Center, *Deconstructing Distrust: How Americans View Government* (Washington: Pew Research Center, 1998), 33. This is an imperfect measure, used here only for heuristic purposes. Such "approval ratings" could simply signify public agreement with the agency's mission and not its performance. Still, it is worth noting that all but a handful of other agencies score materially lower in these polls.
6. P.J. Hilts, *Protecting America's Health: The FDA, Business, and One Hundred Years of Regulation* (New York: Alfred A. Knopf, 2003), chaps. 5 and 10.
7. I acknowledge Susan Moffitt, a doctoral candidate in the University of Michigan Department of Political Science who is writing a dissertation about federal advisory committees, for some of the insight on FDA advisory committees.
8. Alison Lawton, vice-president for regulatory affairs, Genzyme Corporation, interview, 11 June 2003.
9. A. Schmidt, "The FDA Today: Critics, Congress, and Consumerism" (Speech given at the National Press Club, Washington, D.C., 29 October 1974), quoted in H. Grabowski, *Drug Regulation and Innovation* (Washington: AEI Press, 1976), 76.
10. In their book on FDA "founder" Harvey Wiley, Hugh Coppin and Jack High (*The Politics of Purity* [Ann Arbor: University of Michigan Press, 1999]) seem to equate reputation enhancement with selfish, inefficient behavior, and Hilts (*Protecting America's Health*, p. 346) disparages them for criticizing Wiley as selfish. All of these authors fall into the same trap of assuming that altruism cannot possibly have any relation to self-interested behavior. In regulation as in many other fields, reputation enhancement incentives may lead to cooperative, altruistic behavior, and more efficient outcomes.
11. Analysts such as William Niskanen have argued that bureaucracies attempt to maximize their budgets (*Bureaucracy and Representative Government* [Chicago: Aldine-Atherton, 1971]), while others (including myself) have argued that agencies maximize their discretion or autonomy. Although agencies such as the FDA will usually prefer more budget to less, they will generally value reputation over resources. Among the reasons for this are that (1) regulators' personal income is only weakly related to the agency's budget; and (2) budget increases can increase the workload or task diversity of agencies in a way that leaves them "worse off." See J.Q. Wilson, *Bureaucracy: What Government Departments Do and Why They Do It* (New York: Basic Books, 1989), 118–119, 180–181. In addition, statistical tests of the budget maximization hypothesis have not supported Niskanen's theory. See D.P. Carpenter, "Adaptive Signal Processing, Hierarchy, and Budgetary Control in Federal Regulation," *American Political Science Review* (June 1996): 283–302.
12. Memorandum from Paul Leber, director, Division of Neuropharmacological Drugs, to Robert Temple, director, Office of New Drug Evaluation I, Subject: "NDA 20-658, Requip [ropinerole HCl tablets]," 6–7, NDA Public File 20-658, FDA Center for Drug Evaluation and Research.
13. R. Widmark, "Memo regarding Hepatotoxicity of Bromfenac," undated [December 1995], 3, NDA File 20-535, FDA CDER. See also "Wyeth-Ayerst Duract Hepatotoxicity Warning Was Suggested during NDA Review," *Pharmaceutical Approvals Monthly*, F-D-C Reports (April 1998): 34.
14. This is not necessarily the case. If the hazards of drug products are easily discerned and newsworthy, then the assumption is safe. But solid knowledge about product hazards often emerges only many years after market entry and is disseminated more in academic discussions than in the popular news media (the thousands of lost lives attributable to malprescription of arrhythmia drugs such as Tambocor and Encaid). See Hilts, *Protecting America's Health*, 231–232; and C.F.L. Heimann, *Acceptable Risks: Politics, Policy, and Risky*

Technologies (Ann Arbor: University of Michigan Press, 1997).

15. See D.P. Carpenter, "Why Do Bureaucrats Delay? Lessons from a Stochastic Optimal Stopping Model," in *Policy, Politics, and Organizations: Scientific Approaches to Bureaucratic Politics*, ed. G. Krause and K. Meier (Ann Arbor: University of Michigan Press, forthcoming).
16. See D.P. Carpenter, "Protection without Capture: Product Approval by a Politically Responsive, Learning Regulator," Robert Wood Johnson Foundation Scholars in Health Policy Working Paper no. 13 (Princeton, N.J.: RWJF, 2000).
17. F.R. Baumgartner and B.D. Jones, *Agendas and Instability in American Politics* (Chicago: University of Chicago Press, 1993); and J. Walker, *The Mobilization of Interest Groups in America* (Ann Arbor: University of Michigan Press, 1991).
18. As far as one can discern from the data (including Jack Walker's careful 1991 study), the vast majority of these groups neither are for-profit groups nor are funded by the pharmaceutical industry; indeed, most groups diligently avoid the label of "front group" for drug companies. In their analysis of Walker's data, Baumgartner and Jones find that just 26 percent of groups in the health policy field were "for-profit," 43 percent were nonprofit, and another 31 percent were citizens' groups. Only foreign affairs, education, and civil rights had lower ratios of profit-to-citizen involvement. Baumgartner and Jones, *Agendas and Instability in American Politics*, 183.
19. Hilts, *Protecting America's Health*, 246.
20. Agency for Healthcare Research and Quality, *Statistics from the HCUP-3 Nationwide Inpatient Sample for 1997: Principal Diagnoses* (Rockville, Md.: AHRQ, 1998).
21. The *Washington Post* and the Vanderbilt TV News database both have electronically searchable archives. For the methodology used to aggregate stories, see D.P. Carpenter, "Groups, the Media, Agency Waiting Costs, and FDA Drug Approval," *American Journal of Political Science* (July 2002): 490–505.
22. I thank an anonymous reviewer for suggesting this puzzle.
23. A. Feuerstein, "AstraZeneca Scores Comeback Victory on Iressa," www.thestreet.com/_yahoo/tech/adamfeuerstein/10044113.html (12 November 2003). I gathered similar impressions from an interview with Philip Crooker, regulatory affairs director, AstraZeneca, 19 May 2003.
24. R.A. Merrill, "Modernizing the FDA: An Incremental Revolution," *Health Affairs* (Mar/Apr 1999): 96–111.
25. One drawback of the estimations here is that we "observe" only those drugs that have been submitted to the FDA. Strategic firms are likely to anticipate likely regulatory outcomes, and so the sample is highly selected. I am trying to address this problem in ongoing theoretical and empirical research. See D.P. Carpenter and M.M. Ting, "Product Approval with Endogenous Submissions" (Unpublished manuscript, Harvard University, 2003).
26. Kenneth Edmunds, director of electronic submissions, CDER Information Technology Group, interview, May 2002.
27. H.I. Miller, *To America's Health: A Proposal to Reform the Food and Drug Administration* (Stanford, Calif.: Hoover Institution Press, 2000).
28. Consider the possibility that when third-party reviewers commit a Type I error, private or corporate parties could bring suit against them. Whereas Type I errors are more observable than Type II errors now, they would undoubtedly become much more observable under a tort system because the financial payoff to revealing such errors would rise. Hence, privatized review might well suffer from a greater procedural conservatism than FDA review. Although well-functioning insurance markets could smooth this risk, privatized reviewers would still face strong incentives to limit Type I errors.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

DRAFT CONSENSUS GUIDELINE

GUIDELINE FOR ELEMENTAL IMPURITIES

Q3D

Current *Step 2b* version

dated 26 July 2013

At Step 2 of the ICH Process, a consensus draft text or Guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Steering Committee to the regulatory authorities of the three ICH regions (the European Union, Japan and the USA) for internal and external consultation, according to national or regional procedures.

Q3D
Document History

Current *Step 2a* version

Code	History	Date
Q3D	Approval by the Steering Committee under *Step 2a*.	6 June 2013

Current *Step 2b* version

Code	History	Date
Q3D	Approval by the Steering Committee under *Step 2b* and release for public consultation.	6 June 2013
Q3D	Post sign-off corrigendum in: • Table 4.1 W and Al were removed from the list of included elemental impurities in Class 2B and 3 respectively. • Table A.2.1 the Class for Ni was changed to read 3 instead of 2.	14 June 2013
Q3D	Post sign-off minor editorial corrections including: removal of references to Appendix 5 (pgs i & 13); deletion of redundant text (pg 4); change of Option 2 to Option 2a (pg 10); insertion of omitted text under Safety Limiting Toxicity (pg 35); removal of duplicated redundant text (pg 41); replacing references to "metals" in text and "metal" in Table A.4.7 title with "elementals" and "elements" (pg 73); and deletion of header Table A.4.10 (pg 75).	26 July 2013
Q3D	Addition of line numbers to facilitate the provision of comments by stakeholders.	30 September 2013

GUIDELINE FOR ELEMENTAL IMPURITIES

Draft ICH Consensus Guideline
Released for Consultation on 26 July 2013, at *Step 2b* of the ICH Process

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GUIDELINE FOR ELEMENTAL IMPURITIES

Q3D

1. INTRODUCTION

Elemental impurities in drug products may arise from several sources; they may be added intentionally in synthesis, or may be present as contaminants (e.g., through interactions with processing equipment or by being present in components of the drug product) and are consequently detectable in the drug product. Since elemental impurities do not provide any therapeutic benefit to the patient, element impurity levels should be controlled within acceptable limits in the drug product. There are three components of this guideline: the evaluation of the toxicity data for potential elemental impurities, the establishment of a Permitted Daily Exposure (PDE) for each element of toxicological concern, and development of controls designed to limit the inclusion of elemental impurities in drug products to levels at or below the PDE. It is not expected that an applicant tightens the limits based on process capability provided that the elemental impurities in drug products are held at or below the PDE. The PDEs established in this guideline are considered to be protective of public health for all patient populations, including pediatric patients. In some cases, lower levels of elemental impurities may be needed when levels below toxicity thresholds have been shown to have an impact on other quality attributes of the drug product (e.g., element catalyzed degradation of drug substances). In addition, in the case of high PDEs, other limits may have to be considered from a pharmaceutical quality perspective; other guidelines should be consulted.

Developing a strategy to limit elemental impurities in the drug product is consistent with risk management processes identified in ICH Q9. The process is described in this guideline as a four step process to assess and control elemental impurities in the drug product: identify, analyse, evaluate, and control.

The PDE of the elements may change if new safety data become available. The guideline may be updated to include other elemental impurities or other routes of administration as new data become available. Any interested party can make a request and submit the relevant safety data to be considered.

2. SCOPE

The PDEs in this guideline have been established based on acceptable safety limits of potentially toxic elemental impurities. The guideline applies to new finished drug products (as defined in ICH Q6A and Q6B) and new drug products employing existing drug substances. The drug products containing: proteins and polypeptides (produced from recombinant or non-recombinant cell-culture expression systems), their derivatives, and products of which they are components (e.g., conjugates) are in the scope of this guideline. In addition, drug products containing synthetically produced polypeptides, polynucleotides, and oligosaccharides are within scope of this guideline.

This guideline does not apply to herbal products, radiopharmaceuticals, vaccines, cell metabolites, DNA products, allergenic extracts, cells, whole blood, cellular blood components, crude products of animal or plant origin, dialysate solutions not intended for systemic circulation or drug products containing elements that are intentionally included for therapeutic benefit.

This guideline does not apply to drug products used during clinical research stages of development. In the later stages of development, the principles contained in this guideline can be useful in evaluating elemental impurities that may be present in new drug product prepared by the proposed commercial process.

The application of this guideline to existing marketed drug products will be addressed by regional regulatory processes.

3. SAFETY ASSESSMENT OF POTENTIAL ELEMENTAL IMPURITIES

3.1 Principles of the Safety Assessment of Elemental Impurities for Oral, Parenteral and Inhalation Routes of Administration

The method used for establishing the PDE for each element impurity is discussed in detail in Appendix 1. Elements evaluated in this guideline were assessed by reviewing the publicly available data contained in scientific journals, government research reports and studies, international regulatory standards (applicable to drug products) and guidance, and regulatory authority research and assessment reports. This process follows the principles employed in ICH Q3C: Residual Solvents. The available information was reviewed to establish the oral, parenteral and inhalation PDEs provided in the guideline.

A summary safety assessment identifying the critical study for setting a PDE for each element is included in Appendix 3. There are insufficient data to set PDEs by any route of administration for osmium, rhodium, ruthenium and iridium. The PDEs for these elements were established on the basis of their similarity to platinum. The PDEs for each element included in the guideline are summarized in Appendix 2, Table A.2.1.

The factors considered in the safety assessment for establishing the PDE were:

- The oxidation state of the element likely to be present in the drug product;
- Human exposure and safety data when it provided applicable information;
- The most relevant animal study;
- Route of administration;
- Selection of the relevant endpoints or designations (e.g., International Agency for Research on Cancer [IARC] classification, animal carcinogenicity, reproductive toxicology, target organ toxicity, etc);
- The longest duration animal study was generally used to establish the PDE. In some instances, a shorter duration animal study was considered the most relevant study. The rationale for using the shorter duration study is provided in the individual PDE assessment;
- In the absence of data and/or where data were available but were not considered sufficient for a safety assessment for the parenteral and or inhalation route of administration, default factors (see below) were used to derive the PDE from the oral PDE;
- In inhalation drug products, soluble salts are more relevant than particulates to assess elemental impurity toxicity. Therefore, inhalation studies using soluble salts (when available) were preferred over studies using particulates for inhalation assessment and derivation of inhalation PDEs.

In some cases, standards for daily intake for some of the elemental impurities discussed in this guideline exist for food, water, air, and occupational exposure. These standards have developed over time with different regional processes and may use different modifying factors or other estimates (e.g., body weight for an individual). In some cases, these standards are not only safety based, rather, based on practical considerations or

analytical capability. Where appropriate, these standards were considered in the assessment and establishment of the PDEs using the approach as outlined in Appendix 1.

For PDEs established for inhalation (oral or parenteral routes as applicable), doses were normalized to a 24 hour, 7 day exposure. If data were available for local toxicity to the lung, those data were considered in establishing the inhalation PDE.

Where data were available but were not considered sufficient for a safety assessment for the parenteral route of administration, modifying factors were employed as follows:

Oral bioavailability <1% divide by a modifying factor of 100
Oral bioavailability < 50% divide by a modifying factor of 10
Oral bioavailability between 50% and 90% divide by a modifying factor of 2
Oral bioavailability > 90% divide by a modifying factor of 1

Where inhalation and/or parenteral data were available but were not considered sufficient for a safety assessment or Threshold Limit Value (TLV)/Time Weighted Average (TWA) values were not available for the inhalation route of administration, a calculated PDE was used based on the oral PDE divided by a modifying factor of 100 (Ball *et al.* 2007). In cases where the TLV/TWA or a nonclinical inhalation study was used, the dose levels were normalized to a 24 hour, 7 day week.

PDEs for elements of low risk to human health as impurities in drug products were not established. The elements in this category include: Fe, B, Al, W, Zn, K, Ca, Na, Mn, and Mg.

For elements not included in this guideline for which there is limited or insufficient data, the concepts used in this guideline can be used to determine appropriate PDEs.

3.2 Other Routes of Administration

PDEs were only established for oral, parenteral and inhalation routes of administration. Sufficient data to permit the establishment of a PDE for other routes of administration were generally unavailable. However, the concepts applied and described in this guideline can be used to determine appropriate PDEs for other routes of administration. Application of the parenteral PDE can provide the basis of a route-specific safety assessment.

3.3 Justification for Element Impurity Levels Higher than the PDE

Levels of elemental impurities higher than the PDE may be acceptable in certain cases. These cases could include, but are not limited to the following situations:

- less than daily dosing
- short term exposures (i.e., 30 days or less)
- specific indications (e.g., life-threatening, unmet medical needs, rare diseases)

Justification for increased levels in these situations should be made on a case by case basis justifying the proposed level using a risk based approach. ICH Q3C and this guideline use modifying factors for interspecies (Factor F1) and individual (Factor F2) variability. These modifying factors serve as starting points in extrapolating available data to obtain a PDE. The sub-factor approach (WHO, 2009), may be used to justify a higher PDE, where data are available, using knowledge of the mode of action and pharmacokinetic considerations. A justification may also include but is not limited to a consideration of the duration of the study used to set the PDE relative to the intended clinical use (Factor F3), the nature and severity of the toxicity observed, and whether the toxicity was reversible (Factor F4).

An example of the sub-factor approach can be found elsewhere in a risk assessment for boron (US Environmental Protection Agency [EPA], 2004).

3.4 Parenteral Products

The parenteral PDEs are applied irrespective of dose volume.

4. ELEMENT CLASSIFICATION

The elemental impurities included in this guideline have been placed into categories that are intended to facilitate decisions during the risk assessment.

- Class 1 elemental impurities, As, Cd, Hg, and Pb, are significantly toxic across all routes of administration. Typically they have limited or no use in the manufacture of pharmaceuticals but can be present as impurities in commonly used materials (e.g., mined excipients) and can not be readily removed from the material. Because of their unique nature, these four elemental impurities require consideration during the risk assessment across all potential sources of elemental impurities.
- Class 2 elemental impurities are toxic to a greater or lesser extent based on route of administration. In addition, some of the elements present in this category are infrequently observed as impurities in materials used to produce drug products and as such, unless intentionally added have a low probability of inclusion in the drug product and do not present a significant risk. Class 2 elemental impurities are further categorized to establish when they should be considered in the risk assessment and when their contribution can be judged to be negligible.
    - Class 2A: The following elemental impurities require assessment across all potential sources and routes of administration: V, Mo, Se, and Co due to their higher relative natural abundance (US Geological Survey, 2005).
    - Class 2B: The following elemental impurities require assessment across potential elemental impurity sources only if they are intentionally added to the processes used to generate the material under evaluation: Au, Tl, Pd, Pt, Ir, Os, Rh, Ag and Ru.
- Class 3 elemental impurities are impurities with relatively low toxicity (high PDEs) by the oral route administration but require consideration in the risk assessment for other routes of administration (e.g., inhalation and parenteral routes). For oral routes of administration, unless these elements are intentionally added as part of the process generating the material, they do not need to be considered during the risk assessment. For parenteral and inhalation products, the potential for inclusion of these elemental impurities should be evaluated during the risk assessment. The elemental impurities in this class include: Sb, Ba, Li, Cr, Cu, Sn, and Ni.
- Class 4 elemental impurities are elemental impurities that have been evaluated but for which a PDE has not been established due to their low inherent toxicity and/or regional regulations. If these elemental impurities are present or included in the drug product they are addressed following the practices defined by other guidelines and regional regulation. The elements in this class include: Al, B, Fe, Zn, K, Ca, Na, Mn, Mg, and W.

The classification system is summarized in Table 4.1.

183 **Table 4.1: Elemental Impurity Classification**
184

	Included Elemental Impurities	Include in Risk Assessment?
Class 1	As, Pb, Cd, Hg	Yes
Class 2A	V, Mo, Se, and Co	Yes
Class 2B	Ag, Au, Tl, Pd, Pt, Ir, Os, Rh, and Ru	Yes only if intentionally added
Class 3	Sb, Ba, Li, Cr, Cu, Sn, Ni	Dependent upon route of administration – see Class 3 description
Class 4	B, Fe, Zn, K, Ca, Na, Mn, Mg, W, Al	No

185 ## 5. ASSESSMENT AND CONTROL OF ELEMENTAL IMPURITIES

186 In developing the control strategy for elemental impurities in drug products, the
187 principles of quality risk management, described in ICH Q9, should be considered. The
188 risk assessment should be based on scientific knowledge and principles. It should link
189 patient safety considerations with an understanding of the product and its
190 manufacturing process (ICH Q8 and Q11). In the case of elemental impurities, the
191 product risk assessment would therefore be focused on assessing the levels of elemental
192 impurities in a drug product in relation to the PDEs presented in this guidance.
193 Information for this assessment includes but is not limited to: data generated by the
194 applicant, information supplied by drug substance, reagent and/or excipient
195 manufacturers or data available in published literature.

196 The applicant should document the assessment and control approaches in an appropriate
197 manner. The level of effort and formality of the assessment should be proportional to the
198 level of risk. It is neither always appropriate nor always necessary to use a formal risk
199 management process (using recognized tools and/or formal procedures, e.g., standard
200 operating procedures.) The use of informal risk management processes (using empirical
201 tools and/or internal procedures) can also be considered acceptable. Tools to assist in the
202 risk assessment are described in ICH Q9 and will not be presented in this guideline.

203 ### 5.1 General Principles

204 For the purposes of this guideline, the assessment process can be described in four steps:
205 identify, analyse, evaluate and control. In many cases, the steps are considered
206 simultaneously. For example, the analyse and evaluate steps may be iterative steps that
207 initiate adjustments to control elements. The outcome of the assessment may be the
208 result of iterations to develop a final approach to ensure the potential elemental
209 impurities do not exceed the PDE.

210 Identify: Identify known and potential sources of elemental impurities that may
211 find their way into the drug product.

212 Analyze: Determine the probability of observance of a particular elemental impurity
213 in the drug product.

Evaluate: Compare the observed or predicted levels of elemental impurities with the established PDE.

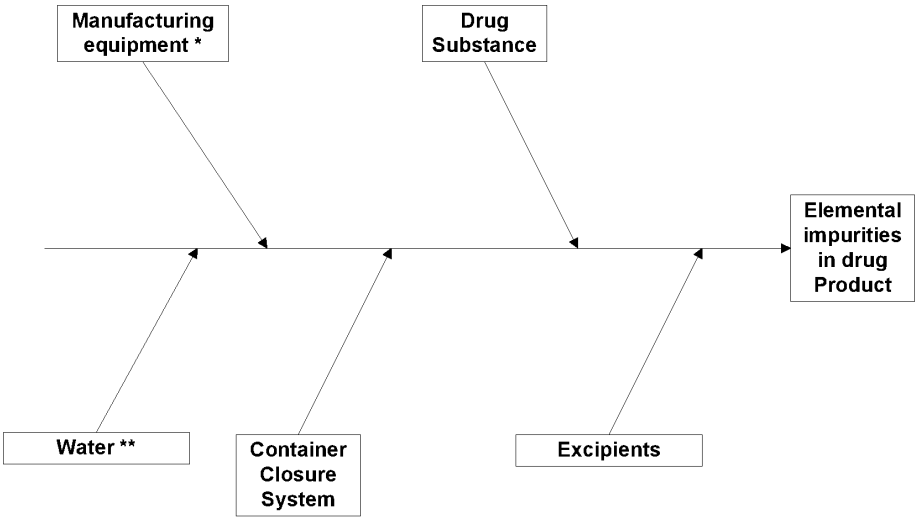
Control: Document and implement a control strategy to limit elemental impurities in the drug product.

5.2 Potential Sources of Elemental Impurities

In considering the production of a drug product, there are several broad categories of potential sources of elemental impurities.

- Residual elemental impurities resulting from elements intentionally added to reactions or processes leading up to the preparation of the drug substance, reagents, starting materials or excipients (e.g., metal catalysts).
- Elemental impurities known or suspected of being present in the drug substance, reagents, water, starting materials or excipients used in the preparation of the drug product.
- Elemental impurities known or suspected of being introduced into the drug substance and/or drug product from manufacturing equipment.
- Elemental impurities that are known or suspected of being leached into the drug substance and drug product from container closure systems.

The following diagram shows an example of typical materials or components used in the production of a drug product. Each of these materials or components may contribute elemental impurities to the drug product, through any individual or any combination of the potential sources listed above. During the assessment, the potential contributions from each of these materials or components should be considered to determine the overall contribution of elemental impurities to the drug product.



* The risk of inclusion of elemental impurities can be reduced through process understanding, equipment selection, equipment qualification and Good Manufacturing Practice (GMP) processes.

** The risk of inclusion of elemental impurities from water can be reduced by complying with compendial (e.g., European Pharmacopoeia, Japanese Pharmacopoeia, US

Pharmacopeial Convention) water quality requirements, if purified water or water for injection is used in the process(es).

5.3 Assessment – Identification of Potential Elemental Impurities

Class 1 elemental impurities: Due to their inherent toxicity, the risk assessment should include an assessment of the Class 1 elemental impurities. All potential sources of elemental impurities should be evaluated for the potential to transfer the Class 1 elemental impurities to the drug product.

Potential elemental impurities derived from intentionally added catalysts or reagents: For this category, the identity of the potential impurities is known and techniques for controlling the elemental impurities are easily characterized and defined. The predominant elemental impurities that comprise this group are the Class 2 and 3 elemental impurities. Table 5.1 shows the suggested consideration in the risk assessment for each of the elemental impurities covered in this guideline. As identified, if any (Class 1, 2, or 3) elemental impurity is added, it should be considered in the risk assessment.

Potential elemental impurities with a relatively high abundance and/or are impurities in excipients or reagents: Elemental impurities known or suspected of being present in the drug substance, reagents, starting materials or excipients used in the preparation of the drug product should be considered. These elemental impurities are often associated with mined materials and excipients. The presence of these impurities can be variable, especially with respect to mined excipients, which can complicate the risk assessment. The variation should be considered when establishing the probability for inclusion in the drug product. The elemental impurities that are of most significant to this potential source include the Class 1 and Class 2A elemental impurities (see Table 4.1). For parenteral and inhalation routes of administration, the risk assessment should evaluate the probability for inclusion of the Class 1 and most 3 elemental impurities as shown in Table 5.1.

Potential elemental impurities derived from manufacturing equipment: The contribution of elemental impurities may be limited and the subset of elemental impurities that should be considered in the risk assessment is relatively small and is dependent on the equipment involved. Application of process knowledge, selection of equipment, equipment qualification and GMP controls ensure a low contribution from manufacturing equipment. The specific elemental impurities of concern should be assessed based on knowledge of the composition of the components of the manufacturing equipment. The assessment of this source of elemental impurities is one that can be utilized potentially for many drug products using similar process trains and processes.

Elemental impurities leached from container closure systems: Identifying the potential elemental impurities extracted from container closure systems should be based on a scientific understanding of likely interactions between a particular drug product type and its packaging. When a review of the materials of construction demonstrates that the container closure system does not contain elemental impurities, no additional assessment needs to be performed. It is recognized that the probability of elemental leaching into solid dosage forms is minimal and does not require further consideration in the assessment. For liquid and semi-solid dosage forms there is a higher probability that elemental impurities could leach from the container closure system into the drug product during the shelf-life of the product. Studies to understand potential extractables and leachables from the final/actual container closure system (after washing sterilization, irradiation) should be performed.

Factors that should be considered (for liquid and semi-solid dosage forms) include but are not limited to:

- Hydrophilicity/hydrophobicity
- Ionic content
- pH
- Temperature (cold chain *vs* room temperature and processing conditions)
- Contact surface area
- Container/component composition
- Terminal sterilization
- Packaging process
- Component sterilization
- Migration potential
- Duration of storage
- Inclusion of metal chelating agents in the formulation (e.g., Ethylenediamine Tetraacetic Acid [EDTA]).

Table 5.1: Recommendation for Consideration During Risk Assessment

Element	Class	If intentionally added (across all routes of administration)	If not intentionally added		
			Oral	Parenteral	Inhalation
As	1	yes	yes	yes	yes
Cd	1	yes	yes	yes	yes
Hg	1	yes	yes	yes	yes
Pb	1	yes	yes	yes	yes
Co	2A	yes	yes	yes	yes
Mo	2A	yes	yes	yes	yes
Se	2A	yes	yes	yes	yes
V	2A	yes	yes	yes	yes
Ag	2B	yes	no	no	no
Au	2B	yes	no	no	no
Ir	2B	yes	no	no	no
Os	2B	yes	no	no	no
Pd	2B	yes	no	no	no
Pt	2B	yes	no	no	no
Rh	2B	yes	no	no	no
Ru	2B	yes	no	no	no
Tl	2B	yes	no	no	no
Ba	3	yes	no	no	yes
Cr	3	yes	no	no	yes
Cu	3	yes	no	yes	yes
Li	3	yes	no	yes	yes
Ni	3	yes	no	yes	yes
Sb	3	yes	no	yes	yes
Sn	3	yes	no	yes	yes

5.4 Assessment – Analysis and Evaluation

As the potential elemental impurity identification process is concluded, there are several possible outcomes: the process and product review does not identify any potential elemental impurities or the process identifies a list of one or more potential elements. When present, the elemental impurities may have a single source or multiple sources. In addition, a number of elemental impurities will be excluded from consideration based on the assessment of their probability of occurrence and their potential to exceed the PDE. In order to accurately complete the assessment, data regarding potential elemental impurity levels may be needed. The data for this assessment can come from a number of sources that include, but are not limited to:

- Prior knowledge
- Published literature
- Data generated from similar processes
- Supplier information or data
- Analysis of the components of the drug product
- Analysis of the drug product

The applicant's risk assessment can be facilitated with information about the potential elemental impurities provided by suppliers of drug substances, excipients, starting materials, reagents, container closure systems, and manufacturing equipment.

Since the PDE is established on the drug product, it is necessary to compare the predicted or known levels of the elemental impurities identified with the established PDE in order to define the appropriate steps to take in developing an approach to control potential elemental impurities in the drug product. This may be done in several different ways and the applicant should consider which option is most appropriate for their use given the elemental impurities identified in combination with the source of the elemental impurity.

5.5 Converting Between PDEs and Concentration Limits

The PDEs, reported in micrograms per day (µg/day) provided in this document give the maximum permitted quantity of each element that may be contained in the maximum daily intake of a drug product. Because the PDE reflects only total exposure from the drug product, it is useful to convert the PDE, into concentrations as a tool in evaluating elemental impurities in drug products or their components. The following options describe some acceptable approaches to establishing concentrations of elemental impurities in drug products or components that would assure that the drug product meets the PDEs. The applicant may select any of these options as long as the resulting permitted concentrations assure that the drug product meets the PDEs for elemental impurities. In the choice of a specific option the applicant must have knowledge of, or make assumptions about, the daily intake of the drug product. In all cases, the PDE should be met. The permitted concentration limits may be used:

- As a tool in the risk assessment to compare the observed or predicted levels to the PDE;
- In discussions with suppliers to help establish upstream controls that would assure that the product meets the PDE;
- To establish concentration targets when developing in-process controls on elemental impurities;
- To convey information regarding the controls on elemental impurities in regulatory submissions.

As discussed in Section 5.2, there are multiple sources for elemental impurities in drug products. When applying any of the options described below, elemental impurities from container closure systems and manufacturing equipment should be taken into account prior to calculating the maximum permitted concentration in the remaining components (excipients and drug substance). If it is determined during the risk assessment that the container closure systems and manufacturing equipment do not contribute to the elemental impurity level in the drug product, they do not need to be considered. Where contributions from container closure systems and manufacturing equipment exist, these contributions may be accounted for by subtracting the estimated daily intake from these sources from the PDE prior to calculation of the allowed concentration in the excipients and drug substance.

Option 1: Common permitted concentration limits of elements across drug product components for drug products with daily intakes of not more than 10 grams:

This option is not intended to imply that all elements are present at the same concentration, but rather provides a simplified approach to the calculations.

The option assumes the daily intake (amount) of the drug product is 10 grams or less, and that elemental impurities identified in the risk assessment (the target elements) are present in all components of the drug product. Using equation (1) below, and a daily intake of 10 grams of drug product, this option calculates a common permissible target elemental concentration for each component in the drug. This approach, for each target element, allows determination of a fixed common maximum concentration in micrograms per gram in each component. The calculated values are provided in Appendix 2 Table A.2.2.

$$Concentration(\mu g/g) = \frac{PDE(\mu g/day)}{daily\ amount\ of\ drug\ \ product(g/day)} \qquad (1)$$

If all the components in a drug product meet the Option 1 concentrations for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product. An example of this calculation is shown in Appendix 4 Table A.4.1. If the permitted concentrations in Appendix 2 Table A.2.2 are not applied, Options 2a, 2b, or 3 must be followed.

Option 2a: Common permitted concentration limits across drug product components for a drug product with a specified daily intake:

This option is similar to Option 1, except that the drug daily intake is not assumed to be 10 grams. The common permitted concentration of each element is determined using Equation 1 and the actual maximum daily intake.

This approach, for each target element, allows determination of a fixed common maximum concentration in micrograms per gram in each component based on the actual daily intake provided. An example of this calculation is provided in Appendix 4 Table A.4.2.

If all components in a drug product meet the Option 2a concentrations for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product.

Option 2b: Permitted concentration limits of elements across drug product component materials for a product with a specified daily intake:

This option requires additional information that the applicant may assemble regarding the potential for specific elemental impurities to be present in specific drug product components. The applicant may set permitted concentrations based on the distribution of elements in the components (e.g., higher concentrations in components with the presence of an element in question). For each element identified as potentially present in the components of the drug product, the total mass of the elemental impurity in the final drug product can be calculated as the sum of the product of the component material masses at the maximum permitted concentrations established by the applicant. The total mass of the elemental impurity in the drug product cannot exceed the PDEs given in Appendix 2 Table A.2.1., as shown in equation 2. If the risk assessment has identified that a specific element is not a potential impurity in a specific component, there is no need to establish a quantitative result for that element in that component. This approach allows that the maximum permitted concentration of an element in certain components of the drug product may be higher than the Option 1 or Option 2a limit, but this should then be compensated by lower allowable concentrations in the other components of the drug product. Equation 2 may be used to set component-specific limits for each element in each component of a drug product.

$$\text{PDE}\left(\mu\text{g/day}\right) \geq \sum_{k=1}^{N} C_k \cdot M_k \qquad (2)$$

k = an index for each of N components in the drug product
C_k = concentration of the elemental impurity in component k (µg/g)
M_k = mass of component k in the maximum daily intake of the drug product (g)

An example of this calculation is provided in Appendix 4 Tables A.4.3 – A.4.5.

Option 3: Finished Product Analysis:

The concentration of each element may be measured in the final drug product. Equation 1 may be used with the maximum total daily dose of the drug product to calculate a maximum permitted concentration of the elemental impurity. An example of this option is provided in Appendix 4 Table A.4.6.

5.6 Assessment Summary

The process described above is intended to enable the applicant to focus on those elements that require additional control elements. The process permits the applicant to utilize information and knowledge gained across products to establish the particular elemental impurities of concern in the specific drug product.

A number of factors can influence the level of the potential impurity in the drug product and should also be considered in the assessment. These include but are not limited to:

- Efficiency of removal of elemental impurities during further processing;
- Natural abundance of elements (especially important for the categories of elements which are not intentionally added);
- Prior knowledge of elemental impurity concentration factors from specific sources.

For elements that are added or are known to be potentially present in excipients or raw materials, the analysis should consider the percentage of the excipient or raw material in the drug product. Assessment of probable concentrations based on this percent of the total composition of the drug product is an additional tool to determine if the contribution is relevant. The analysis may include an assessment of the levels or concentrations that are identified either in each component (including contributions from the container closure system) or in the drug product.

The initial design of the facility and qualification of utilities and equipment, as part of process qualification, would be expected to identify potential elemental impurities and anticipated potential contributions to the drug product. In general, the contribution of elemental impurities from manufacturing equipment and utilities is likely to be negligible and would normally be addressed by implementing appropriate GMP procedures. However, if the assessment demonstrated that the contribution was significant, the anticipated levels of the identified elements should be reviewed as part of the risk evaluation process.

Finally the applicant should consider the significance of the observed level relative to the PDE of the element. As a measure of the significance of the observed elemental impurity level, a control threshold is defined as a level that is 30% of the established PDE in the drug product. This threshold is used to determine if additional controls may be required. If the total elemental impurity level from all sources in the drug product is consistently less than 30% of the PDE, applying appropriate assessment of the data and demonstrating an adequate control strategy, then additional controls are not required.

If the assessment fails to demonstrate that an elemental impurity level is below the control threshold, controls should be established to ensure that the elemental impurity level does not exceed the PDE in the drug product.

In order to apply the control threshold, sources of variability should be understood. Important factors include:

- Variability of the analytical method
- Variability of the elemental impurity level in the specific sources
- Variability of the elemental impurity level in the drug product

There are many acceptable approaches to document the assessment and may include: tables, written summaries of considerations and conclusions of the assessment. The summary should identify the elemental impurities, their sources, and the controls and acceptance criteria as needed.

5.7 Control of Elemental Impurities

Control of elemental impurities includes decision making steps designed to reduce or accept the presence of elemental impurities and their respective concentrations that were identified and evaluated through the assessment process. When the assessment determines that the levels of elemental impurities are below the control threshold, no further control is required but periodic verification testing may be used to confirm that the expected levels are consistent and predictive of future (see Section 5.8). The applicant should provide a justification for the application of periodic verification testing.

When the control threshold is exceeded, the controls established should ensure that the PDE is not exceeded. There are a number of control elements or approaches that an applicant can pursue to control the elemental impurities in drug products. These include but are not limited to:

- Identification of the steps in the manufacturing process that result in the reduction of elemental impurities through specific or non-specific purification steps;
- Implementation of in-process or upstream controls, designed to limit the concentration of the elemental impurity in the drug product;
- Establishment of material (e.g., synthetic intermediates and raw materials) or excipient specifications to limit the level of elemental impurity contributions from those sources;

- Establishment of specification limits for the drug substance;
- Establishment of specification limits for the drug product;
- Reliance on the compliance with compendial standards for materials used in drug product processes;
- Selection of appropriate container closure systems.

Where testing and acceptance criteria are established, periodic verification testing may be appropriate in some cases (see Section 5.8).

An illustration of the risk assessment process described above can be found in Appendix 4.

5.8 Periodic Verification Testing

In situations where a test is recommended to be included in the specification to provide suitable control of elemental impurities, but where routine measurement for release of every batch may not be necessary, it may be possible to apply periodic verification testing (periodic or skip lot testing as described in ICH Q6A). It should be noted that allowance of periodic verification testing is considered to be helpful to provide periodic confirmation that the controls contained within a process perform consistently over the lifecycle of the product. Periodic testing is a means to ensure that the risk assessment assumptions are valid and ensure that unintended or unknown process or material attributes have not changed over time. Application of periodic verification testing should be applied to processes or materials that are under a state of control (i.e., consistently meets specifications and conforms to an appropriately established facility, equipment, processing, and operational control regimen). If upon testing, the elemental impurity level exceeds the PDE, the applicant should investigate the cause of the failure, reassess the controls that are in place and determine if additional controls may be required. Failures observed in periodic verification testing should be reported to the appropriate regulatory authorities following the established procedures.

5.9 Special Considerations for Biotechnologically-Derived Products

For biotechnology-derived products, the risks associated with elemental impurities being present at levels of safety concerns at the drug substance stage are considered low. This is largely due to the following factors: a) elements are not typically used as catalysts or reagents in the manufacturing of biotech products; b) elements are added at trace levels in media feeds during cell culture processes, without accumulation and with significant dilution/removal during further processing; c) typical purification schemes used in biotech manufacturing such as chromatography steps and dialysis or Ultrafiltration-Diafiltration (UF/DF) have the capacity to clear elements introduced in cell culture/fermentation steps or from contact with manufacturing equipment to negligible levels. As such, a specific control strategy that relates to the control of elements up to the biotech drug substance is not generally needed. In cases where the biotechnology derived drug substance contains synthetic elements (such as antibody-drug conjugates), appropriate controls on the small molecule element for elemental impurities should be performed.

However, potential elemental impurity sources included in drug product manufacturing (e.g., excipients) and other environmental sources should be considered for biotechnologically derived drug products. The contribution of these sources to the finished product should be assessed as typically they are introduced in the drug product manufacture at a step in the process where subsequent elemental impurity removal is not generally performed. Risk factors that should be considered in this assessment should include the type of excipients used, the processing conditions and their

susceptibility to contamination by environmental factors (e.g., controlled areas for sterile manufacturing and use of purified water), as well as the overall dosing frequency.

6. SPECIATION

Speciation is defined as the separation of elemental impurities based on oxidation state, organic combination or complexation state. The PDE has been established using the toxicity information on the species expected to be in the drug product.

The applicant is not expected to provide speciation information; however, such information could be used to justify higher levels for the more relevant or less toxic species.

7. ANALYTICAL PROCEDURES

The determination of elemental impurities should be conducted using appropriate procedures suitable for their intended purposes. Unless otherwise justified, the test should be specific for each elemental impurity identified for control during the risk assessment. Pharmacopoeial procedures or suitable validated alternative procedures for determining levels of elemental impurities should be used.

8. LIFE-CYCLE MANAGEMENT OF THE CONTROL STRATEGY FOR ELEMENTAL IMPURITIES

The quality system elements and management responsibilities described in ICH Q10 are intended to encourage the use of science-based and risk-based approaches at each lifecycle stage, thereby promoting continual improvement across the entire product lifecycle. Product and process knowledge should be managed from development through the commercial life of the product up to and including product discontinuation.

The effectiveness of the control strategy should be periodically evaluated throughout the product lifecycle. Knowledge gained from development combined with commercial manufacturing experience and data can be used to further improve process understanding and process performance which can be used to make improvements to the control strategy. It is recognized that the elemental impurity data available for some components is somewhat limited at this time which may direct the applicant to a specific series of control elements. Additional data, if developed, may lead to modifications of the control strategy.

If changes to the drug product process(es) have the potential to change the elemental impurity content of the drug product, the established control elements for elemental impurities should be re-evaluated. Such changes could include but are not limited to: changes in synthetic route, excipient supplier, raw materials, processes, equipment, or facilities. All changes are subject to internal change management process (ICH Q10) and if needed appropriate regional regulatory requirements.

9. RECOMMENDATIONS FOR SUBMISSION OF ELEMENTAL IMPURITIES CONTROL STRATEGY

The information on the control strategy that is provided in a regulatory submission should include the outcome of the risk assessment and a description of the controls established to limit elemental impurities. A good location for the description of the control strategy is Section 3.2.P.5.6. This summary should include appropriate references to the locations of controls on elemental impurities defined in the control strategy (e.g., 3.2.S and 3.2.P). A summary of the approach used to develop the control strategy may be included in the Quality Overall Summary.

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Haxel GB, Hedrick JB, Orris GJ. Rare earth elements-critical resources for high technology. US Geological Survey 2005;Fact Sheet 087-02.

IPCS. Principles and methods for the risk assessment of chemicals in food, chapter 5: dose-response assessment and derivation of health based guidance values. Environmental Health Criteria 240. International Programme on Chemical Safety. World Health Organization, Geneva. 2004; Table 5.5.

US EPA. 0410 Boron and Compounds. Integrated Risk Management System (IRIS). 2004.

GLOSSARY

ATSDR:
Agency for Toxic Substances and Disease Registry.

CEC:
Commission of the European Community.

CFR:
Code of Federal Regulations (USA).

Change Management:
A systematic approach to proposing, evaluating, approving, implementing and reviewing changes. (ICH Q10)

Container Closure System:
The sum of packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components, if the latter are intended to provide additional protection to the drug product. A packaging system is equivalent to a container closure system. (ICH Q1A)

Control Strategy:
A planned set of controls, derived from current product and process understanding, which assures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control. (ICH Q10)

Control Threshold:
A limit that is applied during the assessment of elemental impurities to determine if additional control elements may be required to ensure that the PDE is not exceeded in the drug product. The limit is defined as 30% of the PDE of the specific elemental impurity under consideration.

Daily Dose:
The total mass of drug product that is consumed by a patient on a daily basis.

EFSA:
European Food Safety Agency.

EHC:
Environmental Health Criteria. (WHO)

EU SCOEL:
European Scientific Committee on Occupational Exposure Limits.

IARC:
International Agency for Research on Cancer.

Inhalation Unit Risk:
The upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 µg/L in water, or 1 µg/m^3 in air. The interpretation of inhalation unit risk would be as follows: if unit risk = 2 x 10-6 per µg/L, 2 excess cancer cases (upper bound estimate) are expected to develop per 1,000,000

people if exposed daily for a lifetime to 1 µg of the chemical in 1 liter of drinking water. (US EPA)

IPCS:

International Programme for Chemical Safety.

IUPAC:

International Union of Pure and Applied Chemistry.

IRIS:

Integrated Risk Identification System, United States Environmental Protection Agency.

Lowest-Observed-Adverse-Effect Level (LOAEL):

Lowest concentration or amount of a substance *(*dose*)*, found by experiment or observation, which causes an adverse effect on morphology, functional capacity, growth, development, or life span of a target organism distinguishable from normal (control) organisms of the same species and strain under defined conditions of exposure. (IUPAC)

Limit of Detection (LOD):

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. (ICH Q2)

Lowest-Observed-Effect Level (LOEL):

The lowest dose of substance in a study or group of studies that produces biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

Modifying Factor:

A factor determined by professional judgment of a toxicologist and applied to bioassay data to relate that data to human safety. (Q3C) (See related term Safety Factor)

MRL:

Minimal Risk Level.

No-Observed-Adverse-Effect Level (NOAEL):

Greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure.

No-Observed-Effect Level (NOEL):

The highest dose of substance at which there are no biologically significant increases in frequency or severity of any effects in the exposed humans or animals.

NTP:

National Toxicology Program.

OELV:

Occupational Exposure Limit Value.

OSHA:

Occupational Safety and Health Administration (USA).

PEL:

Permitted Exposure Limit.

687 **Permitted Daily Exposure:**
688 The maximum acceptable intake of elemental impurity in pharmaceutical products per
689 day.

690 **Product Lifecycle**:
691 All phases in the life of the product from the initial development through marketing
692 until the product's discontinuation. (ICH Q9)

693 **Quality**:
694 The degree to which a set of inherent properties of a product, system, or process fulfills
695 requirements (see ICH Q6A definition specifically for *quality* of drug substance and drug
696 products). (ICH Q9)

697 **Quality Risk Management**:
698 A systematic process for the assessment, control, communication, and review of risks to
699 the quality of the drug product across the product lifecycle. (ICH Q9)

700 **Quality System**:
701 The sum of all aspects of a system that implements quality policy and ensures that
702 quality objectives are met. (ICH Q10)

703 **Raw Material:**
704 A general term used to denote starting materials, reagents, and solvents intended for use
705 in the production of intermediates or Active Pharmaceutical Ingredients (APIs). (ICH
706 Q7)

707 **Risk**:
708 The combination of the probability of occurrence of harm and the severity of that harm.
709 (ISO/IEC Guide 51, ICH Q9)

710 **Risk Acceptance:**
711 The decision to accept risk. (ISO Guide 73)

712 **Risk Analysis**:
713 The estimation of the risk associated with the identified hazards. (ICH Q9)

714 **Risk Assessment**:
715 A systematic process of organizing information to support a risk decision to be made
716 within a risk management process. It consists of the identification of hazards and the
717 analysis and evaluation of risks associated with exposure to those hazards. (ICH Q9)

718 **Risk Control:**
719 Actions implementing risk management decisions. (ISO Guide 73)

720 **Risk Identification:**
721 The systematic use of information to identify potential sources of harm (hazards)
722 referring to the risk question or problem description. (ICH Q9)

723 **Risk Management**:
724 The systematic application of quality management policies, procedures, and practices to
725 the tasks of assessing, controlling, communicating, and reviewing risk. (ICH Q9)

726

727

728 **Safety:**

729 Practical certainty that adverse effects will not result from exposure to an agent under
730 defined circumstances. (EHC 240)

731 **Safety Assessment:**

732 An approach that focuses on the scientific understanding and measurement of chemical
733 hazards as well as chemical exposures, and ultimately the risks associated with them.
734 Often (and in this guideline) used synonymously with risk assessment. *Related term*:
735 Risk assessment. (EHC 340)

736 **Safety Factor:**

737 A composite (reductive) factor applied by the risk assessment experts to the No-
738 Observed-Adverse-Effect Level (NOAEL) or other reference point, such as the
739 benchmark dose or benchmark dose lower confidence limit, to derive a reference dose
740 that is considered safe or without appreciable risk, such as an acceptable daily intake or
741 tolerable daily intake (the NOAEL or other reference point is divided by the safety factor
742 to calculate the reference dose). The value of the safety factor depends on the nature of
743 the toxic effect, the size and type of population to be protected, and the quality of the
744 toxicological information available. Related terms: Assessment factor, Uncertainty factor.
745 (EHC 240)

746 **Severity**:

747 A measure of the possible consequences of a hazard. (ICH Q9)

748 **Starting Material**:

749 A material used in the synthesis of a new drug substance that is incorporated as an
750 element into the structure of an intermediate and/or of the new drug substance. Starting
751 materials are normally commercially available and of defined chemical and physical
752 properties and structure. (ICH Q3A)

753 **Threshold Limit Value (TLV):**

754 The concentration in air to which it is believed that most workers can be exposed daily
755 without an adverse effect (i.e., effectively, the threshold between safe and dangerous
756 concentrations). The values were established (and are revised annually) by the ACGIH
757 and are time-weighted concentrations (TWA) for a 7- or 8-hour workday and 40-hour
758 workweek, and thus are related to chronic effects. (IUPAC)

759 **Time Weighted Average (TWA):**

760 As defined by ACGIH, time-weighted average concentration for a conventional 8-hour
761 workday and a 40-hour workweek. (IUPAC)

762 **URF**:

763 Unit Risk Factor.

764 **US DoL:**

765 United States Department of Labor.

766 **US EPA**:

767 United States Environmental Protection Agency.

768 **WHO**:

769 World Health Organization.

770

Appendix 1: Method for Establishing Exposure Limits

The Gaylor-Kodell method of risk assessment (Gaylor DW, Kodell RL. Linear Interpolation algorithm for low dose assessment of toxic substance. J Environ Pathol Toxicol 1980;4:305) is appropriate for carcinogenic elemental impurities. Only in cases where reliable carcinogenicity data are available should extrapolation by the use of mathematical models be applied to setting exposure limits. Exposure limits for carcinogenic elemental impurities could be determined with the use of a large safety factor (i.e., 10,000 to 100,000) with respect to the No-Observed-Effect Level (NOEL).

Acceptable exposure levels for elemental impurities in this guideline were established by calculation of PDE values according to the procedures for setting exposure limits in pharmaceuticals (Pharmacopeial Forum, Nov-Dec 1989), and the method adopted by IPCS for Assessing Human Health Risk of Chemicals (Environmental Health Criteria [EHC] 170, WHO, 1994). These methods are similar to those used by the US EPA (IRIS) and the US FDA (Red Book) and others. The method is outlined here to give a better understanding of the origin of the PDE values. It is not necessary to perform these calculations in order to use the PDE values tabulated in Appendix 2 of this document.

PDE is derived from the NOEL, or the Lowest-Observed-Effect Level (LOEL) in the most relevant animal study as follows:

PDE = NOEL x Mass Adjustment/[F1 x F2 x F3 x F4 x F5] *(1)*

The PDE is derived preferably from a NOEL. If no NOEL is obtained, the LOEL may be used. Modifying factors proposed here, for relating the data to humans, are the same kind of "uncertainty factors" used in Environmental Health Criteria (EHC 170, World Health Organization [WHO], Geneva, 1994), and "modifying factors" or "safety factors" in Pharmacopeial Forum. The assumption of 100% systemic exposure is used in all calculations regardless of route of administration.

The modifying factors are as follows:

F1 = A factor to account for extrapolation between species

F1 = 5 for extrapolation from rats to humans

F1 = 12 for extrapolation from mice to humans

F1 = 2 for extrapolation from dogs to humans

F1 = 2.5 for extrapolation from rabbits to humans

F1 = 3 for extrapolation from monkeys to humans

F1 = 10 for extrapolation from other animals to humans

F1 takes into account the comparative surface area: body mass ratios for the species concerned and for man. Surface area (S) is calculated as:

$S = kM^{0.67}$ (2)

in which M = body mass, and the constant k has been taken to be 10. The body masses used in the equation are those shown below in Table A.1.1

F2 = A factor of 10 to account for variability between individuals

A factor of 10 is generally given for all elemental impurities, and 10 is used consistently in this guideline

F3 = A variable factor to account for toxicity studies of short-term exposure

F3 = 1 for studies that last at least one half lifetime (1 year for rodents or rabbits; 7 years for cats, dogs and monkeys)

F3 = 1 for reproductive studies in which the whole period of organogenesis is covered

F3 = 2 for a 6-month study in rodents, or a 3.5-year study in non-rodents

F3 = 5 for a 3-month study in rodents, or a 2-year study in non-rodents

F3 = 10 for studies of a shorter duration

In all cases, the higher factor has been used for study durations between the time points, e.g., a factor of 2 for a 9-month rodent study.

F4 = A factor that may be applied in cases of severe toxicity, e.g., non-genotoxic carcinogenicity, neurotoxicity or teratogenicity. In studies of reproductive toxicity, the following factors are used:

F4 = 1 for fetal toxicity associated with maternal toxicity

F4 = 5 for fetal toxicity without maternal toxicity

F4 = 5 for a teratogenic effect with maternal toxicity

F4 = 10 for a teratogenic effect without maternal toxicity

F5 = A variable factor that may be applied if the no-effect level was not established

When only an LOEL is available, a factor of up to 10 could be used depending on the severity of the toxicity.

The mass adjustment assumes an arbitrary adult human body mass for either sex of 50 kg. This relatively low mass provides an additional safety factor against the standard masses of 60 kg or 70 kg that are often used in this type of calculation. It is recognized that some adult patients weigh less than 50 kg; these patients are considered to be accommodated by the built-in safety factors used to determine a PDE.

As an example of the application of this equation, consider a toxicity study of cobalt in human volunteers is summarized in Agency for Toxic Substances and Disease Registry (ATSDR, 2004, op/. cit., Davis JE and Fields JP. Proc Soc Exp Biol Med 1958;99:493-5). The Lowest-Observed-Adverse-Effect Level (LOAEL) for polycythemia is 1 mg/kg/day. The PDE for cobalt in this study is calculated as follows:

PDE = 1 mg/kg/day x 50 kg/[1 x 10 x 10 x 1 x 10] = 0.05 mg/day = 50 µg/day

In this example,

F1 = 1 study in humans

F2 = 10 to account for differences between individual humans

F3 = 10 because the duration of the study was only 3 weeks

F4 = 1 because no severe toxicity was encountered

F5 = 10 because a LOAEL was used

849 **Table A.1.1: Values Used in the Calculations in this Document**

Rat body weight	425 g	Mouse respiratory volume	43 L/day
Pregnant rat body weight	330 g	Rabbit respiratory volume	1440 L/day
Mouse body weight	28 g	Guinea pig respiratory volume	430 L/day
Pregnant mouse body weight	30 g	Human respiratory volume	28,800 L/day
Guinea pig body weight	500 g	Dog respiratory volume	9,000 L/day
Rhesus monkey body weight	2.5 kg	Monkey respiratory volume	1,150 L/day
Rabbit body weight (pregnant or not)	4 kg	Mouse water consumption	5 mL/day
Beagle dog body weight	11.5 kg	Rat water consumption	30 mL/day
Rat respiratory volume	290 L/day	Rat food consumption	30 g/day

850

851 **Appendix 2: Established PDEs for Elemental Impurities**

852 **Table A.2.1: Permitted Daily Exposures for Elemental Impurities**[1]

Element	Class[2]	Oral PDE µg/day	Parenteral PDE, µg/day	Inhalation PDE, µg/day
As	1	15	15	1.9
Cd	1	5.0	6.0	3.4
Hg	1	40	4.0	1.2
Pb	1	5.0	5.0	5.0
Co	2A	50	5.0	2.9
Mo	2A	180	180	7.6
Se	2A	170	85	140
V	2A	120	12	1.2
Ag	2B	170	35	6.9
Au	2B	130	130	1.3
Ir[3]	2B	1000	10	1.4
Os[3]	2B	1000	10	1.4
Pd	2B	100	10	1.0
Pt	2B	1000	10	1.4
Rh[3]	2B	1000	10	1.4
Ru[3]	2B	1000	10	1.4
Tl	2B	8.0	8.0	69
Ba	3	13000	1300	340
Cr	3	11000	1100	2.9
Cu	3	1300	130	13
Li	3	780	390	25
Ni	3	600	60	6.0
Sb	3	1200	600	22
Sn	3	6400	640	64

853 [1] PDEs reported in this table are rounded to 2 significant figures (µg/day).
854 [2] Classification as defined in Section 4.
855 [3] Insufficient data to establish an appropriate PDE; the PDE was established based on
856 platinum PDE.
857

858 **Table A.2.2: Permitted Concentrations of Elemental Impurities for Option 1**

859 The values presented in this table represent permitted concentrations in micrograms per
860 gram for elemental impurities in drug products, drug substances and excipients. These
861 concentration limits are intended to be used when Option 1 is selected to assess the
862 elemental impurity content in drug products with daily doses of not more than 10 grams
863 per day. The numbers in this table are based on Table A.2.1.

Element	Class	Oral Concentration µg/g	Parenteral Concentration µg/g	Inhalation Concentration µg/g
As	1	1.5	1.5	0.29
Cd	1	0.50	0.60	0.34
Hg	1	4.0	0.40	0.12
Pb	1	0.50	0.50	0.50
Co	2A	5.0	0.50	0.29

Mo	2A	18	18	0.76
Se	2A	17	8.5	14
V	2A	12	1.2	0.12
Ag	2B	17	3.5	0.69
Au	2B	13	13	0.13
Ir**	2B	100	1.0	0.14
Os**	2B	100	1.0	0.14
Pd	2B	10	1.0	0.10
Pt	2B	100	1.0	0.14
Rh**	2B	100	1.0	0.14
Ru**	2B	100	1.0	0.14
Tl	2B	0.80	0.80	6.9
Ba	3	1300	130	34
Cr	3	1100	110	0.29
Cu	3	130	13	1.3
Li	3	78	39	2.5
Ni	3	60	6.0	0.60
Sb	3	120	60	2.2
Sn	3	640	64	6.4

** Insufficient data to establish an appropriate PDE; the PDE was established based on platinum PDE

Appendix 3: Individual Safety Assessments

ANTIMONY

Summary of PDE for Antimony

Antimony (Sb)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	1200	600	22

Introduction

Antimony (Sb) is a silvery white naturally occurring metalloid element that is used in various manufacturing processes. Small amounts of Sb are found in the earth's crust. It exists in valence states of 3 and 5. Metallic Sb and a few trivalent Sb compounds are the most significant regarding exposure potential and toxicity. Some antimonials, such as Sb potassium tartrate, have been used medicinally as parasiticides. Antimony trioxide is being used as a catalyst (e.g., in the manufacturing of PolyEthylene Terephthalate [PET] used for container closure system components). Antimony is nutritionally not essential and no metabolic function is known (ATSDR, 1992).

Safety Limiting Toxicity

Because of the limited *in vitro* genotoxicity data and the lack of *in vivo* tests, the genotoxicity of Sb cannot be determined (ATSDR, 1992). In humans and animals, the gastrointestinal tract (irritation, diarrhea, vomiting) appears to be the primary target organ after oral exposure. In subchronic studies in rats lower mean body weights and adverse liver findings were the most sensitive endpoints. Inhalation of high levels of Sb over a long period can cause adverse respiratory effects in both humans and animals.

PDE – Oral Exposure

Limited oral data on Sb exposure is available in mice and rats (Schroeder *et al.* 1968; Schroeder *et al.* 1970; Poon *et al.* 1998). The WHO evaluated Sb in drinking water (WHO, 2003). Lynch *et al.* concluded that a NOAEL from a 90 day drinking water rat study using antimony potassium tartrate was 6 mg/kg/day based on lower mean body weight and reduced food consumption (Lynch, 1999). This finding is consistent with the earlier reports from Schroeder *et al.* Thus, the Permitted Daily Exposure (PDE) for oral exposure was determined on the basis of the lowest NOAEL, i.e., 50 mg/L (equivalent to 6.0 mg Sb/kg/day).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as below:

PDE = 6000 µg/kg/day x 50 kg / 5 x 10 x 5 x 1 x 1 = 1200 µg/day.

PDE – Parenteral Exposure

Adverse liver findings were the most sensitive endpoint in rats after repeated intraperitoneal administration. Thus, the PDE for intraperitoneal exposure was determined on the basis of the lowest NOAEL, i.e., 3.0 mg Sb/kg/day. This value was obtained from a 90-day study in rats (based on adverse liver findings at 6 mg/kg in male rats exposed to Sb potassium tartrate *via* intraperitoneal injection) (NTP, 1992).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the human intraperitoneal PDE is calculated as below:

PDE = 3000 µg/kg/day x 50 kg / 5 x 10 x 5 x 1 x 1 = 600 µg/day.

PDE – Inhalation Exposure

Sub chronic and chronic inhalation rat studies have been conducted. The lung effects observed across these studies were consistent. Using the data from a 13 week inhalation rat study using antimony trioxide dust, (Newton *et al.* 1994), a NOAEL of 1.08 mg/m^3 was used to determine the inhalation PDE (~83% Sb). At higher dose levels an increase in mean absolute and relative lung weights were observed, a finding not seen in the one year oncogenicity study.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated as:

$$\text{For continuous dosing} = \frac{0.9 \text{ mg/m}^3 \times 6 \text{ h} \times 5 \text{ d}}{24 \text{ h} \times 7 \text{ d}} = \frac{0.16 \text{ mg/m}^3}{1000 \text{ L/m}^3} = 0.00016 \text{ mg/L}$$

$$\text{Daily dose} = \frac{0.00016 \text{ mg/L} \times 290 \text{ L/d}}{.425 \text{ kg bw}} = 0.11 \text{ mg/kg/d}$$

PDE = 0.11 mg/kg/d x 50 kg / 5 x 10 x 5 x 1 x 1 = 22 µg/d.

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948 **ARSENIC**

949 **Summary of PDE for Arsenic**

Arsenic (As)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	15	15	1.9

950

951 **Introduction**

952 Arsenic (As) is ubiquitous in the environment and present in food, soil, drinking water
953 and in air. Inorganic As occurs in trivalent (e.g., arsenic trioxide, sodium arsenite) or
954 pentavalent forms (e.g., sodium arsenate, arsenic pentoxide, arsenic acid). Arsenic has no
955 known useful biological function in human or mammalian organisms. This assessment
956 focuses on inorganic As, since this is most relevant for drug products.

957 **Safety Limiting Toxicity**

958 Inorganic arsenic has shown to be genotoxic, but not mutagenic and has been
959 acknowledged as a human carcinogen (Group 1; IARC, 2012).

960 Due to its ubiquitous nature and toxicity profile, there have been many risk assessments
961 conducted of arsenic and arsenic compounds, which utilize non-threshold, linear dose
962 response approaches (Meharg and Raab, 2010).

963 The effects of arsenic in humans for the most part have not been reproduced in animals,
964 so the risk assessments have to rely heavily upon epidemiology data in populations with
965 high exposure concentrations (Schuhmacher-Wolz *et al.* 2009). In humans, both cancer
966 and non-cancer effects have been linked to arsenic exposure. Oral exposure has been
967 linked to cancers of the skin, liver, lung, kidney and bladder. Following inhalation
968 exposure there is evidence for an increased risk of lung cancer (ATSDR, 2007; IARC,
969 2012; EU EFSA, 2009; WHO, 2011; US EPA, 2010).

970 The skin (dyspigmentation, palmoplantar keratosis) and gastrointestinal tract (e.g.,
971 nausea) appear to be the most sensitive targets for non-cancer adverse effects after oral
972 ingestion while vascular disease, reproductive effects and neurological effects are also
973 reported as non-cancer endpoints (IARC, 2012; Schuhmacher-Wolz *et al.* 2009; US EPA,
974 2007). Oral exposure studies suggest that skin lesions may appear at levels above 0.02
975 mg As/kg/day; no effects were generally seen at levels from 0.0004 to 0.01 mg As/kg/day
976 (ATSDR, 2007). There are insufficient epidemiological data to set a LOEL or NOEL for
977 other endpoints. The regions of hyperkeratosis may evolve into skin cancers (ATSDR,
978 2007) and can possibly be considered predictive of skin and internal cancers and the non-
979 cancer long-term adverse health effects (Chen *et al.* 2005; Hsu *et al.* 2013; Ahsan and
980 Steinmaus, 2013).

981 Studies of large populations (~40,000) exposed to arsenic concentrations in well water at
982 1000 µg/L and higher in southwestern Chinese Taipei have been the basis of risk
983 assessments of skin cancer, and more recently of bladder and lung cancer (US EPA,
984 2010). Recent meta-analyses of cancer risk have indicated no additional bladder cancer
985 risk at low dose exposure (<100–200 µg/L) (Chu and Crawford-Brown, 2006, 2007; Mink
986 *et al.* 2008). This is consistent with the work of Schuhmacher-Wolz *et al.* (2009).

987 The inhalation unit risk for cancer is 0.0043 per µg/m^3 has been established by the US
988 EPA based on data from two US smelters (US EPA, 2007). The Texas Commission on
989 Environmental Quality provided an update to the US EPA Unit Risk Factor (URF),
990 incorporating additional years of follow-up to the US EPA data and additional data on

991 workers from the United Kingdom and Sweden, and calculated a URF of 0.0015 per
992 µg/m^3. This URF translates to an air concentration of 0.067 µg/m^3 at a risk of 1 in
993 100,000 excess lung cancer mortality (Erraguntla *et al.* 2012).

994 **PDE – Oral Exposure**

995 The oral PDE is based on the chronic effects of As to skin and sets the limit at 15 µg/day
996 based on ATSDR Minimal Risk Level (MRL) and US EPA limit of 0.0003 mg/kg/day
997 (ATSDR, 2007; US EPA 2007; EU EFSA, 2009). The PDE calculated based on the
998 ATSDR MRL is consistent with drinking water standards (WHO, 2011).

999 0.0003 mg/kg/day x 50 kg human = 0.015 mg/day = 15 µg/day.

1000 No modifying factors were applied because they are incorporated into the derivation of
1001 the MRL.

1002 **PDE – Parenteral Exposure**

1003 The oral bioavailability of As is ~95%. The most direct evidence is from a study that
1004 evaluated the 6-day elimination of arsenic in healthy humans who were given water
1005 from a high-arsenic sampling site (arsenic species not specified) and that reported
1006 approximately 95% absorption (Zheng *et al.* 2002). Therefore the PDE is identical to the
1007 oral PDE.

1008 PDE = 15 µg/day.

1009 **PDE – Inhalation Exposure**

1010 Increased risk of lung cancer and other respiratory disorders have been reported
1011 following inhalation exposure to workers in the occupational setting. The rationale for
1012 using a cancer endpoint for inhalation to set the PDE is the relative lack of information
1013 on linear-dose extrapolation, as compared to the oral route. No modifying factors are
1014 needed as the URF were determined for the protection of the general public. Based on
1015 the assessment conducted by Erraguntla *et al.* (2012), based on the risk of 1:100.000, the
1016 inhalation PDE is:

1017 0.067 µg/m^3 ÷ 1000 L/m^3 x 28800 L/d = 1.9 µg/d.

1018 No modifying factors were applied because the PDE is based on the multiplicate relative
1019 risk model described by Erraguntla *et al.* (2012).

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1063

BARIUM

Summary of PDE for Barium

Barium (Ba)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	13000	1300	340

Introduction

Barium (Ba) is a dense, silver-white, soft alkaline earth metal that oxidizes readily in moist air and reacts with water. The Ba^{2+} ion and the water soluble compounds of Ba (chloride, nitrate, hydroxide) are toxic. The insoluble compounds of barium, such as barium sulfate, do not generate free Ba^{2+} ions in the gastrointestinal tract and therefore are generally nontoxic to humans. Ba is nutritionally not essential and no metabolic function is known. Barium sulfate is used as a support for catalyst (e.g., Pd).

Safety Limiting Toxicity

In animals and humans, the kidney appears to be the most sensitive target of toxicity resulting from repeated ingestion of soluble Ba salts. Chronic rodent studies support the evidence for an association between Ba exposure and renal toxicity. In humans, repeated exposure to Ba oxide *via* inhalation may cause bronchitis, including cough, phlegm, and/or shortness of breath.

PDE – Oral Exposure

Mice and rat Ba drinking water studies have been conducted (NTP, 1994). Based on the review of these data, the mouse was determined to be the more sensitive species. The 2-year drinking water study in mice with barium chloride dihydrate was selected as the principal study and compound-related nephropathy was identified as the critical effect for deriving a PDE for Ba and its soluble salts. The lesions were characterized by tubule dilatation, renal tubule atrophy, tubule cell regeneration, hyaline cast formation, multifocal interstitial fibrosis, and the presence of crystals, primarily in the lumen of the renal tubules. These changes were characterized as morphologically distinct from the spontaneous degenerative renal lesions commonly observed in aging mice.

The oral PDE was determined on the basis of the NOAEL of 500 mg/L (equivalent to 30 mg Ba/kg/day), using the modifying factors (F1-F5 as discussed in Appendix 1).

PDE = 30 mg/kg/day x 50 kg / 12 x 10 x 1 x 1 x 1 = 12.5 mg/day ~13.000 µg/day.

PDE – Parenteral Exposure

No relevant data on parenteral exposure to barium compounds were found. The bioavailability of Ba is estimated to be 20 – 60% in adults and infants, respectively (ATSDR, 2007). Thus, a modifying factor of 10 of the oral PDE was used.

PDE = 13.000 µg/day/ 10 = 1300 µg/day.

PDE – Inhalation Exposure

No relevant data on inhalation exposure to barium compounds were found. US DoL (2013) has a reported TWA of 0.5 mg/m^3 based on soluble Ba salts.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated as:

For continuous dosing = $\frac{500\ \mu g/\ m^3 \times 8\ hr/day \times 5\ days/week}{24\ hr/day \times 7\ days/week \times 1000\ L/m^3}$

$= 0.119\ \mu g/L$

Daily dose = $\frac{0.119\ \mu g/L \times 28800\ L}{50\ kg} = 68.6\ \mu g/kg$

PDE = $\frac{68.6\ \mu g/kg \times 50\ kg}{1 \times 10 \times 1 \times 1 \times 1} = 343\ \mu g/day \sim 340\ \mu g/day.$

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CADMIUM

Summary of PDE for Cadmium

Cadmium (Cd)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	5.0	6.0	3.4

Introduction

Cadmium (Cd) is a transition metal whose most abundant naturally-occurring isotope is non-radioactive. It is found in nature in mineral forms and is obtained for commercial uses principally from cadmium ore (ATSDR, 2012). Cadmium exists as a salt form in the +2 oxidation state only. Some cadmium salts are water soluble such as cadmium chloride, cadmium sulfate and cadmium nitrate; other insoluble salts can become more soluble by interaction with acids, light or oxygen. Cadmium, cadmium oxide, cadmium salts on borosilicate carrier are used as catalysts in organic synthesis. Silver cadmium alloy is used in the selective hydrogenation of carbonyl compounds.

Safety Limiting Toxicity

Cadmium has shown to be genotoxic, but not mutagenic and has been acknowledged as a human carcinogen (Group 1; IARC, 2012). Cadmium and cadmium compounds cause cancer of the lung. Also, positive associations have been observed between exposure to cadmium and cadmium compounds and cancer of the kidney and of the prostate.

A sensitive endpoint for oral exposure to cadmium and cadmium salts is renal toxicity (Buchet *et al.* 1990). Skeletal and renal effects are observed at similar exposure levels and are a sensitive marker of cadmium exposure (ATSDR, 2012).

Evidence from numerous epidemiologic studies assessing inhalation exposures to cadmium *via* both occupational and environmental routes has demonstrated an increased risk of developing cancer (primarily lung) that correlates with inhalation exposure to cadmium (IARC, 2012; NTP, 2004).

PDE – Oral Exposure

A sensitive endpoint for oral exposure to cadmium and cadmium salts is renal toxicity (Buchet *et al.* 1990). Skeletal and renal effects are observed at similar exposure levels and are a sensitive marker of cadmium exposure (ATSDR, 2012). A number of oral exposure studies of cadmium in rats and mice showed no evidence of carcinogenicity. Therefore the renal toxicity endpoint was used to establish the oral PDE for cadmium, following the recommendations of ATSDR, a level of 0.1 µg/kg for chronic exposure is used to set the oral PDE. This is in line with the WHO drinking water limit of 0.003 mg/L/day (WHO 2011).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as:

PDE = 0.1 µg/kg/day x 50 kg = 5.0 µg/day.

PDE – Parenteral Exposure

12 week study in rats given daily subcutaneous injections of 0.6 mg/kg Cd, 5 days per week showed renal damage at week 7 and later (Prozialeck, 2009). The LOAEL of this study is 0.6 mg/kg.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the parenteral PDE is calculated as:

PDE = 0.6 mg/kg/day x 50 kg / 5 x 10 x 5 x 10 x 2 = 6.0 µg/day.

F4 was chosen as 10 because cadmium is carcinogenic by the inhalation route. F5 was set at 2, since no NOAEL was identified in this study.

PDE – Inhalation Exposure

The use of 5 µg/m^3 as the PEL (US DoL, 2013) was considered acceptable as cadmium is non-mutagenic. This PDE is similar to the quantitative estimate of carcinogenic risk from inhalation exposure to cadmium (1:10000 risk, US EPA, 1992; EU SCOEL, 2010).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated as:

For continuous dosing = 5 µg/m^3 ÷1000 L/m^3 = 0.005 µg/L

0.005 µg/L x 8 hours x 5 days ÷ 24 hours x 7 days = 0.0012 µg/L

Daily Dose = 0.0012 µg/L x 28800 L/day ÷ 50 kg = 0.69 µg/kg

PDE = 0.69 µg/kg x 50 kg / 1 x 10 x 1 x 1 x 1 = 3.4 µg/day.

A modifying factor F2 of 10 was applied to cover the full population with the data coming from the worker population.

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1203

CHROMIUM

Summary of PDE for Chromium

Chromium (Cr III)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	11000	1100	2.9

Introduction

Chromium (Cr) is found in a variety of oxidation states, the most important being Cr 0 (in stainless steel) Cr II, III and VI. Cr II is readily oxidized and is used as a reducing agent in chemical synthesis. Cr VI is a powerful oxidant, chromate, CrO_4^{2-}, and dichromate, $Cr_2O_7^{2-}$, being the best known oxyanions. Cr III, the most abundant environmental form, is an essential element that plays a role in glucose metabolism. Chromium deficiency causes changes in the metabolism of glucose and lipids and may be associated with maturity-onset diabetes, cardiovascular diseases, and nervous system disorders (Anderson, 1993, 1995). Sources of chromium in pharmaceuticals may include colorants, leaching from equipment or container closure systems, and catalysts. With the exception of use as a catalyst, intake of chromium from pharmaceuticals will be in the form of metallic chromium (Cr 0) or Cr III rather than the more toxic Cr VI; therefore, for drug products, this safety assessment is based on the known toxicity of Cr III and Cr VI is excluded from this assessment. Chromium present as a colorant (e.g., chromium oxide green, chromium hydroxide green; see 21 CFR 72) is intentionally added and thus beyond the scope of this guidance.

Safety Limiting Toxicity

The data was reviewed to identify the safety limiting toxicities based on routes of administration.

PDE – Oral Exposure

No specific target organ toxicities have been identified for the oral intake of chromium. Generally oral intake of 5 mg/kg/day Cr III (US EPA, 1998) is not expected to be associated with adverse health.

The 2 year NTP studies (2010) on the carcinogenicity of Cr (III) picolinate administered in feed to rats and mice provided the most relevant safety information for Cr as present in drug products. The NOAEL was 90 mg/kg Cr (III) picolinate (11.9 weight %; 10.7 mg/kg/day CrIII) in rats based on increase in the incidence of preputial gland adenoma in male rats at 460 mg/kg. This finding was not dose-dependent and was considered an equivocal finding by the study authors. This finding was not observed male mice or in the female counterpart in either species (clitoral gland). In the absence of a treatment-related carcinogenic finding, F4 was set at 1.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as:

PDE = 10.7 mg/kg/day x 50 kg/ 5 x 10 x 1 x 1 x 1 = 10.7 mg/day ~11000 µg/day.

PDE – Parenteral Exposure

Recommendation for the nutritional intravenous administration of Chromium (III) vary per age group between 0.05 µg/kg/day in preterm infants and 15 µg/kg in adults (Moukazel, 2009). There is insufficient information to assess if exceeding these

recommended daily doses may lead to adverse responses e.g., for the kidney especially in newborns and preterm infants.

The safety review for Cr was unable to identify any significant assessments upon which to calculate a PDE for parenteral routes of exposure. On the basis of an oral bioavailability of about 10% for chromium and inorganic chromium compounds (ATSDR, 2012), the recommended PDE for chromium for a parenteral exposure is:

PDE = 11000 µg/day/10 = 1100 µg/day.

PDE – Inhalation Exposure

The study by Deralenko (1999) used inhalation of Cr (III) sulfate particles during 13 weeks (6h/day and 5 days per week) causing predominantly chronic inflammation of the airways (mononuclear infiltrate, particular material) and locally thickening of alveolar walls. The effect was observed at all doses. The LOAEL is 17 mg/m^3 (3 mg CrIII/m^3). A lack of systemic toxicity was noted in a 13 week inhalation study in rats administered soluble or insoluble Cr (III). Based on these data the on these data, the inhalation MRL of 0. 1µg/m^3 was used to set the PDE (ATSDR, 2012).

PDE =0.0001 mg/ m^3 /1000 m^3/L x 28800 L/day = 2.9 µg/day.

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COBALT

Summary of PDE for Cobalt

Cobalt (Co)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	50	5.0	2.9

Introduction

Cobalt (Co) is a naturally-occurring element, often combined with other elements such as oxygen, sulfur, and arsenic. Co is essential in the human body because it is an integral component of Vitamin B-12 and functions as a co-enzyme for several enzymes critical in the synthesis of hemoglobin and the prevention of pernicious anemia. The Recommended Dietary Allowance of vitamin B12 is 2.4 µg/day, which corresponds to 0.1 µg of Co. No essential biological function of inorganic Co in the human body has been identified. Cobalt compounds (e.g., cobalt octoate) are being used as catalysts in selective hydrogenation.

Safety Limiting Toxicity

The IARC (2006) concluded that Co sulphate and other soluble Co (II) salts are possible human carcinogens (Group 2B). The data indicate the location of tumors is limited to the lung in rats and humans.

Polycythemia is considered to be the most sensitive finding after repeated oral exposure to humans. Inhalation exposure of humans to Co has been associated with a severe and progressive respiratory disease known as hard-metal pneumoconiosis, as well as asthma and contact dermatitis.

PDE – Oral Exposure

The oral PDE is based on the available human data. Polycythemia was the most sensitive finding in humans after repeated oral exposure to 150 mg of cobalt chloride (~1 mg Co /kg/day). The oral PDE was determined on the basis of the LOAEL of 1 mg/kg/day in male human volunteers after oral exposure over a period of 22 days (WHO, 2006).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as below:

PDE = 1 mg/kg/day x 50 kg / 1 x 10 x 10 x 1 x 10 = 0.05 mg/day = 50 µg/day.

PDE – Parenteral Exposure

No relevant data on parenteral exposure to cobalt compounds were found. On the basis of the oral bioavailability ranging largely from 18-97% for cobalt and inorganic cobalt compounds (ATSDR, 2004). Using a safety factor of 10 to account for low bioavailability, the PDE for cobalt for parenteral exposure is:

PDE = 50 µg/day / 10 = 5.0 µg/day.

PDE – Inhalation Exposure

Co sulphate and other soluble Co (II) salts are possible human carcinogens (Group 2B) which can induce lung tumors.

Pneumoconiosis, asthma and contact dermatitis were the principal non-carcinogenic effects in humans after chronic inhalation. For the calculation of the inhalation PDE, the chronic inhalation MRL of 0.1 microgram / m^3 was used (ATSDR, 2010).

0.0001 mg/ m^3 /1000 m^3/L x 28800 L/day = 2.9 µg/day.

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COPPER

Summary of PDE for Copper

Copper (Cu)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	1300	130	13

Introduction

Copper (Cu) is a Group 11 element of the first transition series and has two main oxidation states, Cu I and Cu II. It is an essential trace element in both animals and humans. Copper plays a vital role in a number of critical enzyme systems and is closely linked with normal hematopoiesis and cellular metabolism. Copper compounds (e.g., copper chromite) are being used as catalysts in hydrogenolysis and decarboxylation reactions

Safety Limiting Toxicity

A general review of relevant safety data for animals and humans indicates that copper can produce adverse effects to the gastrointestinal tract, liver, and kidney upon ingestion of toxic doses (Araya *et al.* 2003).

PDE – Oral Exposure

Studies on cupric sulfate and copper 8-quinolinolate have been conducted in mice and rats and dogs (EHC, 1998). Rats were determined to be the more sensitive species to effects on liver and kidney. In a 13 week study in rats the NOAEL was 17 mg/kg/day for copper sulfate, equivalent to 6.7 mg Cu/kg/day (Hebert, 1993).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as:

PDE = 6.7 mg/kg/day x 50 kg / 5 x 10 x 5 x 1 x 1 = 1.34 mg/day = 1340 µg/day ~1300 µg/day.

PDE – Parenteral Exposure

The safety review for copper was unable to identify any significant assessments upon which to calculate a PDE for parenteral routes of exposure. The human gastrointestinal system can absorb 30-40% of ingested copper from the typical diets consumed in industrialised countries (Wapnir, 1998). On the basis of limited oral bioavailability of 30%-40% for copper and inorganic copper salts, the recommended PDE for copper for parenteral exposure is:
PDE = 1340 µg/day / 10 = 134 µg/day ~130 µg/day.

PDE – Inhalation Exposure

The available data on the toxicity of inhaled copper were considered inadequate for derivation of acute-, intermediate-, or chronic-duration inhalation MRLs (ATSDR, 2004).

The inhalation PDE was calculated by dividing the oral PDE by 100 (as described in Section 3.1).

1340/100 = 13.4 µg/day ~13 µg/day.

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GOLD

Summary of PDE for Gold

Gold (Au)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	130	130	1.3

Introduction

Gold (Au) exists in metallic form and in oxidation states of +1 to +5, the monovalent and trivalent forms being the most common. Elemental gold is poorly absorbed and consequently is not considered biologically active. Gold is being used on a carrier or in complexes like gold chloride and $L–Au^+$ (where L is a phosphane, phosphite, or an arsine; Telles, 1998), as catalysts in organic synthesis. The only source for gold in drug products comes from the use as catalyst. Gold (I) salts are used therapeutically.

Safety Limiting Toxicity

Most knowledge of gold toxicity is based on therapeutic uses of gold. Currently available therapies are gold salts of monovalent gold (I) with a sulfur ligand (Au-S), but metallic gold has also been studied. No toxicity was seen in 10 patients administered colloidal metallic gold (monoatomic gold) at 30 mg/day for one week followed by 60 mg/day the second week or the reverse schedule. The patients were continued on trial for an additional 2 years at 30 mg/day. There was no evidence of hematologic, renal or hepatic cytotoxicity but some improvement in clinical symptoms of rheumatoid arthritis and in cytokine parameters were noted (Abraham and Himmel, 1997).

Long term animal data are available with Au compounds. However, these studies have been performed with monovalent gold Au I and are not considered sufficiently relevant to assess the potential toxicity of Au in pharmaceutical products.

Au (III) is thought to be the more toxic form and is used in catalysis, e.g., as gold trichloride. There is only limited data on gold (III) complexes. In one study, the gold (III) compound $[Au(en)Cl_2]Cl$ (dichloro(ethylenediamine-aurate(III) ion) caused minimal histological changes in the kidney and liver of rats, and no renal tubular necrosis, at a dose of 32.2 mg/kg in mice administered the compound intraperitoneally for 14 days (Ahmed *et al.* 2012).

PDE – Oral Exposure

The toxicologically significant endpoint for gold exposures is renal toxicity.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as:

PDE = 32.2 mg/kg x 50 kg / 12 x 10 x 10 x 1 x 10 = 134 µg/day ~130 µg/day.

F5 was put at 10 because the NOAEL was not established and the toxicological assessment was not complete.

PDE – Parenteral Exposure

In humans, 50 mg intramuscular (IM) injections of gold sodium thiomalate resulted in >95% bioavailability (Blocka, 1986). In rabbits, ~70 % of the gold sodium thiomalate was absorbed after an IM injection of 2/mg/kg (Melethil, 1987).

Based on high bioavailability, the parenteral PDE is equivalent to the oral PDE.

PDE = 130 µg/day.

PDE – Inhalation Exposure

In the absence of relevant inhalation and parenteral data, a modifying factor of 100 was applied to the oral PDE as described in Section 3.1.

PDE = 134 /100 = 1.34 µg/day ~1.3 µg/day.

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LEAD

Summary of PDE for Lead

Lead (Pb)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	5.0	5.0	5.0

Introduction

Lead (Pb) is the most common heavy element. It occurs in organic and inorganic forms. The generally bivalent Pb compounds include water-soluble salts such as Pb acetate as well as insoluble salts such as Pb oxides. Organic Pb compounds include the gasoline additives tetramethyl- and tetraethyl-lead. Organic Pb compounds undergo fairly rapid degradation in the atmosphere and form persistent inorganic Pb compounds in water and soil. Pb has no known useful biological function in human or mammalian organisms (ATSDR, 2007).

Safety Limiting Toxicity

In humans and animals, exposure to Pb may cause neurological, reproductive, developmental, immune, cardiovascular and renal health effects. In general, sensitivity to Pb toxicity is greater when there is exposure *in utero* and in children compared to adults. A target blood level of 1-2 µg/dL was set, and using modelling programs (US EPA, 2009) that assumed 100% bioavailability and no other exposure, a PDE was obtained. For this reason, the PDEs are the same regardless of the route of administration.

PDE – Oral Exposure

Adverse neurobehavioral effects are considered to be the most sensitive and most relevant endpoint in humans after oral exposure. Data from epidemiological studies show that blood Pb levels <5 µg/dL may be associated with neurobehavioral deficits in children (NTP, 2011).

According to the US EPA model (Integrated Exposure Uptake Biokinetic (IEUBK) Model, 1994) (100% absorption, no other sources of lead), oral intake of 5 µg/day translates into a blood level of 1-2 µg/dL for children age 0-7 years (0-82 months).

PDE = 5.0 µg/day.

PDE – Parenteral Exposure

The oral effects of Pb are based on blood levels. Therefore, the parenteral PDE is equal to the oral PDE of 5.0 µg/day.

PDE – Inhalation Exposure

The oral effects of Pb are based on blood levels. Therefore, the inhalation PDE is equal to the oral PDE of 5.0 µg/day.

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1487

1488 **LITHIUM**

1489 **Summary of PDE for Lithium**

Lithium (Li)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	780	390	25

1490 **Introduction**

1491 Lithium (Li) is a common metal that is present in plant and animal tissues. Lithium is
1492 used as a therapeutic agent to treat bipolar disease. Lithium is being used alone or in
1493 combination with other metals as catalyst. Lithium compounds (e.g., lithium aluminum
1494 hydride) are being used as reagents in organic synthesis.

1495 Lithium exists commonly as a salt in the +1 form oxidation state only.

1496 **Safety Limiting Toxicity**

1497 The data was reviewed to identify the safety limiting toxicities based on routes of
1498 administration.

1499 **PDE – Oral Exposure**

1500 There is a minimal amount of data on the effects of lithium carbonate on the immune
1501 system. A 14 day mouse study was conducted to assess the effects of lithium carbonate
1502 on the immune system (NTP, 1986). Doses were modified to 100, 300 and 400 mg/kg in
1503 repeat and later studies because of a lack of effect at 50 and 200 mg/kg. Findings
1504 included dose-dependent effects on decreased in liver and thymus weight, and changes in
1505 leukocytes and red blood cells and associated parameters.

1506 Using 200 mg/kg/day (18.7 mg Li/kg/day) as the NOAEL and modifying factors (F1-F5 as
1507 discussed in Appendix 1), the PDE is:

1508 PDE = 18.7 mg/kg/day x 50 kg/ 12 x 10 x 10 x 1 x 1 = 0.78 mg/day = 780 µg/day.

1509 **PDE – Parenteral Exposure**

1510 There are no adequate data to develop a parenteral PDE. However, based on oral
1511 bioavailability of 85% (Grandjean, 2009) and using a modifying factor of 2, the parenteral
1512 PDE is calculated as:

1513 PDE = 0.77 mg/day / 2 = 0.39 mg/day =390 µg/day.

1514 **PDE – Inhalation Exposure**

1515 Rabbits were exposed to lithium chloride at 0.6 and1.9 mg/m^3 for 4-8 weeks, 5 days/week
1516 for 6 hours/d (Johansson *et al.* 1988). Lungs were studied by light and electron
1517 microscopy with focus on inflammatory changes. No significant effects were reported, so
1518 the highest dose was used to set the PDE.

1519 Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral
1520 PDE is calculated as:

1521 For continuous dosing: PDE = 1.9 mg/m^3 /1000 L/m^3 = .0019 mg/L

1522 0.0019 mg/L x 6 h/day x 5 days / 24h/day x 7days = 0.000339 mg/L

1523 Daily dose: 0.339 µg/L x 1440 L/day/4 kg = 122.04 µg/kg/day

1524 PDE = 122.04 µg/kg/day x 50kg /2.5x10x10x1x1 = 25 µg/day.

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MERCURY

Summary of PDE for Mercury

Mercury (Hg)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	40	4.0	1.2

Introduction

Mercury (Hg) is an element widely existing in the global environment. Hg exists in three forms: elemental mercury, inorganic mercury and organic mercury. The most likely form of residual mercury in drug products is the inorganic form. Therefore, this safety assessment is based on the relevant toxicological data of elemental or inorganic Hg. This safety assessment and derived PDEs do not apply to organic mercury.

Safety Limiting Toxicity

There is no data to indicate that inorganic mercury is carcinogenic in human. There is limited evidence in experimental animals for the carcinogenicity of mercuric chloride. IARC concluded that inorganic mercury compounds are not classifiable as to their carcinogenicity to humans (Group 3; IARC, 1997).

Inorganic mercury compounds show significantly lower oral bioavailability compared to organic mercury and induce different toxicological effects including neurological, corrosive, hematopoietic, renal effects and cutaneous disease (acrodynia). The safety limiting toxicity for inorganic mercury and salts is renal toxicity.

PDE – Oral Exposure

There were well organized NTP studies of $HgCl_2$ up to 2 years. The 6 month gavage study in rats was selected because it had more detailed clinical pathology assessment and wider range of doses than the 2 year study. Based on adverse renal effects from the 6-months rat study (NTP, 1993), the LOAEL was 0.23 mg/kg/day for mercury (0.16 mg/kg day for mercury when corrected for 7 days of exposure/week).

Using the modifying factors (F1-F5 as discussed in Appendix 1) the oral PDE is calculated as:

PDE = 0.16 mg/kg /day x 50 kg / 5 x 10 x 2 x 1 x 2 = 0.04 mg/day = 40 µg/day.

F5 was set to 2, because no NOAEL was identified in the study and the effect at the LOAEL was a slight increase in incidence of an effect also present in the control animals.

PDE – Parenteral Exposure

Animal studies indicate that the oral bioavailability of inorganic mercury is in the 10-30% range (ATSDR, 1999). Therefore, the oral PDE is divided by a factor of 10 (as described in Section 3.1).

PDE = 40/10 = 4.0 µg/day.

PDE – Inhalation Exposure

Neurobehavioral effects are considered to be the most sensitive endpoint following inhalation exposure in humans as shown in occupational studies at the range of air TWA levels between 14 and 20 $\mu g/m^3$ (US EPA, 1995; EU SCOEL, 2007).

The presence of neurobehavioral effects at low-level mercury exposures (14 µg/m^3) in dentists (Ngim *et al.* 1992) indicates that the TWA needs to be considered as a LOAEL.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated based on the long-term inhalation exposure to elemental mercury vapor:

$$\text{For continuous dosing} = \frac{14\ \mu g/m^3 \times 8\ hr/day \times 6\ days/week}{24\ hr/day \times 7\ days/week \times 1000\ L/m^3}$$

$$= 0.004\ \mu g/L$$

$$\text{Daily dose} = \frac{0.004\ \mu g/L \times 28800\ L}{50\ kg} = 2.30\ \mu g/kg$$

$$PDE = \frac{2.30\ \mu g/kg \times 50\ kg}{1 \times 10 \times 1 \times 1 \times 10} = 1.2\ \mu g/day.$$

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MOLYBDENUM

Summary of PDE for Molybdenum

Molybdenum (Mo)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	180	180	7.6

Introduction

The main oxidation states for Mo are IV and VI, the most common forms of which are oxyanions. The predominant form of Mo occurring in soils and natural waters is the molybdate ion, MoO_4^{2-} which forms soluble compounds with a variety of cations including K^+, NH_4^+ and Ca^{2+}. Mo exists in soil in various forms at concentration of 0.1-10 mg/kg. MoO_2 and MoS_2 are insoluble in water. It is widely present in vegetables, dairy products and meats. Mo combinations (e.g., Bi-Mo, Fe-Mo, molybdenum oxide and Mo-complexes) are being used as catalysts in organic synthesis.

Mo deficiency is characterized by night blindness, nausea, disorientation, coma, tachycardia, tachypnea and associated with various biochemical abnormalities including high plasma methionine. In addition an almost undetectable serum uric acid concentration has been reported in a patient receiving total parenteral nutrition (Abumrad et *al.* 1981).

Safety Limiting Toxicity

Molybdenum as the trioxide was not mutagenic (NTP, 1997). Carcinogenicity has not been evaluated by IARC or US EPA.

Alteration of estrus cycle is the most sensitive effect observed in the various rat studies. Absorption and retention of Mo is markedly influenced by interactions with dietary Cu and sulfate and the typical symptoms from excessive Mo intake were similar to those of copper deficiency including weight loss, growth retardation, anorexia, anemia, diarrhea, achromotrichia, testicular degeneration, poor conception, deficient lactation, dyspnea, incoordination and irritation of mucous membranes (Engel *et al.* 1956).

PDE – Oral Exposure

Fungwe *et al.* (1990) examined the effects on fertility and reproductive performance of sodium molybdenate in female rats given drinking water containing 0, 5, 10, 50 or 100 mg Mo/L. After 6 weeks the effect of Mo on the estrous cycle (3 cycles) and vaginal cytology was determined, and some animals then mated to untreated males. Pregnant dams continued to be dosed to day 21 of gestation with Mo and fetal effects determined. Effects on the estrous cycle, gestational weight gain, and the fetus were observed at 10 mg/L and higher; thus, a dose level of 5 mg/L can be considered a NOAEL. Vyskocil and Viau (1999) calculated this NOAEL to be 0.9 mg Mo/kg/day.

Using modifying factors (F1-F5 as discussed in Appendix 1) the oral PDE is:

PDE = 0.9 mg/kg/day x 50 kg / 5 x 10 x 1 x 5 x 1 = 0.180 mg/day = 180 µg/day.

F4 was selected to be 5 based on the presence of fetal effects.

PDE – Parenteral Exposure

In Vyskocil and Viau (1999), it was reported that oral bioavailability in humans ranged from 28-77%. Turnland *et al.* (2005) report that molybdenum absorption was about 90% in healthy men. Therefore, the parenteral PDE is the same as the oral PDE.

PDE= 180 µg/day.

PDE – Inhalation Exposure

Chronic inflammation in the alveoli was seen in rat and mouse. In addition, a slight trend for bronchiolar alveolar adenoma and carcinoma was observed in male rats exposed to molybdenum trioxide in a 2-year inhalation study (NTP, 1997). Lung neoplasms were not seen in female rats. In mice, bronchiolar alveolar adenoma and carcinoma were observed at the lowest dose of 10 mg/m^3 (6.7 mg/m^3 of Mo).

The inhalation PDE was calculated based on the low dose in the mouse carcinogenicity study, where findings of alveolar and bronchiolar carcinoma were observed, using the modifying factors (F1-F5 as discussed in Appendix 1).

6.7 $mg/m^3 \div 1000\ m^3/L = 0.0067$ mg/L

$$\text{For continuous dosing} = \frac{0.0067\ \text{mg/L} \times 6\ \text{hr} \times 5\ \text{d}}{24\ \text{hr} \times 7\ \text{d}} = 0.0012\ \text{mg/L}$$

$$\text{Daily dose} = \frac{0.0012\ \text{mg/L} \times 43\ \text{L/d}}{0.028\ \text{kg}} = 1.83\text{mg/kg}$$

$$\text{PDE} = \frac{1.83\ \text{mg/kg} \times 50\ \text{kg}}{12 \times 10 \times 1 \times 10 \times 10} = 7.6\ \mu\text{g/day}.$$

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NICKEL

Summary of PDE for Nickel

Nickel (Ni)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	600	60	6.0

Introduction

Nickel (Ni) is a Group 10 element of the first transition series. Although Ni may have valences of 0, I, II and III, its main oxidation state is +2. Ni is a naturally occurring metal existing in various mineral forms. In general, the more soluble Ni compounds, including Ni chloride, Ni sulfate, and Ni nitrate, tend to be more toxic than less soluble forms, such as Ni oxide and Ni subsulfide. Ni is nutritionally not essential for humans, but Ni deficiency may cause adverse effects in animals. Nickel as Ni-Al alloys is being used as catalyst in hydrogenation reactions.

Safety Limiting Toxicity

Nickel is genotoxic, but not mutagenic (IARC 2012). There is no indication of carcinogenicity of Ni salts after oral administration. Depending on the type of salt there was an increase in tumors in some rodent inhalation studies (ATSDR, 2005; EU EFSA, 2005). Combining all forms of Ni, IARC (2012) classified Ni as a human carcinogen (Group 1).

In humans and animals, ingestion of large amounts of Ni may cause stomach pain, depression of body weight and adverse effects on blood and kidneys. Humans generally become sensitised to Ni after prolonged contact with the skin. Chronic inhalation may produce adverse changes in lung and nasal cavity in both humans and animals.

PDE – Oral Exposure

Human sensitisation to Ni was used to establish the oral PDE, because it is the most sensitive endpoint. Human data show that an oral challenge dose of 0.012 mg Ni/kg can induce dermatitis in nickel-sensitized individuals. Exposure to these nickel concentrations did not result in dermatitis in non-sensitized individuals (Nielsen 1999). Similar data were presented for 0.02 mg/kg by ATSDR (2005).

PDE = 0.012 mg/kg/day x 50 kg = 0.60 mg/day = 600 µg/day.

PDE – Parenteral Exposure

A human study using a stable nickel isotope estimated that 29–40% of the ingested label was absorbed (based on fecal excretion data) (Patriarca *et al.* 1997). On the basis of limited oral bioavailability of Ni and water-soluble Ni compound. Therefore, the oral PDE is divided by a factor of 10 (as described in Section 3.1).

PDE = 600 µg/day / 10 = 60 µg/day.

PDE – Inhalation Exposure

For calculation of the inhalation PDE, a relevant form of Ni was selected from the available data. In 2 year studies with nickel oxide (the form commonly used in stainless steel coatings), no tumors were observed in hamsters (Wehner *et al.* 1984) or mice (NTP, 1996), but there was some evidence of carcinogenicity in rats (NTP, 2006) and no evidence of carcinogenicity with inhalation of metallic nickel (Oller, 2008).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated based on the NOAEL in the rat study of 0.5 mg Ni/m^3/day.

For continuous dosing 0.5 mg/m^3 / 1000L/m^3 = 0.0005 mg/L

0.0005 mg/L x 6 hr x 5 d /24 hr x 7 d = 0.000089 mg/L

Daily dose 0.000089 mg/L x 290 L/d / 0.425 kg = 0.060 mg/kg

PDE = 0.060 mg/kg x 50 kg / 5 x 10 x 1 x 10 x 1 = 6.0 µg/day.

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1769 hamsters. IARC Sci Publ 1984;(53):143-51.

1770

1771 **PALLADIUM**

1772 **Summary of PDE for Palladium**

Palladium (Pd)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	100	10	1.0

1773 **Introduction**

1774 Palladium (Pd) is a steel-white, ductile metallic element resembling and occurring with
1775 the other platinum group metals and nickel. It exists in three states: Pd^0 (metallic), Pd^{2+}
1776 and Pd^{4+}. It can form organometallic compounds, only few of which have found industrial
1777 uses. Palladium (on various supports) is being used as catalyst in hydrogenation
1778 reactions. Palladium metal is stable in air and resistant to attack by most reagents
1779 except aqua regia and nitric acid.

1780 Several mutagenicity tests of different palladium compounds with bacterial or
1781 mammalian cells (Ames test with *Salmonella typhimurium*; SOS chromotest with
1782 *Escherichia coli*; micronucleus test with human lymphocytes) *in vitro* gave negative
1783 results.

1784 **Safety Limiting Toxicity**

1785 The data was reviewed to identify the safety limiting toxicities based on routes of
1786 administration.

1787 **PDE – Oral Exposure**

1788 A number of long-term animal studies have been conducted exploring the toxicity and
1789 carcinogenicity of palladium salts. However, none to date have been executed in
1790 accordance with current guidelines for toxicological studies. The available data suggest
1791 potential NOAELs for palladium in the range of 0.8 – 1.5 mg/kg. A lifetime study with
1792 mice given palladium(II) chloride in drinking-water at a dose of about 1.2 mg Pd/kg/day
1793 found a significantly higher incidence of amyloidosis in several inner organs of males and
1794 females and suppressed growth in males, but not in females (Schroeder and Mitchner,
1795 1971; IPCS, 2002). This study also contained a signal that suggested a possible
1796 carcinogenic endpoint; however, the design of the study (single dose level, pooling of the
1797 tumor rates from male and female animals, and a significant increase in the age of the
1798 treated *vs* control animals) limited the utility of the data to assess the carcinogenic
1799 potential.

1800 Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral
1801 PDE is calculated based on the LOEL of 1.2 mg/kg/day.

1802 PDE = 1.2 mg/kg/day x 50 kg / 12 x 10 x 1 x 5x 1 = 0.1 mg/day = 100 µg/day.

1803 **PDE – Parenteral Exposure**

1804 The safety review for Pd was unable to identify any significant assessments upon which
1805 to calculate a PDE for parenteral routes of exposure. Palladium(II) chloride ($PdCl_2$) was
1806 poorly absorbed from the digestive tract (<0.5% of the initial oral dose in adult rats or
1807 about 5% in suckling rats after 3-4 days). Absorption/retention in adult rats was higher
1808 following intratracheal or intravenous exposure, resulting in total body burdens of 5% or
1809 20%, respectively, of the dose administered, 40 days after dosing (IPCS, 2002). On the
1810 basis of an oral bioavailability the PDE for palladium for parenteral exposure is:

PDE = 100 µg/day / 10 = 10 µg/day.

PDE – Inhalation Exposure

There are no adequate inhalation data on Pd. Therefore, the inhalation PDE for palladium was derived from the oral PDE by division by a factor of 100 (as described in Section 3.1).

PDE = 100 µg/day / 100 = 1.0 µg/day.

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PLATINUM

Summary of PDE for Platinum

Platinum (Pt)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	1000	10	1.4

Introduction

Platinum (Pt) is a Group VIII element of the third transition series. It is the most important of the six heaviest of the group VIII elements, collectively called the "platinum group metals" or "platinoids", including palladium, osmium, rhodium, ruthenium and iridium. Platinum and Pd are more chemically reactive than the other platinoids. Metallic Pt has been shown to catalyze many oxidation-reduction and decomposition reactions and the major industrial use of Pt is as a catalyst. Pt complexes exhibiting a range of oxidation states are known, although the principal valences are Pt II and IV. Pt II forms a tetra-coordinate aqua ion $[Pt(H_2O)_4]^{2+}$. The most common Pt IV catalysts are chloroplatinate salts such as tetra and hexachloroplatinate ions.

Safety Limiting Toxicity

The data was reviewed to identify the safety limiting toxicities based on routes of administration.

Chlorinated salts of platinum are responsible for platinum related hypersensitivity and are a major occupational health concern (US EPA, 2009). The hypersensitivity appears to be the most sensitive endpoint of chloroplatinate exposure, at least by the inhalation route. Signs include urticaria, contact dermatitis of the skin, and respiratory disorders ranging from sneezing, shortness of breath, and cyanosis to severe asthma (IPCS, 1991). Exposure reduction was effective in resolving symptoms (Merget *et al.* 2001). Neutral complexes and complexes without halogenated ligands do not appear allergenic (US EPA, 2009; EU SCOEL, 2011). The risk of hypersensitivity appears to be related to sensitizing dose and dose and length of exposure (IPCS, 1991; US EPA, 2009; Arts *et al.* 2006) and cigarette smoking (US EPA, 2009; Merget *et al.* 2000; Caverley, 1995).

PDE – Oral Exposure

No experimental data are available on the carcinogenicity of platinum and platinum compounds, and toxicology data are limited (US EPA, 2009). In one study in male rats administered $PtCl_2$ (relatively insoluble) and $PtCl_4$ (soluble) for 4 weeks, the toxicity of the two platinum salts was investigated. No significant effects on body weight gain or food consumption for either compound, and no effects were observed on hematological parameters for $PtCl_2$. Some hematological parameters were influenced by $PtCl_4$; a reduction of about 13% in hematocrit and erythrocyte parameters was reported at the dose of 50 mg Pt/kg in the diet. Platinum concentration increased in tissues in animals dosed with either compound, particularly the kidney. For this reason plasma creatinine was examined, and found to be increased in animals dosed with $PtCl_4$ when added in the diet at 50 mg Pt/kg diet for 4 weeks, but not $PtCl_2$. This dose corresponded to 21 mg Pt/animal (Reichlmayr-Lais *et al.* 1992). This study was used in the determination of the PDE as one endpoint in the study was renal toxicity (plasma creatinine), a target organ of platinum and a site of accumulation. Renal toxicity is an also an adverse effect of treatment with chemotherapeutic agents such as cisplatin.
Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated based on the NOAEL of 10 mg/kg/day.

PDE = 10 mg/kg/day x 50 kg / 5 x 10 x 10 x 1 x 1 = 1 mg/day = 1000 µg/day.

PDE – Parenteral Exposure

The safety review for platinum identified limited assessments of platinum salt toxicity for parenteral routes of administration. The oral absorption of platinum salts is very low (<1%) (US EPA, 2009). Therefore, the oral PDE is divided by a factor of 100 (as described in section 3.1).

PDE = 1000 µg/day / 100 = 10 µg/day.

PDE – Inhalation Exposure

Due to the use of the chloroplatinates in catalytic converters, numerous animal (Biagini *et al.* 1983) and human (Pepys *et al.* 1972; Pickering 1972; Merget *et al.* 2000; Cristaudo *et al.* 2007) studies have been conducted. The US EPA (1977; 2009) and the EU SCOEL (2011) have also examined the safety of chloroplatinates based on sensitization. The EU SCOEL concluded that the database does not allow for setting an occupational limit for soluble platinum salts. The US DoL (2013) has established an occupational limit for soluble Pt salts at 2 µg/m^3; however, whether this exposure level is completely protective of workers has been questioned (Merget and Rosner, 2001).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated as:

2 µg/m^3 ÷1000 m^3/L = 0.002 µg/L

For continuous dosing = $0.002\ \mu g/L \times \frac{8\ hr \times 5\ d}{24\ hr \times 7\ d} = 0.00048\ \mu g/L$

Daily dose = $\frac{0.00048\ \mu g/L \times 28800\ L/d}{50\ kg} = 0.27\ \mu g/kg/d$

PDE = $\frac{0.27\ \mu g/kg/d \times 50\ kg}{1 \times 10 \times 1 \times 1 \times 1} = 1.37\ \mu g/day \sim 1.4\ \mu g/day.$

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1935

1936

SELENIUM

Summary of PDE for Selenium

Selenium (Se)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	170	85	140

Introduction

Selenium is present in the earth's crust, often in association with sulfur-containing minerals. It can assume four oxidation states (-2, 0, +4, +6) and occurs in many forms, including elemental selenium, selenites and selenates. Selenium is an essential trace element for many species, including humans. Selenium is incorporated into proteins *via* a specific selenocysteine tRNA. Selenium is being used as a catalyst in the manufacture of rubber. Ru-Se catalysts are used in oxygen reduction. Aryl- and alkyl-Selenium reagents have various applications in organic synthesis.

Safety Limiting Toxicity

Selenium was listed as a Group 3 compound by IARC (1987), not classifiable for carcinogenesis. The only selenium compound that has been shown to be carcinogenic in animals is selenium sulfide (NTP, 1980). According to the US EPA, selenium sulfide is in Group B2 (probable human carcinogen) (US EPA, 2002). Other selenium compounds are classified as D; not classifiable as to carcinogenicity in humans.

The most significant toxicity observed in these assessments was hepatotoxicity.

PDE – Oral Exposure

In a rat carcinogenicity study of selenium sulfide, the NOAEL for hepatocellular carcinoma was 3 mg/kg/day (1.7 mg Se/kg/day) (NTP, 1980). There is insufficient data to assess carcinogenicity of other forms of selenium, and the human relevance of the rodent liver tumors has been questioned (IARC, 1999). Some human data are available but only in a limited number of subjects (ATSDR, 2003). The PDE is in line with the MRL of 5 µg/kg/day for Se (ATSDR 2003).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as below.

PDE = 1.7 mg/kg/day x 50 kg / 5 x 10 x 1 x 10 x 1 = 170 µg/day.

PDE – Parenteral Exposure

The safety review for selenium was unable to identify any significant assessments upon which to calculate a PDE for parenteral routes of exposure. Studies in humans and experimental animals indicate that, when ingested, several selenium compounds including selenite, selenate, and selenomethionine are readily absorbed, often to greater than 80% of the administered dose (ATSDR, 2003). On the basis of oral bioavailability of ~80%, the PDE for selenium for parenteral exposure is (as described in section 3.1).

PDE = 170 µg/day / 2 = 85 µg/day.

PDE – Inhalation Exposure

The safety review for selenium was unable to identify any significant animal models or clinical studies of inhalation toxicity. However, occupational limits have established time weighted averages for selenium exposures of 0.2 mg/m^3 (US DoL, 2013).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated as below.

0.2 mg/m^3 /1000 L/m^3= 0.0002 mg/L

For continuous dosing = 0.0002 mg/L x 8 h x 5 d/24 x 7 = 0.0000476 mg/L

Daily dose = 0.0000476 mg/L x 28800 L/50 kg = 0.027 mg/kg

$$\text{PDE} = \frac{0.027 \text{ mg/kg} \times 50 \text{ kg}}{1 \times 10 \times 1 \times 1 \times 1} = 0.135 \text{ mg/day} = 140 \text{ µg/day.}$$

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SILVER

Summary of PDE for Silver

Silver (Ag)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	170	35	6.9

Introduction

Silver (Ag) is present in silver compounds primarily in the oxidation state +1 and less frequently in the oxidation state +2. Ag occurs naturally mainly in the form of very insoluble and immobile oxides, sulfides and some salts. The most important silver compounds in drinking-water are silver nitrate and silver chloride. Most foods contain traces of silver in the 10–100 µg/kg range. Ag is nutritionally not essential and no metabolic function is known. Silver is being used as a catalyst in the oxidation of ethylene to ethyleneoxide. Silver-Cadmium alloy is used in selective hydrogenation of unsaturated carbonyl compounds. Silver oxide is used as a mild oxidizing agent in organic synthesis.

Safety Limiting Toxicity

Silver is not mutagenic. Animal toxicity studies and human occupational studies have not provided sufficient evidence of carcinogenicity. Based on these data Ag is not expected to be carcinogenic in humans (ATSDR, 1990).

Argyria appears to be the most sensitive clinical effect in response to human Ag intake. Silver acetate lozenges are used in smoking cessation (Hymowitz and Eckholdt, 1996). Argyria, a permanent bluish-gray discoloration of the skin, results from the deposition of Ag in the dermis combined with an Ag-induced production of melanin. Inhalation of high levels of silver can result in lung and throat irritation and stomach pains (ATSDR, 1990).

PDE – Oral Exposure

Silver nitrate was added at 0.015% to the drinking water of female mice (0.9 g/mouse; 32.14 mg/kg silver nitrate; 64% silver) for 125 days to examine neurobehavioral activity of the animals based on potential neurotoxicity of silver (Rungby and Danscher, 1984). Treated animals were hypoactive relative to controls; other clinical signs were not noted. In a separate study, silver was shown to be present in the brain after mice were injected with 1 mg/kg ip silver lactate (Rungby and Danscher, 1983). The oral PDE is in line with the reference dose of 5 µg/kg/day (US EPA, 2003).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as below.

20 mg/kg x 50 kg / 12 x 10 x 5 x1 x 10 = 167 µg/d ~170 µg/day.

A factor 10 was chosen for F5 as a NOAEL was not seen in this study and few toxicological endpoints were examined.

PDE – Parenteral Exposure

US EPA (2003) identified a LOAEL of 0.014 mg/kg Ag/d using long-term (2 to 9 years) human iv data based on argyria following colloidal and organic silver medication.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the parenteral PDE is calculated as below.

0.014 mg/kg/d x 50 kg = 700 ug/d/1 x 10 x 1 x 1 x 2 = 35 µg/day.

A factor of 2 was chosen for F5 as the finding of argyria was not considered a serious toxicity and a factor of 10 is used for F2, for a combined modifying factor of 20.

PDE – Inhalation Exposure

Lung and throat irritation and stomach pains were the principal effects in humans after inhalation of high Ag levels.

Using the TLV of 0.01 mg/m^3 for silver metal and soluble compounds (US DoL, 2013), taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated as:

0.01 mg/m^3 /1000 L/m^3= 0.00001 mg/L

For continuous dosing = 0.00001 mg/L x 8 h x 5 d/24 x 7 = 0.00000238 mg/L

$$\text{Daily dose} = \frac{0.00000238 \text{ mg/L} \times 28800 \text{ L/day}}{50 \text{ kg}} = 0.00137 \text{ mg/kg/day}$$

$$\text{PDE} = \frac{0.00137 \text{ mg/kg} \times 50 \text{ kg}}{1 \times 10 \times 1 \times 1 \times 1} = 0.0069 \text{ mg/day} = 6.9 \text{ µg/day.}$$

The factor F2 was set to 10 to extrapolate to the general population.

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THALLIUM

Summary of PDE for Thallium

Thallium (Tl)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	8.0	8.0	69

Introduction

Pure thallium (Tl) is a bluish-white metal. It exists primarily in two valence states: monovalent (thallous) and trivalent (thallic). Monovalent thallium is similar to potassium (K+) in ionic radius and electrical charge, which contribute to its toxic nature. Many of the thallium salts are soluble in water with the exception of the insoluble thallium (III) oxide. Tl sulfate has been used in medicine, primarily as a depilatory agent, but also to treat infections, such as venereal diseases, ringworm of the scalp, typhus, tuberculosis, and malaria. Thallium(III) salts are being used in organic synthesis. Tl is nutritionally not essential and no metabolic function is known (ATSDR, 1992).

Safety Limiting Toxicity

In humans and animals, the skin, especially the hair follicles, appears to be the most sensitive target of toxicity from repeated oral exposure to Tl (US EPA, 2009).

PDE – Oral Exposure

The primary target organ for oral exposure to Tl in humans and animals appears to be the skin, especially the hair follicles, as shown in a 90-day toxicity rat study with Tl sulfate. The NOAEL was defined at 0.04 mg Tl/kg on the basis of an increased incidence of alopecia at the higher doses (Stoltz *et al.* 1986; US EPA, 2009). Thus, the oral PDE was determined on the basis of the NOAEL of 0.04 mg Tl/kg in rat.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as below.

PDE = 0.04 mg/kg/day x 50 kg / 5 x 10 x 5 x 1 x 1 = 0.008 mg/day = 8.0 µg/day.

PDE – Parenteral Exposure

No relevant data on parenteral exposure to thallium compounds were found. The bioavailability of soluble thallium salts is high (> 80%) (US EPA, 2009). Therefore, the parenteral PDE is the same as the oral PDE.

PDE = 8.0 µg/day.

PDE – Inhalation Exposure

No relevant data on inhalation exposure to thallium compounds were found. Using the TLV of 0.1 mg/m^3 for thallium, soluble compounds (US DoL, 2013; CEC, 2000).

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the inhalation PDE is calculated as:

0.1 mg/m^3 /1000 L/m^3= 0.0001 mg/L

For continuous dosing = 0.0001 mg/L x 8 h x 5 d/24 x 7 = 0.0000238 mg/L

Daily dose = $\frac{0.0000238 \text{ mg/L} \times 28800 \text{ L/day}}{}$ = 0.0137 mg/kg/day

$$PDE = \frac{0.0137\ mg/kg \times 50\ kg}{1 \times 10 \times 1 \times 1 \times 1} = 0.069\ mg/day = 69\ \mu g/day.$$

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2130 **TIN**

2131 **Summary of PDE for Tin**

Tin (Sn)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	6400	640	64

2132 **Introduction**

2133 Tin (Sn) is a silvery-white metal that exists in valence states of 2 and 4. The most
2134 important inorganic compounds of tin are its oxides, chlorides, fluorides and halogenated
2135 sodium stannates and stannites. Tin is present in some multi-vitamin and mineral food
2136 supplements (levels up to 10 µg Sn/tablet). Tin is possibly nutritionally essential for
2137 some animals, it has not been shown to be essential for humans. Tin(II) chloride is being
2138 used as a reducing agent, and as a stabilizer of polyvinylchloride (PVC). This safety
2139 assessment focuses on inorganic tin considering that the more frequent occurrence of
2140 inorganic tin is more relevant with respect to metal impurities in drug products than
2141 organic tin compounds.

2142 **Safety Limiting Toxicity**

2143 There is no indication of *in vivo* genotoxicity or carcinogenicity for tin and tin salts. In
2144 several studies in rats, a decrease in hemoglobin as an early sign for anemia, was the
2145 most sensitive endpoint.

2146 **PDE – Oral Exposure**

2147 Anemia was the most sensitive endpoint in rats after repeated oral administration. Thus,
2148 the PDE for oral exposure was determined on the basis of the lowest NOAEL, i.e., 150
2149 ppm (equivalent to 32 mg Sn/kg/day). This value was obtained from a 90-day study in
2150 rats based on signs of anemia starting at 500 ppm in rats exposed to stannous chloride
2151 *via* diet (De Groot *et al.* 1973).

2152 Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral
2153 PDE is calculated as below.

2154 PDE = 32 mg/kg/day x 50 kg / 5 x 10 x 5 x 1 x 1 = 6.4 mg/day = 6400 µg/day.

2155 **PDE – Parenteral Exposure**

2156 The safety review for tin was unable to identify any significant assessments upon which
2157 to calculate a PDE for parenteral routes of exposure. On the basis of an oral
2158 bioavailability of about 5% for tin and inorganic tin compounds (ATSDR, 2005), and
2159 using the default factor of 10, the PDE for tin for a parenteral exposure is (as described
2160 in Section 3.1).

2161 PDE = 6400 µg/day / 10 = 640 µg/day.

2162 **PDE – Inhalation Exposure**

2163 The safety review for tin was unable to identify any significant assessments on inorganic
2164 tin upon which to calculate a PDE for inhalation routes of exposure. Although a TLV is
2165 available for tin (2 mg/m^3; US DoL, 2013), there is insufficient data to set a MRL (ATSDR
2166 2005; EU SCOEL 2003).

2167 Therefore, the PDE for tin is calculated by using a factor of 100 to convert the oral PDE
2168 to the inhalation PDE (as described in Section 3.1).

PDE = 6400 µg/day / 100 = 64 µg/day.

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US DoL (OHSA). 29 CRF 1910.1000 Table Z-1. Limits for air contaminants. U.S. Department of Labor. 2013.

VANADIUM

Summary of PDE for Vanadium

Vanadium (V)			
	Oral	Parenteral	Inhalation
PDE (µg/day)	120	12	1.2

Introduction

Vanadium (V) is present as a trace element in the earth's crust and can exist in a variety of oxidation states (-1, 0, +2, +3, +4 and +5). V is also present in trace quantities in most biological organisms with the principal ions being vanadate, VO_3^- and vanadyl, VO_2^+. Absorption of vanadium from the gastrointestinal tract is poor. Estimates of total dietary intake of vanadium in humans range from 10 to 60 µg/day. Intake from drinking water depends on the water source and estimates are up to 140 µg/day. Human populations have variable serum concentrations of vanadium, with 2 µg/L being the high end of the normal range. Despite its ubiquitous presence in the body, an essential biological role for vanadium in humans has not been established. Vanadium has been reported to have potentially beneficial effects in treatment of osteoporosis, osteopenia, cancer, and diabetes. Oral vanadyl sulfate in amounts up to 20 mg/day is included in some dietary supplements intended to promote muscle growth. Vanadium oxide is used as a catalyst in the manufacturing of sulfuric acid.

Safety Limiting Toxicity

Vanadium is genotoxic, but not mutagenic (ATSDR, 2009). Vanadium pentoxide is classified as a possible human carcinogen (Group 2B; IARC, 2012).

PDE – Oral Exposure

Following oral administration to animals and humans the gastrointestinal tract, cardiovascular, and hematological system are the primary targets of toxicity. The most appropriate study to assess vanadium toxicity through oral administration was conducted in humans exposed to vanadium for 12 weeks. In these studies, no significant alterations in hematological parameters, liver function (as measured by serum enzymes), cholesterol and triglyceride levels, kidney function (as measured by blood urea nitrogen), body weight, or blood pressure were observed in subjects administered *via* capsule 0.12 or 0.19 mg vanadium as ammonium vanadyl tartrate or vanadyl sulfate for 6–12 weeks (ATSDR, 2012). The oral NOAEL of 0.12 mg vanadium/kg/day for hematological and blood pressure effects was used to calculate the oral PDE.

Taking into account the modifying factors (F1-F5 as discussed in Appendix 1), the oral PDE is calculated as below.

PDE = 0.12 mg/kg/day x 50 kg / 1 x 10 x 5 x 1 x 1 = 0.12 mg/day = 120 µg/day.

PDE – Parenteral Exposure

The safety review for vanadium was unable to identify any significant assessments upon which to calculate a PDE for parenteral routes of exposure. On the basis of an approximate oral bioavailability of <1–10% for vanadium and inorganic vanadium compounds (ATSDR, 2012), the oral PDE was divided by 10 (as described in Section 3.1).

PDE = 120 µg/day / 10 = 12 µg/day.

PDE – Inhalation Exposure

A two year chronic inhalation exposure study in rats was considered for use for the inhalation PDE for vanadium. In this study, carcinogenic effects were observed to the lowest dose tested, 0.5 mg/m^3 vanadium pentoxide (Ress *et al.* 2003). Vanadium pentoxide is a caustic agent and is not considered to be present in drug products. Therefore, the inhalation PDE for vanadium was derived from the oral PDE by division by a factor of 100 (as described in Section 3.1).

PDE = 120/100 = 1.2 µg/day.

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Appendix 4: Illustrative Example – Calculation Options for Converting PDEs to Concentrations

Examples for Converting PDEs into Permitted Elemental Impurity Concentrations

Option 1: Permitted common concentration limits of elemental impurities across drug product component materials for products with daily intakes of not more than 10 grams.

For this example, consider a solid oral drug product with a maximum daily intake of 2.5 grams, containing 9 components (1 drug substance and 8 excipients, see Table A.4.1). Because this drug product does not exceed a maximum daily intake of 10 grams, the concentrations in Table A.2.2 may be used. As Option 1 has a common permitted concentration, each of the 9 components can be used at any level in the formulation. The drug substance synthesis uses Pd and Ni catalysts, and the applicant is also concerned about Pb, As, Cd, Hg, and V on the basis of the risk assessment. The maximum daily intake of each elemental impurity in the drug product is given in Table A.4.2 assuming that each elemental impurity is present at the concentration given in Table A.2.2. The maximum potential daily intake of an elemental impurity is determined using the actual drug product daily intake and the concentration limit for the elemental impurity in Table A.2.2 (concentration multiplied by the actual daily intake of the drug product of 2.5 grams). The maximum daily intake given for each elemental impurity is not a summation of values found in the individual columns.

This calculation demonstrates that no elemental impurities exceed their PDEs. Thus if these concentrations in each component are not exceeded, the drug product is assured to meet the PDEs for each identified elemental impurity.

Table A.4.1: Maximum Daily Intake of Components of the Drug Product

Component	Daily Intake, g
Drug Substance	0.200
MCC	1.100
Lactose	0.450
Ca Phosphate	0.350
Crospovidone	0.265
Mg Stearate	0.035
HPMC	0.060
Titanium Dioxide	0.025
Iron Oxide	0.015
Drug Product	2.500

2267 **Table A.4.2: Permitted Concentrations from Table A.2.2 (assuming uniform**
2268 **concentrations and 10 grams daily intake)**

Component	Maximum Permitted Concentration (µg/g)						
	Pb	As	Cd	Hg	Pd	V	Ni
Drug Substance	0.5	1.5	0.5	4	10	12	60
MCC	0.5	1.5	0.5	4	10	12	60
Lactose	0.5	1.5	0.5	4	10	12	60
Ca Phosphate	0.5	1.5	0.5	4	10	12	60
Crospovidone	0.5	1.5	0.5	4	10	12	60
Mg Stearate	0.5	1.5	0.5	4	10	12	60
HPMC	0.5	1.5	0.5	4	10	12	60
Titanium Dioxide	0.5	1.5	0.5	4	10	12	60
Iron Oxide	0.5	1.5	0.5	4	10	12	60
Maximum Daily intake, µg	**1.25**	**3.75**	**1.25**	**10**	**25**	**30**	**150**
PDE, µg/day	**5.0**	**15**	**5.0**	**40**	**100**	**120**	**600**

2269

2270 **Option 2a:** Permitted common concentration limits across drug product component
2271 materials for a product with a specified daily intake:

2272 For this example, consider the same solid oral drug product with a maximum daily
2273 intake of 2.5 grams, containing 9 components (1 drug substance and 8 excipients, see
2274 Table A.4.1) used in Option 1. As Option 2a has a common permitted concentration,
2275 each of the 9 components can be used at any level in the formulation. The drug
2276 substance synthesis uses Pd and Ni catalysts, and the applicant is also concerned about
2277 Pb, As, Cd, Hg, and V on the basis of the risk assessment. The concentration of each
2278 elemental impurity identified in the risk assessment can be calculated using the PDEs in
2279 Table A.2.1 and equation 1.

2280 The maximum potential daily intake of an elemental impurity is determined using the
2281 actual drug product daily intake and the concentration limit for the elemental impurity
2282 in Table A.4.3 (concentration multiplied by the actual daily intake of the drug product of
2283 2.5 grams). The maximum daily intake given for each elemental impurity is not a
2284 summation of values found in the individual columns.

2285 This calculation also demonstrates that no elemental impurities exceed their PDEs. Thus
2286 if these concentrations in each component are not exceeded, the drug product is assured
2287 to meet the PDEs for each identified elemental impurity.

2288 The factor of 4 increase in Option 2a for permitted concentration seen when comparing
2289 Option 1 and Option 2a concentration limits is due to the use of 10 grams and 2.5 grams
2290 respectively as daily intake of the drug product.

2291

Table A.4.3: Calculation of Maximum Permitted Concentrations Assuming Uniform Concentrations in a Product with a Specified Daily Intake:

Component	Maximum Permitted Concentration (µg/g)						
	Pb	As	Cd	Hg	Pd	V	Ni
Drug Substance	2	6	2	16	40	48	240
MCC	2	6	2	16	40	48	240
Lactose	2	6	2	16	40	48	240
Ca Phosphate	2	6	2	16	40	48	240
Crospovidone	2	6	2	16	40	48	240
Mg Stearate	2	6	2	16	40	48	240
HPMC	2	6	2	16	40	48	240
Titanium Dioxide	2	6	2	16	40	48	240
Iron Oxide	2	6	2	16	40	48	240
Maximum Daily intake, µg	**5.0**	**15**	**5.0**	**40**	**100**	**120**	**600**
PDE, µg/day	**5.0**	**15**	**5.0**	**40**	**100**	**120**	**600**

Option 2b: Permitted concentration limits of elemental impurities across drug product component materials for a product with a specified daily intake:

For this example, consider the same solid oral drug product with a maximum daily intake of 2.5 grams, containing 9 components (1 drug substance and 8 excipients, see Table A.4.1) used in Option 1 and 2a. The drug substance synthesis uses Pd and Ni catalysts, and the applicant is also concerned about Pb, As, Cd, Hg, and V on the basis of the risk assessment. To use Option 2b, the applicant must use the composition of the drug product and have additional knowledge regarding the content of each elemental impurity in the components. The applicant has generated the following data on elemental impurities in the components of the drug product:

Table A.4.4: Measured Concentrations of Elemental Impurities (µg/g) in the Components

Component	Measured Concentration (µg/g)						
	Pb	As	Cd	Hg	Pd	V	Ni
Drug Substance	ND	0.5	ND	ND	20	ND	50
MCC	0.1	0.1	0.1	0.1	*	ND	ND
Lactose	0.1	0.1	0.1	0.1	*	ND	ND
Ca Phosphate	1	1	1	1	*	10	5
Crospovidone	0.1	0.1	0.1	0.1	*	ND	ND
Mg Stearate	0.5	0.5	0.5	0.5	*	ND	0.5
HPMC	0.1	0.1	0.1	0.1	*	ND	ND
Titanium Dioxide	20	1	1	1	*	1	ND
Iron Oxide	10	10	10	10	*	2000	50

ND = Below the detection limit

* = The risk assessment identified that Pd was not a potential elemental impurity; a quantitative result was not obtained.

The applicant also knows the maximum daily intake of the drug product is 2.5 grams and determines the maximum daily intake for each component as shown in Table A.4.5.

Based on the observed levels (see Table A.4.4), the applicant evaluated the potential maximum permitted concentrations of each elemental impurity in the components. The concentrations selected (see Table A.4.5) were set at levels that would ensure the PDE is met if the maximum permitted concentration was reached for each component. The maximum daily intake in Table A.4.5 is the summation of the values obtained by multiplying the actual weight of the component by the maximum permitted concentration for each elemental impurity across all components.

Table A.4.5: Maximum Permitted Concentrations of Elemental Impurities in the Components

Component	Maximum Permitted Concentration (µg/g)						
	Pb	As	Cd	Hg	Pd	V	Ni
Drug Substance	**	5	**	**	500	**	2000
MCC	0.5	5	1	10	*	**	**
Lactose	0.5	5	1	10	*	**	**
Ca Phosphate	5	5	5	40	*	125	475
Crospovidone	0.5	5	1	10	*	**	**
Mg Stearate	5	10	5	100	*	**	50
HPMC	2.5	5	1	10	*	**	**
Titanium Dioxide	40	20	10	25	*	50	**
Iron Oxide	20	100	50	200	*	5000	2000
Maximum Daily intake, µg	**4.3**	**14.5**	**4.8**	**39.9**	**100**	**120**	**598**
PDE, µg/day	**5.0**	**15**	**5.0**	**40**	**100**	**120**	**600**

* The risk assessment identified that Pd was not a potential elemental impurity; a quantitative result was not obtained.

** Quantitative results demonstrated less than the limit of detection.

Option 3: Finished Product Analysis

For this example, consider the same solid oral drug product with a maximum daily intake of 2.5 grams, containing 9 components (1 drug substance and 8 excipients) used in Option 1, 2a and 2b. The drug substance synthesis uses Pd and Ni catalysts, and the applicant is also concerned about Pb, As, Cd, Hg, and V on the basis of the risk assessment. The maximum concentration of each elemental impurity in the drug product may be calculated using the daily intake of drug product and the PDE of the elemental impurity using equation 1. The total mass of each elemental impurity should be not more than the PDE.

$$Concentration(\mu g / g) = \frac{PDE(\mu g / day)}{2.5(g / day)}$$

Table A.4.6: Calculation of Concentrations for the Finished Product

		Maximum Permitted Concentration (µg/g)						
	Daily Intake (g)	Pb	As	Cd	Hg	Pd	V	Ni
Drug Product	2.5	2	6	2	16	40	40	800
Maximum Daily Intake (µg)		5	15	5	40	100	120	600

Illustrative Example – Elemental Impurities Assessment

The following example is intended as illustration of an elemental impurities risk assessment. This example is intended for illustrative purposes and not as the only way to document the assessment. There are many different ways to approach the risk assessment process and its documentation.

This example relies on the oral drug product described in Appendix 4. Consider a solid oral drug product with a maximum daily intake of 2.5 grams, containing 9 components (1 drug substance and 8 excipients). The drug substance synthesis uses Pd and Ni catalysts.

The applicant conducts the risk assessment starting with the identification of potential elemental impurities following the process described in Section 5. Since the applicant had limited historical data for the excipients used in the drug product, the applicant determined that the Class 1 elementals (As, Cd, Hg, Pb) would be taken through the evaluation phase. The table below shows a summary of the findings of the identification stage of the assessment.

Table A.4.7: Identification of Potential Elemental Impurities

	Potential Elemental Impurities			
Component	Intentionally added	Potential elemental impurities with a relatively high abundance and/or are impurities in excipients or reagents	Potential elemental impurities from manufacturing equipment	Potential elemental impurities from container closure systems
Drug Substance	Pd, Ni	As	Ni	None
MCC	None	As, Cd, Hg, Pb		None
Lactose	None	As, Cd, Hg, Pb		None
Ca Phosphate	None	As, Cd, Hg, Pb	V, Ni	None
Crospovidone	None	As, Cd, Hg, Pb		None
Mg stearate	None	As, Cd, Hg, Pb	Ni	None
HPMC	None	As, Cd, Hg, Pb		None
Titanium Dioxide	None	As, Cd, Hg, Pb	V	None
Iron Oxide	None	As, Cd, Hg, Pb	V, Ni	None

The identification phase of the assessment identified seven potential elemental impurities requiring additional evaluation. Three of the identified elemental impurities were found in multiple components. The applicant continued the risk assessment collecting information from the vendor and available development data. The summary of the results can be found in Table A.4.3. The application of the individual component data to the evaluation in the assessment process is shown below in Table A.4.8.

2357 **Table A.4.8: Elemental Impurity Assessment – Evaluation of Daily Contribution to the Total Mass of Elemental Impurities in the Drug Product**

Component	Daily intake, g	Measured Concentration (µg/g)							Total Daily Mass of Elemental Impurity, µg						
		Pb	As	Cd	Hg	Pd	V	Ni	Pb	As	Cd	Hg	Pd	V	Ni
Drug Substance	0.2	ND	0.5	ND	ND	20	ND	50	0	0.1	0	0	4	0	10
MCC	1.1	0.1	0.1	0.1	0.1	*	ND	ND	0.11	0.11	0.11	0.11	0	0	0
Lactose	0.45	0.1	0.1	0.1	0.1	*	ND	ND	0.045	0.045	0.045	0.045	0	0	0
Ca Phosphate	0.35	1	1	1	1	*	10	5	0.35	0.35	0.35	0.35	0	3.5	1.75
Crospovidone	0.265	0.1	0.1	0.1	0.1	*	ND	ND	0.0265	0.0265	0.0265	0.0265	0	0	0
Mg stearate	0.035	0.5	0.5	0.5	0.5	*	ND	0.5	0.0175	0.0175	0.0175	0.0175	0	0	0.0175
HPMC	0.06	0.1	0.1	0.1	0.1	*	ND	ND	0.006	0.006	0.006	0.006	0	0	0
Titanium Dioxide	0.025	20	1	1	1	*	1	ND	0.5	0.025	0.025	0.025	0	0.025	0
Iron Oxide	0.015	10	10	10	10	*	400	50	0.15	0.15	0.15	0.15	0	6	0.75
							total daily mass, µg/day		1.2	0.8	0.7	0.7	4.0	9.5	12.5

2358

2359 **Table A.4.9: Assessment Example – Data Entry Descriptions**

2360 Column 1: Review the components of drug product for any elements intentionally added in the production (the primary source is the
2361 drug substance). For those used, record the elements for further consideration in the assessment.
2362 Column 2: Identify any potential elements or impurities that are associated with excipients or reagents used in the preparation of the
2363 drug product. Record the source(s) for further consideration in the assessment.
2364 Column 3: Identify any elemental impurities known or expected to be leached from the manufacturing equipment. Record the specific
2365 elemental impurities for further consideration in the assessment.
2366 Column 4: Identify any elemental impurities known or expected to be leached from the container closure system. Record the specific
2367 elemental impurities for further consideration in the assessment.
2368 Column 5: Calculate the total contribution of the potential elemental impurity by summing the contributions across the components
2369 of the drug product.

2370 Column 6: Assess the variability of the elemental impurity level(s) in the components
2371 Column 7: Enter the control threshold of each potential elemental impurity identified. If the variability is known and it is within
2372 acceptable limits, the control threshold (30% of the PDE) for each elemental impurity can be applied.
2373 Column 8: Describe action taken – none if the value in column 6 is less than or equal to the control threshold (column 7). Define
2374 control element if material variability is high or control threshold is exceeded.
2375

	1	2	3	4	5	6	7	8
Element	**Intentionally added (if used in the process)**	**Elemental impurities with a relatively high abundance and/or are impurities in excipients or reagents**	**Manufacturing equipment**	**Leached from container closure systems**	**Total elemental impurity contribution µg/day**	**Acceptable variability of elemental impurity contribution**	**Control threshold**	**Action**
As	No	Observed contaminant in all excipients and drug substance	No	No	0.8	yes	4.5	no further controls required
Cd	No	Observed contaminant in all excipients	No	No	0.7	yes	1.5	no further controls required
Hg	No	Observed contaminant in all excipients	No	No	0.7	yes	12	no further controls required
Pb	No	Observed contaminant in all excipients	No	No	1.2	yes	1.5	no further controls required
Pd	API catalyst	No	No	No	4.0	yes	30	no further controls required
Ni	API catalyst	Observed in 3 excipients	No	No	12.5	yes	180	no further controls required
V	No	Observed in 3 excipients	No	No	9.5	yes	36	no further controls required

2376

Guidance for Industry

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U.S. Department of Health and Human Services
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Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
August 2001
ICH

Guidance for Industry

Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
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Guidance for Industry[1]
Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION (1)

A. Objective (1.1)

This document is intended to provide guidance regarding good manufacturing practice (GMP) for the manufacturing of active pharmaceutical ingredients (APIs) under an appropriate system for managing quality. It is also intended to help ensure that APIs meet the quality and purity characteristics that they purport, or are represented, to possess.

In this guidance, the term *manufacturing* is defined to include all operations of receipt of materials, production, packaging, repackaging, labeling, relabeling, quality control, release, storage and distribution of APIs and the related controls. In this guidance, the term *should* identifies recommendations that, when followed, will ensure compliance with CGMPs. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes. For the purposes of this guidance, the terms *current good manufacturing practices* and *good manufacturing practices* are equivalent.

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[1] This guidance was developed within the Expert Working Group (Q7A) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Steering Committee at *Step 4* of the ICH process, November 2000. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and the United States.

Arabic numbers in subheadings reflect the organizational breakdown in the document endorsed by the ICH Steering Committee at Step 4 of the ICH process, November 2000.

The guidance as a whole does not cover safety aspects for the personnel engaged in manufacturing, nor aspects related to protecting the environment. These controls are inherent responsibilities of the manufacturer and are governed by national laws.

This guidance is not intended to define registration and/or filing requirements or modify pharmacopoeial requirements. This guidance does not affect the ability of the responsible regulatory agency to establish specific registration/filing requirements regarding APIs within the context of marketing/manufacturing authorizations or drug applications. All commitments in registration/filing documents should be met.

B. Regulatory Applicability (1.2)

Within the world community, materials may vary as to their legal classification as an API. When a material is classified as an API in the region or country in which it is manufactured or used in a drug product, it should be manufactured according to this guidance.

C. Scope (1.3)

This guidance applies to the manufacture of APIs for use in human drug (medicinal) products. It applies to the manufacture of sterile APIs only up to the point immediately prior to the APIs being rendered sterile. The sterilization and aseptic processing of sterile APIs are not covered by this guidance, but should be performed in accordance with GMP guidances for drug (medicinal) products as defined by local authorities.

This guidance covers APIs that are manufactured by chemical synthesis, extraction, cell culture/fermentation, recovery from natural sources, or any combination of these processes. Specific guidance for APIs manufactured by cell culture/fermentation is described in Section XVIII (18).

This guidance excludes all vaccines, whole cells, whole blood and plasma, blood and plasma derivatives (plasma fractionation), and gene therapy APIs. However, it does include APIs that are produced using blood or plasma as raw materials. Note that cell substrates (mammalian, plant, insect or microbial cells, tissue or animal sources including transgenic animals) and early process steps may be subject to GMP but are not covered by this guidance. In addition, the guidance does not apply to medical gases, bulk-packaged drug (medicinal) products (e.g., tablets or capsules in bulk containers), or radiopharmaceuticals.

Section XIX (19) contains guidance that only applies to the manufacture of APIs used in the production of drug (medicinal) products specifically for clinical trials (investigational medicinal products).

An *API starting material* is a raw material, an intermediate, or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API. An API starting material can be an article of commerce, a material

purchased from one or more suppliers under contract or commercial agreement, or produced in-house. API starting materials normally have defined chemical properties and structure.

The company should designate and document the rationale for the point at which production of the API begins. For synthetic processes, this is known as the point at which API starting materials are entered into the process. For other processes (e.g., fermentation, extraction, purification), this rationale should be established on a case-by-case basis. Table 1 gives guidance on the point at which the API starting material is normally introduced into the process.

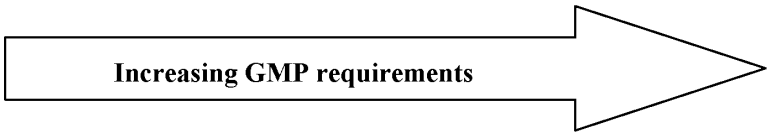
From this point on, appropriate GMP as defined in this guidance should be applied to these intermediate and/or API manufacturing steps. This would include the validation of critical process steps determined to impact the quality of the API. However, it should be noted that the fact that a company chooses to validate a process step does not necessarily define that step as critical.

The guidance in this document would normally be applied to the steps shown in gray in Table 1. However, all steps shown may not need to be completed. The stringency of GMP in API manufacturing should increase as the process proceeds from early API steps to final steps, purification, and packaging. Physical processing of APIs, such as granulation, coating or physical manipulation of particle size (e.g., milling, micronizing) should be conducted according to this guidance.

This GMP guidance does not apply to steps prior to the introduction of the defined API starting material.

Table 1: Application of this Guidance to API Manufacturing

Type of Manufacturing	Application of this guidance to steps (shown in gray) used in this type of manufacturing				
Chemical Manufacturing	Production of the API starting material	Introduction of the API starting material into process	Production of Intermediate(s)	Isolation and purification	Physical processing, and packaging
API derived from animal sources	Collection of organ, fluid, or tissue	Cutting, mixing, and/or initial processing	Introduction of the API starting material into process	Isolation and purification	Physical processing, and packaging
API extracted from plant sources	Collection of plant	Cutting and initial extraction(s)	Introduction of the API starting material into process	Isolation and purification	Physical processing, and packaging
Herbal extracts used as API	Collection of plants	Cutting and initial extraction		Further extraction	Physical processing, and packaging
API consisting of comminuted or powdered herbs	Collection of plants and/or cultivation and harvesting	Cutting/ comminuting			Physical processing, and packaging
Biotechnology: fermentation/ cell culture	Establish-ment of master cell bank and working cell bank	Maintenance of working cell bank	Cell culture and/or fermentation	Isolation and purification	Physical processing, and packaging
"Classical" Fermentation to produce an API	Establish-ment of cell bank	Maintenance of the cell bank	Introduction of the cells into fermentation	Isolation and purification	Physical processing, and packaging



II. QUALITY MANAGEMENT (2)

A. Principles (2.1)

Quality should be the responsibility of all persons involved in manufacturing.

Each manufacturer should establish, document, and implement an effective system for managing quality that involves the active participation of management and appropriate manufacturing personnel.

The system for managing quality should encompass the organizational structure, procedures, processes and resources, as well as activities to ensure confidence that the API will meet its intended specifications for quality and purity. All quality-related activities should be defined and documented.

There should be a quality unit(s) that is independent of production and that fulfills both quality assurance *(QA)* and quality control *(QC)* responsibilities. The quality unit can be in the form of separate QA and QC units or a single individual or group, depending upon the size and structure of the organization.

The persons authorized to release intermediates and APIs should be specified.

All quality-related activities should be recorded at the time they are performed.

Any deviation from established procedures should be documented and explained. Critical deviations should be investigated, and the investigation and its conclusions should be documented.

No materials should be released or used before the satisfactory completion of evaluation by the quality unit(s) unless there are appropriate systems in place to allow for such use (e.g., release under quarantine as described in Section X (10) or the use of raw materials or intermediates pending completion of evaluation).

Procedures should exist for notifying responsible management in a timely manner of regulatory inspections, serious GMP deficiencies, product defects and related actions (e.g., quality-related complaints, recalls, and regulatory actions).

B. Responsibilities of the Quality Unit(s) (2.2)

The quality unit(s) should be involved in all quality-related matters.

The quality unit(s) should review and approve all appropriate quality-related documents.

The main responsibilities of the independent quality unit(s) should not be delegated. These responsibilities should be described in writing and should include, but not necessarily be limited to:

1. Releasing or rejecting all APIs. Releasing or rejecting intermediates for use outside the control of the manufacturing company

2. Establishing a system to release or reject raw materials, intermediates, packaging, and labeling materials

3. Reviewing completed batch production and laboratory control records of critical process steps before release of the API for distribution

4. Making sure that critical deviations are investigated and resolved

5. Approving all specifications and master production instructions

6. Approving all procedures affecting the quality of intermediates or APIs

7. Making sure that internal audits (self-inspections) are performed

8. Approving intermediate and API contract manufacturers

9. Approving changes that potentially affect intermediate or API quality

10. Reviewing and approving validation protocols and reports

11. Making sure that quality-related complaints are investigated and resolved

12. Making sure that effective systems are used for maintaining and calibrating critical equipment

13. Making sure that materials are appropriately tested and the results are reported

14. Making sure that there is stability data to support retest or expiry dates and storage conditions on APIs and/or intermediates, where appropriate

15. Performing product quality reviews (as defined in Section 2.5)

C. Responsibility for Production Activities (2.3)

The responsibility for production activities should be described in writing and should include, but not necessarily be limited to:

1. Preparing, reviewing, approving, and distributing the instructions for the production of intermediates or APIs according to written procedures

2. Producing APIs and, when appropriate, intermediates according to pre-approved instructions

3. Reviewing all production batch records and ensuring that these are completed and signed

4. Making sure that all production deviations are reported and evaluated and that critical deviations are investigated and the conclusions are recorded

5. Making sure that production facilities are clean and, when appropriate, disinfected

6. Making sure that the necessary calibrations are performed and records kept

7. Making sure that the premises and equipment are maintained and records kept

8. Making sure that validation protocols and reports are reviewed and approved

9. Evaluating proposed changes in product, process or equipment

10. Making sure that new and, when appropriate, modified facilities and equipment are qualified

D. Internal Audits (Self Inspection) (2.4)

To verify compliance with the principles of GMP for APIs, regular internal audits should be performed in accordance with an approved schedule.

Audit findings and corrective actions should be documented and brought to the attention of responsible management of the firm. Agreed corrective actions should be completed in a timely and effective manner.

E. Product Quality Review (2.5)

Regular quality-reviews of APIs should be conducted with the objective of verifying the consistency of the process. Such reviews should normally be conducted and documented annually and should include at least:

- A review of critical in-process control and critical API test results
- A review of all batches that failed to meet established specification(s)
- A review of all critical deviations or nonconformances and related investigations
- A review of any changes carried out to the processes or analytical methods;
- A review of results of the stability monitoring program

- A review of all quality-related returns, complaints and recalls
- A review of adequacy of corrective actions

The results of this review should be evaluated and an assessment made of whether corrective action or any revalidation should be undertaken. Reasons for such corrective action should be documented. Agreed corrective actions should be completed in a timely and effective manner.

III. PERSONNEL (3)

A. Personnel Qualifications (3.1)

There should be an adequate number of personnel qualified by appropriate education, training, and/or experience to perform and supervise the manufacture of intermediates and APIs.

The responsibilities of all personnel engaged in the manufacture of intermediates and APIs should be specified in writing.

Training should be regularly conducted by qualified individuals and should cover, at a minimum, the particular operations that the employee performs and GMP as it relates to the employee's functions. Records of training should be maintained. Training should be periodically assessed.

B. Personnel Hygiene (3.2)

Personnel should practice good sanitation and health habits.

Personnel should wear clean clothing suitable for the manufacturing activity with which they are involved and this clothing should be changed, when appropriate. Additional protective apparel, such as head, face, hand, and arm coverings, should be worn, when necessary, to protect intermediates and APIs from contamination.

Personnel should avoid direct contact with intermediates or APIs.

Smoking, eating, drinking, chewing and the storage of food should be restricted to certain designated areas separate from the manufacturing areas.

Personnel suffering from an infectious disease or having open lesions on the exposed surface of the body should not engage in activities that could result in compromising the quality of APIs. Any person shown at any time (either by medical examination or supervisory observation) to have an apparent illness or open lesions should be excluded from activities where the health condition could adversely affect the quality of the APIs until the condition is corrected or qualified medical personnel determine that the person's inclusion would not jeopardize the safety or quality of the APIs.

C. Consultants (3.3)

Consultants advising on the manufacture and control of intermediates or APIs should have sufficient education, training, and experience, or any combination thereof, to advise on the subject for which they are retained.

Records should be maintained stating the name, address, qualifications, and type of service provided by these consultants.

IV. BUILDINGS AND FACILITIES (4)

A. Design and Construction (4.1)

Buildings and facilities used in the manufacture of intermediates and APIs should be located, designed, and constructed to facilitate cleaning, maintenance, and operations as appropriate to the type and stage of manufacture. Facilities should also be designed to minimize potential contamination. Where microbiological specifications have been established for the intermediate or API, facilities should also be designed to limit exposure to objectionable microbiological contaminants, as appropriate.

Buildings and facilities should have adequate space for the orderly placement of equipment and materials to prevent mix-ups and contamination.

Where the equipment itself (e.g., closed or contained systems) provides adequate protection of the material, such equipment can be located outdoors.

The flow of materials and personnel through the building or facilities should be designed to prevent mix-ups or contamination.

There should be defined areas or other control systems for the following activities:

- Receipt, identification, sampling, and quarantine of incoming materials, pending release or rejection
- Quarantine before release or rejection of intermediates and APIs
- Sampling of intermediates and APIs
- Holding rejected materials before further disposition (e.g., return, reprocessing or destruction)
- Storage of released materials
- Production operations
- Packaging and labeling operations
- Laboratory operations

Adequate and clean washing and toilet facilities should be provided for personnel. These facilities should be equipped with hot and cold water, as appropriate, soap or detergent, air

dryers, or single service towels. The washing and toilet facilities should be separate from, but easily accessible to, manufacturing areas. Adequate facilities for showering and/or changing clothes should be provided, when appropriate.

Laboratory areas/operations should normally be separated from production areas. Some laboratory areas, in particular those used for in-process controls, can be located in production areas, provided the operations of the production process do not adversely affect the accuracy of the laboratory measurements, and the laboratory and its operations do not adversely affect the production process, intermediate, or API.

B. Utilities (4.2)

All utilities that could affect product quality (e.g., steam, gas, compressed air, heating, ventilation, and air conditioning) should be qualified and appropriately monitored and action should be taken when limits are exceeded. Drawings for these utility systems should be available.

Adequate ventilation, air filtration and exhaust systems should be provided, where appropriate. These systems should be designed and constructed to minimize risks of contamination and cross-contamination and should include equipment for control of air pressure, microorganisms (if appropriate), dust, humidity, and temperature, as appropriate to the stage of manufacture. Particular attention should be given to areas where APIs are exposed to the environment.

If air is recirculated to production areas, appropriate measures should be taken to control risks of contamination and cross-contamination.

Permanently installed pipework should be appropriately identified. This can be accomplished by identifying individual lines, documentation, computer control systems, or alternative means. Pipework should be located to avoid risks of contamination of the intermediate or API.

Drains should be of adequate size and should be provided with an air break or a suitable device to prevent back-siphonage, when appropriate.

C. Water (4.3)

Water used in the manufacture of APIs should be demonstrated to be suitable for its intended use.

Unless otherwise justified, process water should, at a minimum, meet World Health Organization (WHO) guidelines for drinking (potable) water quality.

If drinking (potable) water is insufficient to ensure API quality and tighter chemical and/or microbiological water quality specifications are called for, appropriate specifications for physical/chemical attributes, total microbial counts, objectionable organisms, and/or endotoxins should be established.

Where water used in the process is treated by the manufacturer to achieve a defined quality, the treatment process should be validated and monitored with appropriate action limits.

Where the manufacturer of a nonsterile API either intends or claims that it is suitable for use in further processing to produce a sterile drug (medicinal) product, water used in the final isolation and purification steps should be monitored and controlled for total microbial counts, objectionable organisms, and endotoxins.

D. Containment (4.4)

Dedicated production areas, which can include facilities, air handling equipment and/or process equipment, should be employed in the production of highly sensitizing materials, such as penicillins or cephalosporins.

The use of dedicated production areas should also be considered when material of an infectious nature or high pharmacological activity or toxicity is involved (e.g., certain steroids or cytotoxic anti-cancer agents) unless validated inactivation and/or cleaning procedures are established and maintained.

Appropriate measures should be established and implemented to prevent cross-contamination from personnel and materials moving from one dedicated area to another.

Any production activities (including weighing, milling, or packaging) of highly toxic nonpharmaceutical materials, such as herbicides and pesticides, should not be conducted using the buildings and/or equipment being used for the production of APIs. Handling and storage of these highly toxic nonpharmaceutical materials should be separate from APIs.

E. Lighting (4.5)

Adequate lighting should be provided in all areas to facilitate cleaning, maintenance, and proper operations.

F. Sewage and Refuse (4.6)

Sewage, refuse, and other waste (e.g., solids, liquids, or gaseous by-products from manufacturing) in and from buildings and the immediate surrounding area should be disposed of in a safe, timely, and sanitary manner. Containers and/or pipes for waste material should be clearly identified.

G. Sanitation and Maintenance (4.7)

Buildings used in the manufacture of intermediates and APIs should be properly maintained and repaired and kept in a clean condition.

Written procedures should be established assigning responsibility for sanitation and describing the cleaning schedules, methods, equipment, and materials to be used in cleaning buildings and facilities.

When necessary, written procedures should also be established for the use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents to prevent the contamination of equipment, raw materials, packaging/labeling materials, intermediates, and APIs.

V. PROCESS EQUIPMENT (5)

A. Design and Construction (5.1)

Equipment used in the manufacture of intermediates and APIs should be of appropriate design and adequate size, and suitably located for its intended use, cleaning, sanitation (where appropriate), and maintenance.

Equipment should be constructed so that surfaces that contact raw materials, intermediates, or APIs do not alter the quality of the intermediates and APIs beyond the official or other established specifications.

Production equipment should only be used within its qualified operating range.

Major equipment (e.g., reactors, storage containers) and permanently installed processing lines used during the production of an intermediate or API should be appropriately identified.

Any substances associated with the operation of equipment, such as lubricants, heating fluids or coolants, should not contact intermediates or APIs so as to alter the quality of APIs or intermediates beyond the official or other established specifications. Any deviations from this practice should be evaluated to ensure that there are no detrimental effects on the material's fitness for use. Wherever possible, food grade lubricants and oils should be used.

Closed or contained equipment should be used whenever appropriate. Where open equipment is used, or equipment is opened, appropriate precautions should be taken to minimize the risk of contamination.

A set of current drawings should be maintained for equipment and critical installations (e.g., instrumentation and utility systems).

B. Equipment Maintenance and Cleaning (5.2)

Schedules and procedures (including assignment of responsibility) should be established for the preventative maintenance of equipment.

Written procedures should be established for cleaning equipment and its subsequent release for use in the manufacture of intermediates and APIs. Cleaning procedures should contain sufficient details to enable operators to clean each type of equipment in a reproducible and effective manner. These procedures should include:

- Assignment of responsibility for cleaning of equipment
- Cleaning schedules, including, where appropriate, sanitizing schedules
- A complete description of the methods and materials, including dilution of cleaning agents used to clean equipment
- When appropriate, instructions for disassembling and reassembling each article of equipment to ensure proper cleaning
- Instructions for the removal or obliteration of previous batch identification
- Instructions for the protection of clean equipment from contamination prior to use
- Inspection of equipment for cleanliness immediately before use, if practical
- Establishing the maximum time that may elapse between the completion of processing and equipment cleaning, when appropriate

Equipment and utensils should be cleaned, stored, and, where appropriate, sanitized or sterilized to prevent contamination or carry-over of a material that would alter the quality of the intermediate or API beyond the official or other established specifications.

Where equipment is assigned to continuous production or campaign production of successive batches of the same intermediate or API, equipment should be cleaned at appropriate intervals to prevent build-up and carry-over of contaminants (e.g., degradants or objectionable levels of microorganisms).

Nondedicated equipment should be cleaned between production of different materials to prevent cross-contamination.

Acceptance criteria for residues and the choice of cleaning procedures and cleaning agents should be defined and justified.

Equipment should be identified as to its contents and its cleanliness status by appropriate means.

C. Calibration (5.3)

Control, weighing, measuring, monitoring, and testing equipment critical for ensuring the quality of intermediates or APIs should be calibrated according to written procedures and an established schedule.

Equipment calibrations should be performed using standards traceable to certified standards, if they exist.

Records of these calibrations should be maintained.

The current calibration status of critical equipment should be known and verifiable.

Instruments that do not meet calibration criteria should not be used.

Deviations from approved standards of calibration on critical instruments should be investigated to determine if these could have had an effect on the quality of the intermediate(s) or API(s) manufactured using this equipment since the last successful calibration.

D. Computerized Systems (5.4)

GMP-related computerized systems should be validated. The depth and scope of validation depends on the diversity, complexity, and criticality of the computerized application.

Appropriate installation and operational qualifications should demonstrate the suitability of computer hardware and software to perform assigned tasks.

Commercially available software that has been qualified does not require the same level of testing. If an existing system was not validated at time of installation, a retrospective validation could be conducted if appropriate documentation is available.

Computerized systems should have sufficient controls to prevent unauthorized access or changes to data. There should be controls to prevent omissions in data (e.g., system turned off and data not captured). There should be a record of any data change made, the previous entry, who made the change, and when the change was made.

Written procedures should be available for the operation and maintenance of computerized systems.

Where critical data are being entered manually, there should be an additional check on the accuracy of the entry. This can be done by a second operator or by the system itself.

Incidents related to computerized systems that could affect the quality of intermediates or APIs or the reliability of records or test results should be recorded and investigated.

Changes to computerized systems should be made according to a change procedure and should be formally authorized, documented, and tested. Records should be kept of all changes, including modifications and enhancements made to the hardware, software, and any other critical component of the system. These records should demonstrate that the system is maintained in a validated state.

If system breakdowns or failures would result in the permanent loss of records, a back-up system should be provided. A means of ensuring data protection should be established for all computerized systems.

Data can be recorded by a second means in addition to the computer system.

VI. DOCUMENTATION AND RECORDS (6)

A. Documentation System and Specifications (6.1)

All documents related to the manufacture of intermediates or APIs should be prepared, reviewed, approved, and distributed according to written procedures. Such documents can be in paper or electronic form.

The issuance, revision, superseding, and withdrawal of all documents should be controlled by maintaining revision histories.

A procedure should be established for retaining all appropriate documents (e.g., development history reports, scale-up reports, technical transfer reports, process validation reports, training records, production records, control records, and distribution records). The retention periods for these documents should be specified.

All production, control, and distribution records should be retained for at least 1 year after the expiry date of the batch. For APIs with retest dates, records should be retained for at least 3 years after the batch is completely distributed.

When entries are made in records, these should be made indelibly in spaces provided for such entries, directly after performing the activities, and should identify the person making the entry. Corrections to entries should be dated and signed and leave the original entry still legible.

During the retention period, originals or copies of records should be readily available at the establishment where the activities described in such records occurred. Records that can be promptly retrieved from another location by electronic or other means are acceptable.

Specifications, instructions, procedures, and records can be retained either as originals or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records. Where reduction techniques such as microfilming or electronic records are used, suitable retrieval equipment and a means to produce a hard copy should be readily available.

Specifications should be established and documented for raw materials, intermediates where necessary, APIs, and labeling and packaging materials. In addition, specifications may be appropriate for certain other materials, such as process aids, gaskets, or other materials used during the production of intermediates or APIs that could critically affect quality. Acceptance criteria should be established and documented for in-process controls.

If electronic signatures are used on documents, they should be authenticated and secure.

B. Equipment Cleaning and Use Record (6.2)

Records of major equipment use, cleaning, sanitation, and/or sterilization and maintenance should show the date, time (if appropriate), product, and batch number of each batch processed in the equipment and the person who performed the cleaning and maintenance.

If equipment is dedicated to manufacturing one intermediate or API, individual equipment records are not necessary if batches of the intermediate or API follow in traceable sequence. In cases where dedicated equipment is employed, the records of cleaning, maintenance, and use can be part of the batch record or maintained separately.

C. Records of Raw Materials, Intermediates, API Labeling and Packaging Materials (6.3)

Records should be maintained including:

- The name of the manufacturer, identity, and quantity of each shipment of each batch of raw materials, intermediates, or labeling and packaging materials for API's; the name of the supplier; the supplier's control number(s), if known, or other identification number; the number allocated on receipt; and the date of receipt
- The results of any test or examination performed and the conclusions derived from this
- Records tracing the use of materials
- Documentation of the examination and review of API labeling and packaging materials for conformity with established specifications
- The final decision regarding rejected raw materials, intermediates, or API labeling and packaging materials

Master (approved) labels should be maintained for comparison to issued labels.

D. Master Production Instructions (Master Production and Control Records) (6.4)

To ensure uniformity from batch to batch, master production instructions for each intermediate and API should be prepared, dated, and signed by one person and independently checked, dated, and signed by a person in the quality unit(s).

Master production instructions should include:

- The name of the intermediate or API being manufactured and an identifying document reference code, if applicable
- A complete list of raw materials and intermediates designated by names or codes sufficiently specific to identify any special quality characteristics
- An accurate statement of the quantity or ratio of each raw material or intermediate to be used, including the unit of measure. Where the quantity is not fixed, the calculation for

each batch size or rate of production should be included. Variations to quantities should be included where they are justified

- The production location and major production equipment to be used
- Detailed production instructions, including the:

  - sequences to be followed

  - ranges of process parameters to be used

  - sampling instructions and in-process controls with their acceptance criteria, where appropriate

  - time limits for completion of individual processing steps and/or the total process, where appropriate

  - expected yield ranges at appropriate phases of processing or time
- Where appropriate, special notations and precautions to be followed, or cross-references to these
- The instructions for storage of the intermediate or API to ensure its suitability for use, including the labelling and packaging materials and special storage conditions with time limits, where appropriate.

E. Batch Production Records (Batch Production and Control Records) (6.5)

Batch production records should be prepared for each intermediate and API and should include complete information relating to the production and control of each batch. The batch production record should be checked before issuance to ensure that it is the correct version and a legible accurate reproduction of the appropriate master production instruction. If the batch production record is produced from a separate part of the master document, that document should include a reference to the current master production instruction being used.

These records should be numbered with a unique batch or identification number, dated and signed when issued. In continuous production, the product code together with the date and time can serve as the unique identifier until the final number is allocated.

Documentation of completion of each significant step in the batch production records (batch production and control records) should include:

- Dates and, when appropriate, times
- Identity of major equipment (e.g., reactors, driers, mills, etc.) used
- Specific identification of each batch, including weights, measures, and batch numbers of raw materials, intermediates, or any reprocessed materials used during manufacturing
- Actual results recorded for critical process parameters
- Any sampling performed

- Signatures of the persons performing and directly supervising or checking each critical step in the operation
- In-process and laboratory test results
- Actual yield at appropriate phases or times
- Description of packaging and label for intermediate or API
- Representative label of API or intermediate if made commercially available
- Any deviation noted, its evaluation, investigation conducted (if appropriate) or reference to that investigation if stored separately
- Results of release testing

Written procedures should be established and followed for investigating critical deviations or the failure of a batch of intermediate or API to meet specifications. The investigation should extend to other batches that may have been associated with the specific failure or deviation.

F. Laboratory Control Records (6.6)

Laboratory control records should include complete data derived from all tests conducted to ensure compliance with established specifications and standards, including examinations and assays, as follows:

- A description of samples received for testing, including the material name or source, batch number or other distinctive code, date sample was taken, and, where appropriate, the quantity and date the sample was received for testing
- A statement of or reference to each test method used
- A statement of the weight or measure of sample used for each test as described by the method; data on or cross-reference to the preparation and testing of reference standards, reagents and standard solutions
- A complete record of all raw data generated during each test, in addition to graphs, charts and spectra from laboratory instrumentation, properly identified to show the specific material and batch tested
- A record of all calculations performed in connection with the test, including, for example, units of measure, conversion factors, and equivalency factors
- A statement of the test results and how they compare with established acceptance criteria
- The signature of the person who performed each test and the date(s) the tests were performed
- The date and signature of a second person showing that the original records have been reviewed for accuracy, completeness, and compliance with established standards

Complete records should also be maintained for:

- Any modifications to an established analytical method
- Periodic calibration of laboratory instruments, apparatus, gauges, and recording devices

- All stability testing performed on APIs
- Out-of-specification (OOS) investigations

G. Batch Production Record Review (6.7)

Written procedures should be established and followed for the review and approval of batch production and laboratory control records, including packaging and labeling, to determine compliance of the intermediate or API with established specifications before a batch is released or distributed.

Batch production and laboratory control records of critical process steps should be reviewed and approved by the quality unit(s) before an API batch is released or distributed. Production and laboratory control records of noncritical process steps can be reviewed by qualified production personnel or other units following procedures approved by the quality unit(s).

All deviation, investigation, and OOS reports should be reviewed as part of the batch record review before the batch is released.

The quality unit(s) can delegate to the production unit the responsibility and authority for release of intermediates, except for those shipped outside the control of the manufacturing company.

VII. MATERIALS MANAGEMENT (7)

A. General Controls (7.1)

There should be written procedures describing the receipt, identification, quarantine, storage, handling, sampling, testing, and approval or rejection of materials.

Manufacturers of intermediates and/or APIs should have a system for evaluating the suppliers of critical materials.

Materials should be purchased against an agreed specification, from a supplier, or suppliers, approved by the quality unit(s).

If the supplier of a critical material is not the manufacturer of that material, the name and address of that manufacturer should be known by the intermediate and/or API manufacturer.

Changing the source of supply of critical raw materials should be treated according to Section 13, Change Control.

B. Receipt and Quarantine (7.2)

Upon receipt and before acceptance, each container or grouping of containers of materials should be examined visually for correct labeling (including correlation between the name used by the

supplier and the in-house name, if these are different), container damage, broken seals and evidence of tampering or contamination. Materials should be held under quarantine until they have been sampled, examined, or tested, as appropriate, and released for use.

Before incoming materials are mixed with existing stocks (e.g., solvents or stocks in silos), they should be identified as correct, tested, if appropriate, and released. Procedures should be available to prevent discharging incoming materials wrongly into the existing stock.

If bulk deliveries are made in nondedicated tankers, there should be assurance of no cross-contamination from the tanker. Means of providing this assurance could include one or more of the following:

- certificate of cleaning
- testing for trace impurities
- audit of the supplier

Large storage containers and their attendant manifolds, filling, and discharge lines should be appropriately identified.

Each container or grouping of containers (batches) of materials should be assigned and identified with a distinctive code, batch, or receipt number. This number should be used in recording the disposition of each batch. A system should be in place to identify the status of each batch.

C. Sampling and Testing of Incoming Production Materials (7.3)

At least one test to verify the identity of each batch of material should be conducted, with the exception of the materials described below. A *supplier's certificate of analysis* can be used in place of performing other tests, provided that the manufacturer has a system in place to evaluate suppliers.

Supplier approval should include an evaluation that provides adequate evidence (e.g., past quality history) that the manufacturer can consistently provide material meeting specifications. Complete analyses should be conducted on at least three batches before reducing in-house testing. However, as a minimum, a complete analysis should be performed at appropriate intervals and compared with the certificates of analysis. Reliability of certificates of analysis should be checked at regular intervals.

Processing aids, hazardous or highly toxic raw materials, other special materials, or materials transferred to another unit within the company's control do not need to be tested if the manufacturer's certificate of analysis is obtained, showing that these raw materials conform to established specifications. Visual examination of containers, labels, and recording of batch numbers should help in establishing the identity of these materials. The lack of on-site testing for these materials should be justified and documented.

Samples should be representative of the batch of material from which they are taken. Sampling methods should specify the number of containers to be sampled, which part of the container to sample, and the amount of material to be taken from each container. The number of containers to sample and the sample size should be based on a sampling plan that takes into consideration the criticality of the material, material variability, past quality history of the supplier, and the quantity needed for analysis.

Sampling should be conducted at defined locations and by procedures designed to prevent contamination of the material sampled and contamination of other materials.

Containers from which samples are withdrawn should be opened carefully and subsequently reclosed. They should be marked to indicate that a sample has been taken.

D. Storage (7.4)

Materials should be handled and stored in a manner to prevent degradation, contamination, and cross-contamination.

Materials stored in fiber drums, bags, or boxes should be stored off the floor and, when appropriate, suitably spaced to permit cleaning and inspection.

Materials should be stored under conditions and for a period that have no adverse effect on their quality, and should normally be controlled so that the oldest stock is used first.

Certain materials in suitable containers can be stored outdoors, provided identifying labels remain legible and containers are appropriately cleaned before opening and use.

Rejected materials should be identified and controlled under a quarantine system designed to prevent their unauthorized use in manufacturing.

E. Re-evaluation (7.5)

Materials should be re-evaluated, as appropriate, to determine their suitability for use (e.g., after prolonged storage or exposure to heat or humidity).

VIII. PRODUCTION AND IN-PROCESS CONTROLS (8)

A. Production Operations (8.1)

Raw materials for intermediate and API manufacturing should be weighed or measured under appropriate conditions that do not affect their suitability for use. Weighing and measuring devices should be of suitable accuracy for the intended use.

If a material is subdivided for later use in production operations, the container receiving the material should be suitable and should be so identified that the following information is available:

- Material name and/or item code
- Receiving or control number
- Weight or measure of material in the new container
- Re-evaluation or retest date if appropriate

Critical weighing, measuring, or subdividing operations should be witnessed or subjected to an equivalent control. Prior to use, production personnel should verify that the materials are those specified in the batch record for the intended intermediate or API.

Other critical activities should be witnessed or subjected to an equivalent control.

Actual yields should be compared with expected yields at designated steps in the production process. Expected yields with appropriate ranges should be established based on previous laboratory, pilot scale, or manufacturing data. Deviations in yield associated with critical process steps should be investigated to determine their impact or potential impact on the resulting quality of affected batches.

Any deviation should be documented and explained. Any critical deviation should be investigated.

The processing status of major units of equipment should be indicated either on the individual units of equipment or by appropriate documentation, computer control systems, or alternative means.

Materials to be reprocessed or reworked should be appropriately controlled to prevent unauthorized use.

B. Time Limits (8.2)

If time limits are specified in the master production instruction (see 6.40), these time limits should be met to ensure the quality of intermediates and APIs. Deviations should be documented and evaluated. Time limits may be inappropriate when processing to a target value (e.g., pH adjustment, hydrogenation, drying to predetermined specification) because completion of reactions or processing steps are determined by in-process sampling and testing.

Intermediates held for further processing should be stored under appropriate conditions to ensure their suitability for use.

C. In-process Sampling and Controls (8.3)

Written procedures should be established to monitor the progress and control the performance of processing steps that cause variability in the quality characteristics of intermediates and APIs. In-process controls and their acceptance criteria should be defined based on the information gained during the developmental stage or from historical data.

The acceptance criteria and type and extent of testing can depend on the nature of the intermediate or API being manufactured, the reaction or process step being conducted, and the degree to which the process introduces variability in the product's quality. Less stringent in-process controls may be appropriate in early processing steps, whereas tighter controls may be appropriate for later processing steps (e.g., isolation and purification steps).

Critical in-process controls (and critical process monitoring), including control points and methods, should be stated in writing and approved by the quality unit(s).

In-process controls can be performed by qualified production department personnel and the process adjusted without prior quality unit(s) approval if the adjustments are made within pre-established limits approved by the quality unit(s). All tests and results should be fully documented as part of the batch record.

Written procedures should describe the sampling methods for in-process materials, intermediates, and APIs. Sampling plans and procedures should be based on scientifically sound sampling practices.

In-process sampling should be conducted using procedures designed to prevent contamination of the sampled material and other intermediates or APIs. Procedures should be established to ensure the integrity of samples after collection.

Out-of-specification (OOS) investigations are not normally needed for in-process tests that are performed for the purpose of monitoring and/or adjusting the process.

D. Blending Batches of Intermediates or APIs (8.4)

For the purpose of this document, blending is defined as the process of combining materials within the same specification to produce a homogeneous intermediate or API. In-process mixing of fractions from single batches (e.g., collecting several centrifuge loads from a single crystallization batch) or combining fractions from several batches for further processing is considered to be part of the production process and is not considered to be blending.

Out-of-specification batches should not be blended with other batches for the purpose of meeting specifications. Each batch incorporated into the blend should have been manufactured using an established process and should have been individually tested and found to meet appropriate specifications prior to blending.

Acceptable blending operations include, but are not limited to:

- Blending of small batches to increase batch size
- Blending of tailings (i.e., relatively small quantities of isolated material) from batches of the same intermediate or API to form a single batch

Blending processes should be adequately controlled and documented, and the blended batch should be tested for conformance to established specifications, where appropriate.

The batch record of the blending process should allow traceability back to the individual batches that make up the blend.

Where physical attributes of the API are critical (e.g., APIs intended for use in solid oral dosage forms or suspensions), blending operations should be validated to show homogeneity of the combined batch. Validation should include testing of critical attributes (e.g., particle size distribution, bulk density, and tap density) that may be affected by the blending process.

If the blending could adversely affect stability, stability testing of the final blended batches should be performed.

The expiry or retest date of the blended batch should be based on the manufacturing date of the oldest tailings or batch in the blend.

E. Contamination Control (8.5)

Residual materials can be carried over into successive batches of the same intermediate or API if there is adequate control. Examples include residue adhering to the wall of a micronizer, residual layer of damp crystals remaining in a centrifuge bowl after discharge, and incomplete discharge of fluids or crystals from a processing vessel upon transfer of the material to the next step in the process. Such carryover should not result in the carryover of degradants or microbial contamination that may adversely alter the established API impurity profile.

Production operations should be conducted in a manner that prevents contamination of intermediates or APIs by other materials.

Precautions to avoid contamination should be taken when APIs are handled after purification.

IX. PACKAGING AND IDENTIFICATION LABELING OF APIs AND INTERMEDIATES (9)

A. General (9.1)

There should be written procedures describing the receipt, identification, quarantine, sampling, examination, and/or testing, release, and handling of packaging and labeling materials.

Packaging and labeling materials should conform to established specifications. Those that do not comply with such specifications should be rejected to prevent their use in operations for which they are unsuitable.

Records should be maintained for each shipment of labels and packaging materials showing receipt, examination, or testing, and whether accepted or rejected.

B. Packaging Materials (9.2)

Containers should provide adequate protection against deterioration or contamination of the intermediate or API that may occur during transportation and recommended storage.

Containers should be clean and, where indicated by the nature of the intermediate or API, sanitized to ensure that they are suitable for their intended use. These containers should not be reactive, additive, or absorptive so as to alter the quality of the intermediate or API beyond the specified limits.

If containers are reused, they should be cleaned in accordance with documented procedures, and all previous labels should be removed or defaced.

C. Label Issuance and Control (9.3)

Access to the label storage areas should be limited to authorized personnel.

Procedures should be established to reconcile the quantities of labels issued, used, and returned and to evaluate discrepancies found between the number of containers labeled and the number of labels issued. Such discrepancies should be investigated, and the investigation should be approved by the quality unit(s).

All excess labels bearing batch numbers or other batch-related printing should be destroyed. Returned labels should be maintained and stored in a manner that prevents mix-ups and provides proper identification.

Obsolete and out-dated labels should be destroyed.

Printing devices used to print labels for packaging operations should be controlled to ensure that all imprinting conforms to the print specified in the batch production record.

Printed labels issued for a batch should be carefully examined for proper identity and conformity to specifications in the master production record. The results of this examination should be documented.

A printed label representative of those used should be included in the batch production record.

D. Packaging and Labeling Operations (9.4)

There should be documented procedures designed to ensure that correct packaging materials and labels are used.

Labeling operations should be designed to prevent mix-ups. There should be physical or spatial separation from operations involving other intermediates or APIs.

Labels used on containers of intermediates or APIs should indicate the name or identifying code, batch number, and storage conditions when such information is critical to ensure the quality of intermediate or API.

If the intermediate or API is intended to be transferred outside the control of the manufacturer's material management system, the name and address of the manufacturer, quantity of contents, special transport conditions, and any special legal requirements should also be included on the label. For intermediates or APIs with an expiry date, the expiry date should be indicated on the label and certificate of analysis. For intermediates or APIs with a retest date, the retest date should be indicated on the label and/or certificate of analysis.

Packaging and labeling facilities should be inspected immediately before use to ensure that all materials not needed for the next packaging operation have been removed. This examination should be documented in the batch production records, the facility log, or other documentation system.

Packaged and labeled intermediates or APIs should be examined to ensure that containers and packages in the batch have the correct label. This examination should be part of the packaging operation. Results of these examinations should be recorded in the batch production or control records.

Intermediate or API containers that are transported outside of the manufacturer's control should be sealed in a manner such that, if the seal is breached or missing, the recipient will be alerted to the possibility that the contents may have been altered.

X. STORAGE AND DISTRIBUTION (10)

A. Warehousing Procedures (10.1)

Facilities should be available for the storage of all materials under appropriate conditions (e.g., controlled temperature and humidity when necessary). Records should be maintained of these conditions if they are critical for the maintenance of material characteristics.

Unless there is an alternative system to prevent the unintentional or unauthorized use of quarantined, rejected, returned, or recalled materials, separate storage areas should be assigned for their temporary storage until the decision as to their future use has been made.

B. Distribution Procedures (10.2)

APIs and intermediates should only be released for distribution to third parties after they have been released by the quality unit(s). APIs and intermediates can be transferred under quarantine to another unit under the company's control when authorized by the quality unit(s) and if appropriate controls and documentation are in place.

APIs and intermediates should be transported in a manner that does not adversely affect their quality.

Special transport or storage conditions for an API or intermediate should be stated on the label.

The manufacturer should ensure that the contract acceptor (contractor) for transportation of the API or intermediate knows and follows the appropriate transport and storage conditions.

A system should be in place by which the distribution of each batch of intermediate and/or API can be readily determined to permit its recall.

XI. LABORATORY CONTROLS (11)

A. General Controls (11.1)

The independent quality unit(s) should have at its disposal adequate laboratory facilities.

There should be documented procedures describing sampling, testing, approval, or rejection of materials and recording and storage of laboratory data. Laboratory records should be maintained in accordance with Section 6.6.

All specifications, sampling plans, and test procedures should be scientifically sound and appropriate to ensure that raw materials, intermediates, APIs, and labels and packaging materials conform to established standards of quality and/or purity. Specifications and test procedures should be consistent with those included in the registration/filing. There can be specifications in addition to those in the registration/filing. Specifications, sampling plans, and test procedures, including changes to them, should be drafted by the appropriate organizational unit and reviewed and approved by the quality unit(s).

Appropriate specifications should be established for APIs in accordance with accepted standards and consistent with the manufacturing process. The specifications should include control of impurities (e.g., organic impurities, inorganic impurities, and residual solvents). If the API has a specification for microbiological purity, appropriate action limits for total microbial counts and objectionable organisms should be established and met. If the API has a specification for endotoxins, appropriate action limits should be established and met.

Laboratory controls should be followed and documented at the time of performance. Any departures from the above-described procedures should be documented and explained.

Any out-of-specification result obtained should be investigated and documented according to a procedure. This procedure should include analysis of the data, assessment of whether a significant problem exists, allocation of the tasks for corrective actions, and conclusions. Any resampling and/or retesting after OOS results should be performed according to a documented procedure.

Reagents and standard solutions should be prepared and labeled following written procedures. *Use by* dates should be applied, as appropriate, for analytical reagents or standard solutions.

Primary reference standards should be obtained, as appropriate, for the manufacture of APIs. The source of each primary reference standard should be documented. Records should be maintained of each primary reference standard's storage and use in accordance with the supplier's recommendations. Primary reference standards obtained from an officially recognized source are normally used without testing if stored under conditions consistent with the supplier's recommendations.

Where a primary reference standard is not available from an officially recognized source, an *in-house primary standard* should be established. Appropriate testing should be performed to establish fully the identity and purity of the primary reference standard. Appropriate documentation of this testing should be maintained.

Secondary reference standards should be appropriately prepared, identified, tested, approved, and stored. The suitability of each batch of secondary reference standard should be determined prior to first use by comparing against a primary reference standard. Each batch of secondary reference standard should be periodically requalified in accordance with a written protocol.

B. Testing of Intermediates and APIs (11.2)

For each batch of intermediate and API, appropriate laboratory tests should be conducted to determine conformance to specifications.

An impurity profile describing the identified and unidentified impurities present in a typical batch produced by a specific controlled production process should normally be established for each API. The impurity profile should include the identity or some qualitative analytical designation (e.g., retention time), the range of each impurity observed, and classification of each identified impurity (e.g., inorganic, organic, solvent). The impurity profile is normally dependent upon the production process and origin of the API. Impurity profiles are normally not necessary for APIs from herbal or animal tissue origin. Biotechnology considerations are covered in ICH guidance Q6B.

The impurity profile should be compared at appropriate intervals against the impurity profile in the regulatory submission or compared against historical data to detect changes to the API

resulting from modifications in raw materials, equipment operating parameters, or the production process.

Appropriate microbiological tests should be conducted on each batch of intermediate and API where microbial quality is specified.

C. Validation of Analytical Procedures - See Section 12. (11.3)

D. Certificates of Analysis (11.4)

Authentic certificates of analysis should be issued for each batch of intermediate or API on request.

Information on the name of the intermediate or API including, where appropriate, its grade, the batch number, and the date of release should be provided on the certificate of analysis. For intermediates or APIs with an expiry date, the expiry date should be provided on the label and certificate of analysis. For intermediates or APIs with a retest date, the retest date should be indicated on the label and/or certificate of analysis.

The certificate should list each test performed in accordance with compendial or customer requirements, including the acceptance limits, and the numerical results obtained (if test results are numerical).

Certificates should be dated and signed by authorized personnel of the quality unit(s) and should show the name, address, and telephone number of the original manufacturer. Where the analysis has been carried out by a repacker or reprocessor, the certificate of analysis should show the name, address, and telephone number of the repacker/reprocessor and reference the name of the original manufacturer.

If new certificates are issued by or on behalf of repackers/reprocessors, agents or brokers, these certificates should show the name, address and telephone number of the laboratory that performed the analysis. They should also contain a reference to the name and address of the original manufacturer and to the original batch certificate, a copy of which should be attached.

E. Stability Monitoring of APIs (11.5)

A documented, on-going testing program should be established to monitor the stability characteristics of APIs, and the results should be used to confirm appropriate storage conditions and retest or expiry dates.

The test procedures used in stability testing should be validated and be stability indicating.

Stability samples should be stored in containers that simulate the market container. For example, if the API is marketed in bags within fiber drums, stability samples can be packaged in bags of

the same material and in small-scale drums of similar or identical material composition to the market drums.

Normally, the first three commercial production batches should be placed on the stability monitoring program to confirm the retest or expiry date. However, where data from previous studies show that the API is expected to remain stable for at least 2 years, fewer than three batches can be used.

Thereafter, at least one batch per year of API manufactured (unless none is produced that year) should be added to the stability monitoring program and tested at least annually to confirm the stability.

For APIs with short shelf-lives, testing should be done more frequently. For example, for those biotechnological/biologic and other APIs with shelf-lives of one year or less, stability samples should be obtained and should be tested monthly for the first 3 months, and at 3-month intervals after that. When data exist that confirm that the stability of the API is not compromised, elimination of specific test intervals (e.g., 9-month testing) can be considered.

Where appropriate, the stability storage conditions should be consistent with the ICH guidances on stability.

F. Expiry and Retest Dating (11.6)

When an intermediate is intended to be transferred outside the control of the manufacturer's material management system and an expiry or retest date is assigned, supporting stability information should be available (e.g., published data, test results).

An API expiry or retest date should be based on an evaluation of data derived from stability studies. Common practice is to use a retest date, not an expiration date.

Preliminary API expiry or retest dates can be based on pilot scale batches if (1) the pilot batches employ a method of manufacture and procedure that simulates the final process to be used on a commercial manufacturing scale and (2) the quality of the API represents the material to be made on a commercial scale.

A representative sample should be taken for the purpose of performing a retest.

G. Reserve/Retention Samples (11.7)

The packaging and holding of reserve samples is for the purpose of potential future evaluation of the quality of batches of API and not for future stability testing purposes.

Appropriately identified reserve samples of each API batch should be retained for 1 year after the expiry date of the batch assigned by the manufacturer, or for 3 years after distribution of the

batch, whichever is longer. For APIs with retest dates, similar reserve samples should be retained for 3 years after the batch is completely distributed by the manufacturer.

The reserve sample should be stored in the same packaging system in which the API is stored or in one that is equivalent to or more protective than the marketed packaging system. Sufficient quantities should be retained to conduct at least two full compendial analyses or, when there is no pharmacopoeial monograph, two full specification analyses.

XII. VALIDATION (12)

A. Validation Policy (12.1)

The company's overall policy, intentions, and approach to validation, including the validation of production processes, cleaning procedures, analytical methods, in-process control test procedures, computerized systems, and persons responsible for design, review, approval, and documentation of each validation phase, should be documented.

The critical parameters/attributes should normally be identified during the development stage or from historical data, and the necessary ranges for the reproducible operation should be defined. This should include:

- Defining the API in terms of its critical product attributes
- Identifying process parameters that could affect the critical quality attributes of the API
- Determining the range for each critical process parameter expected to be used during routine manufacturing and process control

Validation should extend to those operations determined to be critical to the quality and purity of the API.

B. Validation Documentation (12.2)

A written validation protocol should be established that specifies how validation of a particular process will be conducted. The protocol should be reviewed and approved by the quality unit(s) and other designated units.

The validation protocol should specify critical process steps and acceptance criteria as well as the type of validation to be conducted (e.g., retrospective, prospective, concurrent) and the number of process runs.

A validation report that cross-references the validation protocol should be prepared, summarizing the results obtained, commenting on any deviations observed, and drawing the appropriate conclusions, including recommending changes to correct deficiencies.

Any variations from the validation protocol should be documented with appropriate justification.

C. Qualification (12.3)

Before initiating process validation activities, appropriate qualification of critical equipment and ancillary systems should be completed. Qualification is usually carried out by conducting the following activities, individually or combined:

- Design Qualification (DQ): documented verification that the proposed design of the facilities, equipment, or systems is suitable for the intended purpose

- Installation Qualification (IQ): documented verification that the equipment or systems, as installed or modified, comply with the approved design, the manufacturer's recommendations and/or user requirements

- Operational Qualification (OQ): documented verification that the equipment or systems, as installed or modified, perform as intended throughout the anticipated operating ranges

- Performance Qualification (PQ): documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly based on the approved process method and specifications

D. Approaches to Process Validation (12.4)

Process Validation (PV) is the documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce an intermediate or API meeting its predetermined specifications and quality attributes.

There are three approaches to validation. Prospective validation is the preferred approach, but there are situations where the other approaches can be used. These approaches and their applicability are discussed here.

Prospective validation should normally be performed for all API processes as defined in 12.1. Prospective validation of an API process should be completed before the commercial distribution of the final drug product manufactured from that API.

Concurrent validation can be conducted when data from replicate production runs are unavailable because only a limited number of API batches have been produced, API batches are produced infrequently, or API batches are produced by a validated process that has been modified. Prior to the completion of concurrent validation, batches can be released and used in final drug product for commercial distribution based on thorough monitoring and testing of the API batches.

An exception can be made for retrospective validation of well-established processes that have been used without significant changes to API quality due to changes in raw materials, equipment, systems, facilities, or the production process. This validation approach may be used where:

1. Critical quality attributes and critical process parameters have been identified

2. Appropriate in-process acceptance criteria and controls have been established

3. There have not been significant process/product failures attributable to causes other than operator error or equipment failures unrelated to equipment suitability

4. Impurity profiles have been established for the existing API

Batches selected for retrospective validation should be representative of all batches produced during the review period, including any batches that failed to meet specifications, and should be sufficient in number to demonstrate process consistency. Retained samples can be tested to obtain data to retrospectively validate the process.

E. Process Validation Program (12.5)

The number of process runs for validation should depend on the complexity of the process or the magnitude of the process change being considered. For prospective and concurrent validation, three consecutive successful production batches should be used as a guide, but there may be situations where additional process runs are warranted to prove consistency of the process (e.g., complex API processes or API processes with prolonged completion times). For retrospective validation, generally data from 10 to 30 consecutive batches should be examined to assess process consistency, but fewer batches can be examined if justified.

Critical process parameters should be controlled and monitored during process validation studies. Process parameters unrelated to quality, such as variables controlled to minimize energy consumption or equipment use, need not be included in the process validation.

Process validation should confirm that the impurity profile for each API is within the limits specified. The impurity profile should be comparable to, or better than, historical data and, where applicable, the profile determined during process development or for batches used for pivotal clinical and toxicological studies.

F. Periodic Review of Validated Systems (12.6)

Systems and processes should be periodically evaluated to verify that they are still operating in a valid manner. Where no significant changes have been made to the system or process, and a quality review confirms that the system or process is consistently producing material meeting its specifications, there is normally no need for revalidation.

G. Cleaning Validation (12.7)

Cleaning procedures should normally be validated. In general, cleaning validation should be directed to situations or process steps where contamination or carryover of materials poses the greatest risk to API quality. For example, in early production it may be unnecessary to validate equipment cleaning procedures where residues are removed by subsequent purification steps.

Validation of cleaning procedures should reflect actual equipment usage patterns. If various APIs or intermediates are manufactured in the same equipment and the equipment is cleaned by the same process, a representative intermediate or API can be selected for cleaning validation. This selection should be based on the solubility and difficulty of cleaning and the calculation of residue limits based on potency, toxicity, and stability.

The cleaning validation protocol should describe the equipment to be cleaned, procedures, materials, acceptable cleaning levels, parameters to be monitored and controlled, and analytical methods. The protocol should also indicate the type of samples to be obtained and how they are collected and labeled.

Sampling should include swabbing, rinsing, or alternative methods (e.g., direct extraction), as appropriate, to detect both insoluble and soluble residues. The sampling methods used should be capable of quantitatively measuring levels of residues remaining on the equipment surfaces after cleaning. Swab sampling may be impractical when product contact surfaces are not easily accessible due to equipment design and/or process limitations (e.g., inner surfaces of hoses, transfer pipes, reactor tanks with small ports or handling toxic materials, and small intricate equipment such as micronizers and microfluidizers).

Validated analytical methods having sensitivity to detect residues or contaminants should be used. The detection limit for each analytical method should be sufficiently sensitive to detect the established acceptable level of the residue or contaminant. The method's attainable recovery level should be established. Residue limits should be practical, achievable, verifiable, and based on the most deleterious residue. Limits can be established based on the minimum known pharmacological, toxicological, or physiological activity of the API or its most deleterious component.

Equipment cleaning/sanitation studies should address microbiological and endotoxin contamination for those processes where there is a need to reduce total microbiological count or endotoxins in the API, or other processes where such contamination could be of concern (e.g., non-sterile APIs used to manufacture sterile products).

Cleaning procedures should be monitored at appropriate intervals after validation to ensure that these procedures are effective when used during routine production. Equipment cleanliness can be monitored by analytical testing and visual examination, where feasible. Visual inspection can allow detection of gross contamination concentrated in small areas that could otherwise go undetected by sampling and/or analysis.

H. Validation of Analytical Methods (12.8)

Analytical methods should be validated unless the method employed is included in the relevant pharmacopoeia or other recognized standard reference. The suitability of all testing methods used should nonetheless be verified under actual conditions of use and documented.

Methods should be validated to include consideration of characteristics included within the ICH guidances on validation of analytical methods. The degree of analytical validation performed should reflect the purpose of the analysis and the stage of the API production process.

Appropriate qualification of analytical equipment should be considered before initiating validation of analytical methods.

Complete records should be maintained of any modification of a validated analytical method. Such records should include the reason for the modification and appropriate data to verify that the modification produces results that are as accurate and reliable as the established method.

XIII. CHANGE CONTROL (13)

A formal change control system should be established to evaluate all changes that could affect the production and control of the intermediate or API.

Written procedures should provide for the identification, documentation, appropriate review, and approval of changes in raw materials, specifications, analytical methods, facilities, support systems, equipment (including computer hardware), processing steps, labeling and packaging materials, and computer software.

Any proposals for GMP relevant changes should be drafted, reviewed, and approved by the appropriate organizational units and reviewed and approved by the quality unit(s).

The potential impact of the proposed change on the quality of the intermediate or API should be evaluated. A classification procedure may help in determining the level of testing, validation, and documentation needed to justify changes to a validated process. Changes can be classified (e.g., as minor or major) depending on the nature and extent of the changes, and the effects these changes may impart on the process. Scientific judgment should determine what additional testing and validation studies are appropriate to justify a change in a validated process.

When implementing approved changes, measures should be taken to ensure that all documents affected by the changes are revised.

After the change has been implemented, there should be an evaluation of the first batches produced or tested under the change.

The potential for critical changes to affect established retest or expiry dates should be evaluated. If necessary, samples of the intermediate or API produced by the modified process can be placed on an accelerated stability program and/or can be added to the stability monitoring program.

Current dosage form manufacturers should be notified of changes from established production and process control procedures that can affect the quality of the API.

XIV. REJECTION AND RE-USE OF MATERIALS (14)

A. Rejection (14.1)

Intermediates and APIs failing to meet established specifications should be identified as such and quarantined. These intermediates or APIs can be reprocessed or reworked as described below. The final disposition of rejected materials should be recorded.

B. Reprocessing (14.2)

Introducing an intermediate or API, including one that does not conform to standards or specifications, back into the process and reprocessing by repeating a crystallization step or other appropriate chemical or physical manipulation steps (e.g., distillation, filtration, chromatography, milling) that are part of the established manufacturing process is generally considered acceptable. However, if such reprocessing is used for a majority of batches, such reprocessing should be included as part of the standard manufacturing process.

Continuation of a process step after an in-process control test has shown that the step is incomplete is considered to be part of the normal process. This is not considered to be reprocessing.

Introducing unreacted material back into a process and repeating a chemical reaction is considered to be reprocessing unless it is part of the established process. Such reprocessing should be preceded by careful evaluation to ensure that the quality of the intermediate or API is not adversely affected due to the potential formation of by-products and over-reacted materials.

C. Reworking (14.3)

Before a decision is taken to rework batches that do not conform to established standards or specifications, an investigation into the reason for nonconformance should be performed.

Batches that have been reworked should be subjected to appropriate evaluation, testing, stability testing if warranted, and documentation to show that the reworked product is of equivalent quality to that produced by the original process. Concurrent validation is often the appropriate validation approach for rework procedures. This allows a protocol to define the rework procedure, how it will be carried out, and the expected results. If there is only one batch to be reworked, a report can be written and the batch released once it is found to be acceptable.

Procedures should provide for comparing the impurity profile of each reworked batch against batches manufactured by the established process. Where routine analytical methods are inadequate to characterize the reworked batch, additional methods should be used.

D. Recovery of Materials and Solvents (14.4)

Recovery (e.g., from mother liquor or filtrates) of reactants, intermediates, or the API is considered acceptable, provided that approved procedures exist for the recovery and the recovered materials meet specifications suitable for their intended use.

Solvents can be recovered and reused in the same processes or in different processes, provided that the recovery procedures are controlled and monitored to ensure that solvents meet appropriate standards before reuse or commingling with other approved materials.

Fresh and recovered solvents and reagents can be combined if adequate testing has shown their suitability for all manufacturing processes in which they may be used.

The use of recovered solvents, mother liquors, and other recovered materials should be adequately documented.

E. Returns (14.5)

Returned intermediates or APIs should be identified as such and quarantined.

If the conditions under which returned intermediates or APIs have been stored or shipped before or during their return or the condition of their containers casts doubt on their quality, the returned intermediates or APIs should be reprocessed, reworked, or destroyed, as appropriate.

Records of returned intermediates or APIs should be maintained. For each return, documentation should include:

- Name and address of the consignee
- Intermediate or API, batch number, and quantity returned
- Reason for return
- Use or disposal of the returned intermediate or API

XV. COMPLAINTS AND RECALLS (15)

All quality-related complaints, whether received orally or in writing, should be recorded and investigated according to a written procedure.

Complaint records should include:

- Name and address of complainant
- Name (and, where appropriate, title) and phone number of person submitting the complaint
- Complaint nature (including name and batch number of the API)
- Date complaint is received
- Action initially taken (including dates and identity of person taking the action);
- Any follow-up action taken
- Response provided to the originator of complaint (including date response sent)
- Final decision on intermediate or API batch or lot

Records of complaints should be retained to evaluate trends, product-related frequencies, and severity with a view to taking additional, and if appropriate, immediate corrective action.

There should be a written procedure that defines the circumstances under which a recall of an intermediate or API should be considered.

The recall procedure should designate who should be involved in evaluating the information, how a recall should be initiated, who should be informed about the recall, and how the recalled material should be treated.

In the event of a serious or potentially life-threatening situation, local, national, and/or international authorities should be informed and their advice sought.

XVI. CONTRACT MANUFACTURERS (INCLUDING LABORATORIES) (16)

All contract manufacturers (including laboratories) should comply with the GMP defined in this guidance. Special consideration should be given to the prevention of cross-contamination and to maintaining traceability.

Companies should evaluate any contractors (including laboratories) to ensure GMP compliance of the specific operations occurring at the contractor sites.

There should be a written and approved contract or formal agreement between a company and its contractors that defines in detail the GMP responsibilities, including the quality measures, of each party.

A contract should permit a company to audit its contractor's facilities for compliance with GMP.

Where subcontracting is allowed, a contractor should not pass to a third party any of the work entrusted to it under the contract without the company's prior evaluation and approval of the arrangements.

Manufacturing and laboratory records should be kept at the site where the activity occurs and be readily available.

Changes in the process, equipment, test methods, specifications, or other contractual requirements should not be made unless the contract giver is informed and approves the changes.

XVII. AGENTS, BROKERS, TRADERS, DISTRIBUTORS, REPACKERS, AND RELABELLERS (17)

A. Applicability (17.1)

This section applies to any party other than the original manufacturer who may trade and/or take possession, repack, relabel, manipulate, distribute, or store an API or intermediate.

All agents, brokers, traders, distributors, repackers, and relabelers should comply with GMP as defined in this guidance.

B. Traceability of Distributed APIs and Intermediates (17.2)

Agents, brokers, traders, distributors, repackers, or relabelers should maintain complete traceability of APIs and intermediates that they distribute. Documents that should be retained and available include:

- Identity of original manufacturer
- Address of original manufacturer
- Purchase orders
- Bills of lading (transportation documentation)
- Receipt documents
- Name or designation of API or intermediate
- Manufacturer's batch number
- Transportation and distribution records
- All authentic Certificates of Analysis, including those of the original manufacturer
- Retest or expiry date

C. Quality Management (17.3)

Agents, brokers, traders, distributors, repackers, or relabelers should establish, document and implement an effective system of managing quality, as specified in Section 2.

D. Repackaging, Relabeling, and Holding of APIs and Intermediates (17.4)

Repackaging, relabeling, and holding APIs and intermediates should be performed under appropriate GMP controls, as stipulated in this guidance, to avoid mix-ups and loss of API or intermediate identity or purity.

Repackaging should be conducted under appropriate environmental conditions to avoid contamination and cross-contamination.

E. Stability (17.5)

Stability studies to justify assigned expiration or retest dates should be conducted if the API or intermediate is repackaged in a different type of container than that used by the API or intermediate manufacturer.

F. Transfer of Information (17.6)

Agents, brokers, distributors, repackers, or relabelers should transfer all quality or regulatory information received from an API or intermediate manufacturer to the customer, and from the customer to the API or intermediate manufacturer.

The agent, broker, trader, distributor, repacker, or relabeler who supplies the API or intermediate to the customer should provide the name of the original API or intermediate manufacturer and the batch number(s) supplied.

The agent should also provide the identity of the original API or intermediate manufacturer to regulatory authorities upon request. The original manufacturer can respond to the regulatory authority directly or through its authorized agents, depending on the legal relationship between the authorized agents and the original API or intermediate manufacturer. (In this context *authorized* refers to authorized by the manufacturer.)

The specific guidance for certificate of analysis included in Section 11.4 should be met.

G. Handling of Complaints and Recalls (17.7)

Agents, brokers, traders, distributors, repackers, or relabelers should maintain records of complaints and recalls, as specified in Section 15, for all complaints and recalls that come to their attention.

If the situation warrants, the agents, brokers, traders, distributors, repackers, or relabelers should review the complaint with the original API or intermediate manufacturer to determine whether any further action, either with other customers who may have received this API or intermediate or with the regulatory authority, or both, should be initiated. The investigation into the cause for the complaint or recall should be conducted and documented by the appropriate party.

Where a complaint is referred to the original API or intermediate manufacturer, the record maintained by the agents, brokers, traders, distributors, repackers, or relabelers should include any response received from the original API or intermediate manufacturer (including date and information provided).

H. Handling of Returns (17.8)

Returns should be handled as specified in Section 14.5. The agents, brokers, traders, distributors, repackers, or relabelers should maintain documentation of returned APIs and intermediates.

XVIII. SPECIFIC GUIDANCE FOR APIs MANUFACTURED BY CELL CULTURE/FERMENTATION (18)

A. General (18.1)

Section 18 is intended to address specific controls for APIs or intermediates manufactured by cell culture or fermentation using natural or recombinant organisms and that have not been covered adequately in the previous sections. It is not intended to be a stand-alone section. In general, the GMP principles in the other sections of this document apply. Note that the principles of fermentation for *classical* processes for production of small molecules and for processes using recombinant and nonrecombinant organisms for production of proteins and/or polypeptides are the same, although the degree of control will differ. Where practical, this section will address these differences. In general, the degree of control for biotechnological processes used to produce proteins and polypeptides is greater than that for classical fermentation processes.

The term *biotechnological process* (biotech) refers to the use of cells or organisms that have been generated or modified by recombinant DNA, hybridoma, or other technology to produce APIs. The APIs produced by biotechnological processes normally consist of high molecular weight substances, such as proteins and polypeptides, for which specific guidance is given in this Section. Certain APIs of low molecular weight, such as antibiotics, amino acids, vitamins, and carbohydrates, can also be produced by recombinant DNA technology. The level of control for these types of APIs is similar to that employed for classical fermentation.

The term *classical fermentation* refers to processes that use microorganisms existing in nature and/or modified by conventional methods (e.g., irradiation or chemical mutagenesis) to produce APIs. APIs produced by *classical fermentation* are normally low molecular weight products such as antibiotics, amino acids, vitamins, and carbohydrates.

Production of APIs or intermediates from cell culture or fermentation involves biological processes such as cultivation of cells or extraction and purification of material from living organisms. Note that there may be additional process steps, such as physicochemical modification, that are part of the manufacturing process. The raw materials used (media, buffer components) may provide the potential for growth of microbiological contaminants. Depending on the source, method of preparation, and the intended use of the API or intermediate, control of bioburden, viral contamination, and/or endotoxins during manufacturing and monitoring of the process at appropriate stages may be necessary.

Appropriate controls should be established at all stages of manufacturing to ensure intermediate and/or API quality. While this guidance starts at the cell culture/fermentation step, prior steps

(e.g., cell banking) should be performed under appropriate process controls. This guidance covers cell culture/fermentation from the point at which a vial of the cell bank is retrieved for use in manufacturing.

Appropriate equipment and environmental controls should be used to minimize the risk of contamination. The acceptance criteria for determining environmental quality and the frequency of monitoring should depend on the step in production and the production conditions (open, closed, or contained systems).

In general, process controls should take into account:

- Maintenance of the working cell bank (where appropriate)
- Proper inoculation and expansion of the culture
- Control of the critical operating parameters during fermentation/cell culture
- Monitoring of the process for cell growth, viability (for most cell culture processes) and productivity, where appropriate
- Harvest and purification procedures that remove cells, cellular debris and media components while protecting the intermediate or API from contamination (particularly of a microbiological nature) and from loss of quality
- Monitoring of bioburden and, where needed, endotoxin levels at appropriate stages of production
- Viral safety concerns as described in ICH guidance Q5A *Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*

Where appropriate, the removal of media components, host cell proteins, other process-related impurities, product-related impurities and contaminants should be demonstrated.

B. Cell Bank Maintenance and Record Keeping (18.2)

Access to cell banks should be limited to authorized personnel.

Cell banks should be maintained under storage conditions designed to maintain viability and prevent contamination.

Records of the use of the vials from the cell banks and storage conditions should be maintained.

Where appropriate, cell banks should be periodically monitored to determine suitability for use.

See ICH guidance Q5D *Quality of Biotechnological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products* for a more complete discussion of cell banking.

C. Cell Culture/Fermentation (18.3)

Where cell substrates, media, buffers, and gases are to be added under aseptic conditions, closed or contained systems should be used where possible. If the inoculation of the initial vessel or subsequent transfers or additions (media, buffers) are performed in open vessels, there should be controls and procedures in place to minimize the risk of contamination.

Where the quality of the API can be affected by microbial contamination, manipulations using open vessels should be performed in a biosafety cabinet or similarly controlled environment.

Personnel should be appropriately gowned and take special precautions handling the cultures.

Critical operating parameters (for example temperature, pH, agitation rates, addition of gases, pressure) should be monitored to ensure consistency with the established process. Cell growth, viability (for most cell culture processes), and, where appropriate, productivity should also be monitored. Critical parameters will vary from one process to another, and for classical fermentation, certain parameters (cell viability, for example) may not need to be monitored.

Cell culture equipment should be cleaned and sterilized after use. As appropriate, fermentation equipment should be cleaned, sanitized, or sterilized.

Culture media should be sterilized before use, when necessary, to protect the quality of the API.

Appropriate procedures should be in place to detect contamination and determine the course of action to be taken. Procedures should be available to determine the impact of the contamination on the product and to decontaminate the equipment and return it to a condition to be used in subsequent batches. Foreign organisms observed during fermentation processes should be identified, as appropriate, and the effect of their presence on product quality should be assessed, if necessary. The results of such assessments should be taken into consideration in the disposition of the material produced.

Records of contamination events should be maintained.

Shared (multi-product) equipment may warrant additional testing after cleaning between product campaigns, as appropriate, to minimize the risk of cross-contamination.

D. Harvesting, Isolation and Purification (18.4)

Harvesting steps, either to remove cells or cellular components or to collect cellular components after disruption should be performed in equipment and areas designed to minimize the risk of contamination.

Harvest and purification procedures that remove or inactivate the producing organism, cellular debris and media components (while minimizing degradation, contamination, and loss of quality) should be adequate to ensure that the intermediate or API is recovered with consistent quality.

All equipment should be properly cleaned and, as appropriate, sanitized after use. Multiple successive batching without cleaning can be used if intermediate or API quality is not compromised.

If open systems are used, purification should be performed under environmental conditions appropriate for the preservation of product quality.

Additional controls, such as the use of dedicated chromatography resins or additional testing, may be appropriate if equipment is to be used for multiple products.

E. Viral Removal/Inactivation steps (18.5)

See ICH guidance Q5A *Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* for more specific information.

Viral removal and viral inactivation steps are critical processing steps for some processes and should be performed within their validated parameters.

Appropriate precautions should be taken to prevent potential viral contamination from previral to postviral removal/inactivation steps. Therefore, open processing should be performed in areas that are separate from other processing activities and have separate air handling units.

The same equipment is not normally used for different purification steps. However, if the same equipment is to be used, the equipment should be appropriately cleaned and sanitized before reuse. Appropriate precautions should be taken to prevent potential virus carry-over (e.g., through equipment or environment) from previous steps.

XIX. APIs FOR USE IN CLINICAL TRIALS (19)

A. General (19.1)

Not all the controls in the previous sections of this guidance are appropriate for the manufacture of a new API for investigational use during its development. Section XIX (19) provides specific guidance unique to these circumstances.

The controls used in the manufacture of APIs for use in clinical trials should be consistent with the stage of development of the drug product incorporating the API. Process and test procedures should be flexible to provide for changes as knowledge of the process increases and clinical testing of a drug product progresses from pre-clinical stages through clinical stages. Once drug development reaches the stage where the API is produced for use in drug products intended for clinical trials, manufacturers should ensure that APIs are manufactured in suitable facilities using appropriate production and control procedures to ensure the quality of the API.

B. Quality (19.2)

Appropriate GMP concepts should be applied in the production of APIs for use in clinical trials with a suitable mechanism for approval of each batch.

A quality unit(s) independent from production should be established for the approval or rejection of each batch of API for use in clinical trials.

Some of the testing functions commonly performed by the quality unit(s) can be performed within other organizational units.

Quality measures should include a system for testing of raw materials, packaging materials, intermediates, and APIs.

Process and quality problems should be evaluated.

Labeling for APIs intended for use in clinical trials should be appropriately controlled and should identify the material as being for investigational use.

C. Equipment and Facilities (19.3)

During all phases of clinical development, including the use of small-scale facilities or laboratories to manufacture batches of APIs for use in clinical trials, procedures should be in place to ensure that equipment is calibrated, clean, and suitable for its intended use.

Procedures for the use of facilities should ensure that materials are handled in a manner that minimizes the risk of contamination and cross-contamination.

D. Control of Raw Materials (19.4)

Raw materials used in production of APIs for use in clinical trials should be evaluated by testing, or received with a supplier's analysis and subjected to identity testing. When a material is considered hazardous, a supplier's analysis should suffice.

In some instances, the suitability of a raw material can be determined before use based on acceptability in small-scale reactions (i.e., use testing) rather than on analytical testing alone.

E. Production (19.5)

The production of APIs for use in clinical trials should be documented in laboratory notebooks, batch records, or by other appropriate means. These documents should include information on the use of production materials, equipment, processing, and scientific observations.

Expected yields can be more variable and less defined than the expected yields used in commercial processes. Investigations into yield variations are not expected.

F. Validation (19.6)

Process validation for the production of APIs for use in clinical trials is normally inappropriate, where a single API batch is produced or where process changes during API development make batch replication difficult or inexact. The combination of controls, calibration, and, where appropriate, equipment qualification ensures API quality during this development phase.

Process validation should be conducted in accordance with Section 12 when batches are produced for commercial use, even when such batches are produced on a pilot or small scale.

G. Changes (19.7)

Changes are expected during development, as knowledge is gained and the production is scaled up. Every change in the production, specifications, or test procedures should be adequately recorded.

H. Laboratory Controls (19.8)

While analytical methods performed to evaluate a batch of API for clinical trials may not yet be validated, they should be scientifically sound.

A system for retaining reserve samples of all batches should be in place. This system should ensure that a sufficient quantity of each reserve sample is retained for an appropriate length of time after approval, termination, or discontinuation of an application.

Expiry and retest dating as defined in Section 11.6 applies to existing APIs used in clinical trials. For new APIs, Section 11.6 does not normally apply in early stages of clinical trials.

I. Documentation (19.9)

A system should be in place to ensure that information gained during the development and the manufacture of APIs for use in clinical trials is documented and available.

The development and implementation of the analytical methods used to support the release of a batch of API for use in clinical trials should be appropriately documented.

A system for retaining production and control records and documents should be used. This system should ensure that records and documents are retained for an appropriate length of time after the approval, termination, or discontinuation of an application.

GLOSSARY (20)

Acceptance Criteria: Numerical limits, ranges, or other suitable measures for acceptance of test results.

Active Pharmaceutical Ingredient (API) ***(or Drug Substance):*** Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body.

API Starting Material: A raw material, intermediate, or an API that is used in the production of an API and that is incorporated as a significant structural fragment into the structure of the API. An API starting material can be an article of commerce, a material purchased from one or more suppliers under contract or commercial agreement, or produced in-house. API starting materials are normally of defined chemical properties and structure.

Batch (or Lot): A specific quantity of material produced in a process or series of processes so that it is expected to be homogeneous within specified limits. In the case of continuous production, a batch may correspond to a defined fraction of the production. The batch size can be defined either by a fixed quantity or by the amount produced in a fixed time interval.

Batch Number (or Lot Number): A unique combination of numbers, letters, and/or symbols that identifies a batch (or lot) and from which the production and distribution history can be determined.

Bioburden: The level and type (e.g., objectionable or not) of microorganisms that can be present in raw materials, API starting materials, intermediates or APIs. Bioburden should not be considered contamination unless the levels have been exceeded or defined objectionable organisms have been detected.

Calibration: The demonstration that a particular instrument or device produces results within specified limits by comparison with results produced by a reference or traceable standard over an appropriate range of measurements.

Computer System: A group of hardware components and associated software designed and assembled to perform a specific function or group of functions.

Computerized System: A process or operation integrated with a computer system.

Contamination: The undesired introduction of impurities of a chemical or microbiological nature, or of foreign matter, into or onto a raw material, intermediate, or API during production, sampling, packaging, or repackaging, storage or transport.

Contract Manufacturer: A manufacturer who performs some aspect of manufacturing on behalf of the original manufacturer.

Critical: Describes a process step, process condition, test requirement, or other relevant parameter or item that must be controlled within predetermined criteria to ensure that the API meets its specification.

Cross-Contamination: Contamination of a material or product with another material or product.

Deviation: Departure from an approved instruction or established standard.

Drug (Medicinal) Product: The dosage form in the final immediate packaging intended for marketing. (Reference Q1A)

Drug Substance: See Active Pharmaceutical Ingredient.

Expiry Date (or Expiration Date): The date placed on the container/labels of an API designating the time during which the API is expected to remain within established shelf life specifications if stored under defined conditions and after which it should not be used.

Impurity: Any component present in the intermediate or API that is not the desired entity.

Impurity Profile: A description of the identified and unidentified impurities present in an API.

In-Process Control (or Process Control): Checks performed during production to monitor and, if appropriate, to adjust the process and/or to ensure that the intermediate or API conforms to its specifications.

Intermediate: A material produced during steps of the processing of an API that undergoes further molecular change or purification before it becomes an API. Intermediates may or may not be isolated. (Note: this guidance only addresses those intermediates produced after the point that a company has defined as the point at which the production of the API begins.)

Lot: See Batch

Lot Number: See *Batch Number*

Manufacture: All operations of receipt of materials, production, packaging, repackaging, labeling, relabeling, quality control, release, storage, and distribution of APIs and related controls.

Material: A general term used to denote raw materials (starting materials, reagents, solvents), process aids, intermediates, APIs, and packaging and labeling materials.

Mother Liquor: The residual liquid that remains after the crystallization or isolation processes. A mother liquor may contain unreacted materials, intermediates, levels of the API, and/or impurities. It can be used for further processing.

Packaging Material: Any material intended to protect an intermediate or API during storage and transport.

Procedure: A documented description of the operations to be performed, the precautions to be taken, and measures to be applied directly or indirectly related to the manufacture of an intermediate or API.

Process Aids: Materials, excluding solvents, used as an aid in the manufacture of an intermediate or API that do not themselves participate in a chemical or biological reaction (e.g., filter aid, activated carbon).

Process Control: See ***In-Process Control.***

Production: All operations involved in the preparation of an API from receipt of materials through processing and packaging of the API.

Qualification: Action of proving and documenting that equipment or ancillary systems are properly installed, work correctly, and actually lead to the expected results. Qualification is part of validation, but the individual qualification steps alone do not constitute process validation.

Quality Assurance (QA): The sum total of the organized arrangements made with the object of ensuring that all APIs are of the quality required for their intended use and that quality systems are maintained.

Quality Control (QC): Checking or testing that specifications are met.

Quality Unit(s): An organizational unit independent of production that fulfills both quality assurance and quality control responsibilities. This can be in the form of separate QA and QC units or a single individual or group, depending upon the size and structure of the organization.

Quarantine: The status of materials isolated physically or by other effective means pending a decision on their subsequent approval or rejection.

Raw Material: A general term used to denote starting materials, reagents, and solvents intended for use in the production of intermediates or APIs.

Reference Standard, Primary: A substance that has been shown by an extensive set of analytical tests to be authentic material that should be of high purity. This standard can be: (1) obtained from an officially recognized source, (2) prepared by independent synthesis, (3) obtained from existing production material of high purity, or (4) prepared by further purification of existing production material.

Reference Standard, Secondary: A substance of established quality and purity, as shown by comparison to a primary reference standard, used as a reference standard for routine laboratory analysis.

Reprocessing: Introducing an intermediate or API, including one that does not conform to standards or specifications, back into the process and repeating a crystallization step or other appropriate chemical or physical manipulation steps (e.g., distillation, filtration, chromatography, milling) that are part of the established manufacturing process. Continuation of a process step after an in-process control test has shown that the step is incomplete, is considered to be part of the normal process, and is not reprocessing.

Retest Date: The date when a material should be re-examined to ensure that it is still suitable for use.

Reworking: Subjecting an intermediate or API that does not conform to standards or specifications to one or more processing steps that are different from the established manufacturing process to obtain acceptable quality intermediate or API (e.g., recrystallizing with a different solvent).

Signature (signed): See definition for signed.

Signed (signature): The record of the individual who performed a particular action or review. This record can be initials, full handwritten signature, personal seal, or authenticated and secure electronic signature.

Solvent: An inorganic or organic liquid used as a vehicle for the preparation of solutions or suspensions in the manufacture of an intermediate or API.

Specification: A list of tests, references to analytical procedures, and appropriate acceptance criteria that are numerical limits, ranges, or other criteria for the test described. It establishes the set of criteria to which a material should conform to be considered acceptable for its intended use. *Conformance to specification* means that the material, when tested according to the listed analytical procedures, will meet the listed acceptance criteria.

Validation: A documented program that provides a high degree of assurance that a specific process, method, or system will consistently produce a result meeting predetermined acceptance criteria.

Validation Protocol: A written plan stating how validation will be conducted and defining acceptance criteria. For example, the protocol for a manufacturing process identifies processing equipment, critical process parameters and/or operating ranges, product characteristics, sampling, test data to be collected, number of validation runs, and acceptable test results.

Yield, Expected: The quantity of material or the percentage of theoretical yield anticipated at any appropriate phase of production based on previous laboratory, pilot scale, or manufacturing data.

Yield, Theoretical: The quantity that would be produced at any appropriate phase of production based upon the quantity of material to be used, in the absence of any loss or error in actual production.

Diethylstilbestrol Exposure

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Diethylstilbestrol is a synthetic nonsteroidal estrogen that was used to prevent miscarriage and other pregnancy complications between 1938 and 1971 in the United States. In 1971, the U.S. Food and Drug Administration issued a warning about the use of diethylstilbestrol during pregnancy after a relationship between exposure to this synthetic estrogen and the development of clear cell adenocarcinoma of the vagina and cervix was found in young women whose mothers had taken diethylstilbestrol while they were pregnant. Although diethylstilbestrol has not been given to pregnant women in the United States for more than 30 years, its effects continue to be seen. Women who took diethylstilbestrol during pregnancy have a slightly higher risk of breast cancer than the general population and therefore should be encouraged to have regular mammography. Women who were exposed to diethylstilbestrol in utero may have structural reproductive tract anomalies, an increased infertility rate, and poor pregnancy outcomes. However, the majority of these women have been able to deliver successfully. Recommendations for gynecologic examinations include vaginal and cervical digital palpation, which may provide the only evidence of clear cell adenocarcinoma. Initial colposcopic examination should be considered; if the findings are abnormal, colposcopy should be repeated annually. If the initial colposcopic examination is normal, annual cervical and vaginal cytology is recommended. Because of the higher risk of spontaneous abortion, ectopic pregnancy, and preterm delivery, obstetric consultation may be required for pregnant women who had in utero diethylstilbestrol exposure. The male offspring of women who took diethylstilbestrol during pregnancy have an increased incidence of genital abnormalities and a possibly increased risk of prostate and testicular cancer. Routine prostate cancer screening and testicular self-examination should be encouraged. (Am Fam Physician 2004:69:2395-400,2401-2.

A patient information handout on diethylstilbestrol, written by the authors of this article, is provided on page 2401.

See page 2291 for definitions of strength-of-recommendation labels.

Between 1938 and 1971, as many as 4 million women in the United States took diethylstilbestrol (DES), an oral synthetic nonsteroidal estrogen, for the purpose of improving pregnancy outcomes.[1,2] In 1953, it was demonstrated that DES did not prevent miscarriage and other pregnancy complications. However, physicians continued to prescribe DES to pregnant women until at least 1971, when a connection was established between in utero DES exposure and the development of clear cell adenocarcinoma of the vagina and cervix in the daughters of women who had taken DES during pregnancy.[3] In 1971, the U.S. Food and Drug Administration issued a warning against the use of DES in pregnant women.[4] DES continued to be used in various European countries until the early 1980s.

In 1971, the U.S. Food and Drug Administration warned against the use of diethylstilbestrol in pregnant women because of an increased risk of clear cell adenocarcinoma in female offspring.

The association between in utero DES exposure and vaginal clear cell adenocarcinoma has been well documented. Other adverse associations have been identified in DES-exposed women and their offspring, and animal studies have shown effects in the next generation (grandchildren).[5,6] The Centers for Disease Control and Prevention has instituted a campaign to educate health care professionals and patients about the risks associated with exposure to this synthetic estrogen.

It is difficult to determine the number of persons with DES exposure. However, physicians should be alert for patients who may have been exposed to this agent and should be aware of the possible consequences of such exposure.

Dosages of DES varied greatly, as did the time during pregnancy that DES was taken. These factors may contribute to the wide range of adverse effects in the offspring of women who took DES while they were pregnant.

Illustrative Case

A 37-year-old woman who had been trying to conceive for two years came to her physician's office to discuss fertility issues. Her basal body temperature charts illustrated presumed ovulatory cycles, and her husband had a normal semen analysis. She had an abnormal Papanicolaou (Pap) smear 15 years previously, but all subsequent Pap smears had been normal. However, her previous physician had noted that her cervix "looked funny." The patient was the oldest of four siblings; her mother had two miscarriages before the patient was born.

The patient's general physical examination was normal. On pelvic examination, her vagina was normal, but her cervix had a pseudopolyp. Because of the patient's history of infertility and the consideration that she might have been exposed to DES in utero, hysterosalpingography was ordered, and the patient was asked to discuss the possibility of DES exposure with her mother.

The patient's mother accompanied her to the follow-up visit. The hysterosalpingogram showed that the patient had a T-shaped uterus. Her mother vaguely remembered taking medication to prevent another miscarriage when she was pregnant with her daughter.

Subsequent to a follow-up visit, the patient's mother contacted her physician for a copy of her obstetric records. The patient was referred to a reproductive endocrinologist for evaluation of infertility.

TABLE 1
Identifying DES-Exposed Patients

Patient	*Approach to identifying DES exposure, and subsequent actions*
Woman who may have taken DES during pregnancy*	Questions: Have you ever had a miscarriage? More than one miscarriage? Did you take any prescription medicines while you were pregnant? If so, what medicine and for what reason? Actions: If the patient is not sure about the medications that she took, try to obtain her obstetric records. If DES exposure is documented or surmised from the history, counsel all of the patient's offspring.
Daughter or son who may have been exposed to DES in utero†	Questions: Did your mother have one or more miscarriages? Did your mother take any prescription medicines while she was pregnant with you? Actions: If the patient has reproductive tract anomalies consistent with those seen in DES-exposed offspring, attempt to obtain the mother's obstetric records. If the records cannot be obtained, consider the patient to have been exposed to DES.

DES = diethylstilbestrol.

**—Although DES was not used in pregnant women in the United States after 1971, it continued to be used in other countries until the early 1980s.*

†—Born in the United States from 1938 through 1971, or born outside the United States from 1938 through the early 1980s.

Information from Centers for Disease Control and Prevention. DES update. Accessed online February 19, 2004, at: http://www.cdc.gov/DES.

Identifying DES Exposure

It is important to include questions about DES in the routine medical history of women who gave birth between 1938 and 1971, and of patients who were born during those years[3,7] *(Table 1)*.[2] In persons born outside the United States, there is a chance of DES exposure if they gave birth or were born as late as the 1980s. Many women may not be aware that they received DES during pregnancy, in part because the synthetic estrogen was marketed under many different names.[2,8]

One recent study[9] found that an office system intervention was successful in increasing awareness of DES exposure among clinical staff. The intervention entailed the addition of questions about DES exposure to the routine health history form.

Women Who Took DES During Pregnancy

Women who took DES while they were pregnant have a slightly higher incidence of

TABLE 2
Structural Abnormalities in Women with in Utero DES Exposure

Cervix	**Vagina**
Hypoplastic cervix	Clear cell adenocarcinoma
Cockscomb cervix	Adenosis
Cervical collar	**Uterus**
Pseudopolyp	T-shaped uterus

DES = diethylstilbestrol

Women who took diethylstilbestrol during pregnancy have a slightly increased risk of breast cancer.

breast cancer compared with the general population. The relative risk ranges from 1.27 to 1.35 in several studies.[10] In comparison, the relative risk of breast cancer is 1.3 in women who have taken hormone therapy for more than five years,[11] and 2.1 in women with a family history of breast cancer.[12] Women who were prescribed DES during pregnancy should have annual mammography and clinical breast examinations after the age of 50.[12] [Strength of recommendation (SOR) A, evidence-based guideline]

No increased risk of other hormone-dependent cancers has been found in women with DES exposure during pregnancy. Therefore, other preventive and screening measures should be based on standard guidelines.

Daughters with in Utero DES Exposure

In the daughters of women who took DES during pregnancy, the incidence of clear cell adenocarcinoma of the vagina and cervix ranges from 1.4 cases per 1,000 exposed persons to one case per 10,000 exposed persons.[13] Clear cell adenocarcinoma is most likely to develop when women with in utero DES exposure are between 17 and 22 years of age. However, cases have been diagnosed in women in their 30s and 40s, and there is concern about a possible second age-incidence peak of clear cell adenocarcinoma as women with in utero DES exposure grow older.[14] Clear cell adenocarcinoma of the vagina and cervix is rare in women without in utero DES exposure; in such cases, the cancer usually develops in the postmenopausal period.[15]

Many women who were exposed to DES in utero are just beginning to reach menopause. Because of the concern about a second peak in the incidence of clear cell adenocarcinoma, continued surveillance for this cancer is warranted in these women.[16]

Women with in utero DES exposure do not have a higher documented incidence of any other cancer. Data from several studies[17,18] suggest that these women may have a higher incidence of high-grade cervical intraepithelial neoplasia, but not invasive cervical carcinoma. However, the findings of these studies have been questioned, in that women with in utero DES exposure may receive increased cytologic screening. A link with breast cancer is under investigation.[2]

Many women who were exposed to DES in utero have a range of structural reproductive tract abnormalities[19,20] *(Table 2)*. The National Collaborative Diethylstilbestrol Adenosis project[19] followed approximately 4,500 DES-exposed women for almost 20 years and found an 18 percent incidence of structural uterine, cervical, or vaginal abnormalities. The incidence of these abnormalities may be as high as 33 percent in women with in utero DES exposure.[2]

DES can cause changes in the vaginal epithelium, including adenosis (columnar epithelium located in the upper one third of the vagina). Although vaginal adenosis is benign, it sometimes causes abnormal bleeding. The degree of adenosis depends on the DES dosage and the stage during the pregnancy that the agent was taken. The most severe changes occur in the daughters of

TABLE 3

Clinical Recommendation for Women with in Utero DES Exposure

Perform colposcopy as part of the first pelvic examination. If the colposcopic examination is normal, no further screening is needed. If the examination is abnormal, repeat colposcopy annually along with cervical and vaginal (four-quadrant) cytology.
Perform annual cervical cytology, four-quadrant vaginal cytology, and careful digital palpation for adenosis and vaginal clear cell adenocarcinoma.
Provide counseling about increased risk of infertility and poor pregnancy outcome.
Refer pregnant patients for high-risk obstetric management.

women who took DES during the first trimester.[20] Although vaginal clear cell adenocarcinoma generally develops in areas of adenosis, whether individual areas of adenosis progress to this cancer remains unknown.

Performance of colposcopy (to assess for abnormal epithelium) frequently is recommended during the first pelvic examination in all women with in utero DES exposure *(Table 3)*.[21] If the initial colposcopic examination is normal, annual pelvic examinations and annual cervical Pap smears and four-quadrant vaginal Pap smears are adequate, and colposcopy does not need to be repeated.[21,22] [Reference 22: SOR C, consensus practice guideline based on expert opinion] If the initial colposcopic examination demonstrates any abnormalities, annual colposcopy with cytology is indicated.

For the four-quadrant Pap smear, cells are obtained from all four walls of the upper vagina. Cells first are obtained from the two lateral walls; then the speculum is rotated 90 degrees, and specimens are obtained from the anterior and posterior walls. The four-quadrant Pap smear should be performed annually to screen for adenosis and clear cell adenocarcinoma in women with in utero DES exposure.

Routine cervical cytology also should be performed annually in women who were exposed to DES in utero. In addition, the cervix and upper vaginal walls should be palpated carefully during the bimanual examination to feel for thickening that might indicate adenosis or clear cell adenocarcinoma.[22]

Women with in utero DES exposure should be counseled about their slightly increased risk of infertility and a possibly increased risk of adverse pregnancy outcome. Infertility is most common in women with underlying structural abnormalities and usually is caused by uterine or tubal factors.[23] Women who were exposed to DES in utero should be monitored closely during pregnancy.[24-26]

Although most women with in utero DES exposure have normal pregnancies, there is evidence for an increased risk of first- and second-trimester spontaneous abortion, ectopic pregnancy, and preterm delivery.[26] The most comprehensive study[26] to date found that 64.5 percent of women with in utero DES exposure had full-term infants, compared with 84.5 percent of matched women who had not been exposed to DES. In addition, the DES-exposed women had higher rates of preterm delivery (19.4 percent versus 7.5 percent), ectopic pregnancy (4.2 percent versus 0.77 percent), and second-trimester spontaneous abortion (6.3 percent versus 1.6 percent). Consequently, high-risk obstetric care

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may be indicated for pregnant women who were exposed to DES in utero.

Contraceptive management may be complicated in women with in utero DES exposure. Use of intrauterine devices is controversial because of the high incidence of structural uterine abnormalities, as well as possible changes in the elasticity of endometrial tissue. Because of cervical abnormalities, diaphragms and cervical caps may be difficult to fit.[27] No evidence indicates that oral contraceptive pills are not safe for use in women with in utero DES exposure, although some experts are reluctant to prescribe hormonal contraception of any type to these women.[3]

Sons with in Utero DES Exposure

The sons of women who took DES during pregnancy are three times more likely to have genital structural abnormalities than men without such exposure.[28] The most common abnormalities are epididymal cysts, undescended testes, and small testes. Epididymal cysts have no clinical implications, but undescended testes and small testes are associated with an increased risk of testicular cancer.[29] Men with in utero DES exposure also have sperm and semen abnormalities but do not have an increased risk of infertility or sexual dysfunction.[30]

There is some concern about the effects of DES on the prostate.[31] One study[32] that examined the prostatic utricle of male stillborns who were exposed to DES in utero showed a significantly higher incidence of squamous metaplasia in this müllerian-derived tissue.

A recent study[33] showed a possibly increased incidence of testicular cancer in men with in utero DES exposure. Although this finding was not statistically significant, the investigators concluded that the connection between DES and testicular cancer "remains uncertain," and suggested that ongoing clinical surveillance would be prudent. Therefore, the sons of women who took DES during pregnancy should be encouraged to practice routine testicular self-examination.

The sons of women who took diethylstilbestrol during pregnancy have an increased incidence of genital structural abnormalities, testicular cancer, and sperm and semen abnormalities.

Future Considerations

An increased susceptibility to reproductive tract tumors has been demonstrated in mice that are descended from parents with prenatal DES exposure (i.e., multigenerational effect),[6] but this relationship has yet to be observed in humans. To date, no studies have shown an increased risk of cancer in the offspring of men and women who were exposed to DES in utero. Two studies[34,35] of "DES granddaughters" (third-generation females) have found no health effects related to DES exposure. However, one small study[36] of "DES grandsons" showed an increased risk of hypospadias.

DES currently is being studied as an experimental hormonal treatment (i.e., a type of estrogen therapy) in men with refractory prostate cancer.[37]

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NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for SACCHARIN

FINAL
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Proposed Report on Carcinogens Delisting for Saccharin[1]

Saccharin is currently listed in the Report on Carcinogens, 8th Edition as *reasonably anticipated to be a human carcinogen.* The basis for this listing was sufficient evidence of carcinogenicity in experimental animals. The Calorie Control Council has petitioned the NTP to consider delisting saccharin from its Report on Carcinogens based upon mechanistic data related to development of urinary bladder cancers in rats.

Carcinogenicity

In four studies of up to 30 months duration, sodium saccharin was carcinogenic in Charles River CD and Sprague-Dawley male rats as evidenced by a dose-related increased incidence of benign or malignant urinary bladder neoplasms at dietary concentrations of 1% or greater (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980; Schoenig et al., 1985). Non-statistically significant increases in urinary bladder cancer have also been seen in saccharin-treated female rats from studies showing a positive effect in males (Arnold et al., 1980; Taylor et al., 1980). Furthermore, several initiation/promotion studies in different rat strains have shown a reduced latency and/or increased incidence of similar urinary bladder cancers in male and female rats fed sodium saccharin subsequent to treatment with different urinary bladder initiators (e.g., Hicks and Chowaniec, 1977; Cohen et al., 1979; Nakanishi et al., 1980b; West et al., 1986; Fukushima et al., 1990). Several additional rat studies in which sodium saccharin was administered either in the diet or in drinking water were negative for tumorigenicity (Fitzhugh et al., 1951; Lessel, 1971; Schmähl, 1973; cited by IARC, 1980; Chowaniec and Hicks, 1979; Hooson et al., 1980; Schmähl and Habs, 1984).

Three mouse studies have reported positive carcinogenicity following exposure to saccharin. Two of these studies involved surgical implantation of saccharin-containing cholesterol pellets into the urinary bladders and resulted in development of malignant urothelial neoplasms (Allen et al., 1957; Bryan et al., 1970). In the third study, dietary sodium saccharin resulted in increased incidences of malignant thyroid neoplasms (Prasad and Rai, 1986). While the mouse data cannot be discounted, some of these studies had methodological flaws, provided limited information, did not show a dose-response, or had unexpected outcomes that may be species or strain-specific and should be verified by additional studies. Four studies in mice were judged negative for tumorigenesis (Roe et al., 1970; Kroes et al., 1977; Homberger, 1978; Frederick et al., 1989) as were studies in nonhuman primates (McChesney et al., 1977 abstr.; Sieber and Adamson, 1978; both cited by IARC, 1980; Thorgiersson et al., 1994; Cohen et al., 1996 abstr.) and a single hamster study (Althoff et al., 1975).

Much of the epidemiology has examined associations between urinary bladder cancer and artificial sweeteners, rather than saccharin per se. The time trend data for bladder cancer are essentially noninformative with no clear indication that the increased use of saccharin or artificial sweeteners commencing in the 1940s is associated with a general increase in bladder cancer when controlled for confounding factors, chiefly smoking. Risk of bladder cancer in diabetics, who presumably consume greater amounts of artificial sweeteners compared to the general population,

[1]Saccharin is produced commercially as calcium and sodium salts as well as the free acid, and the name saccharin has been applied to all three.

is not greater than risks in the general population (Armstrong and Doll, 1975). Based upon several case-control studies there is no overall association between use of artificial sweeteners and bladder cancer (reviewed by IARC, 1980; IARC, 1987b; JECFA, 1993). It is harder to reject an association between use of artificial sweeteners and bladder cancer in some case-control subgroups, even though the numbers are small[2] (Howe et al., 1980; Hoover and Strasser, 1980; Morrison and Buring, 1980; Cartwright et al., 1981; Morrison et al., 1982; Mommsen et al., 1983). Taken together, while the available epidemiology data show no consistent evidence that saccharin is associated with increased bladder cancer in general, a small increased risk in some subgroups, such as heavy users of artificial sweeteners, cannot be unequivocally excluded. With regard to the general population, if sodium saccharin is a risk factor, it is weak and cannot be proven or disproved due to lack of actual exposure data and intrinsic limitations of existing epidemiology studies.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Extensive studies of the mutagenicity and genotoxicity of saccharin have shown generally negative but occasionally conflicting results. Sodium saccharin is essentially nonmutagenic in conventional bacterial systems but is weakly clastogenic or genotoxic in short-term *in vitro* and *in vivo* test systems (reviewed by Ashby, 1985; IARC, 1987a,b; Whysner and Williams, 1996) with evidence that equimolar ionic solutions of sodium chloride *in vitro* produce a comparable cytotoxic response (Garland et al., 1989a). Urine from mice treated with sodium saccharin was mutagenic in the Ames test (Batzinger et al., 1977). Saccharin does not covalently bind to DNA and does not induce unscheduled DNA synthesis in bladder urothelium.

Saccharin-induced carcinogenesis in rats shows a sex predilection for males (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980), an organ specificity for urinary bladder (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980; Fukushima et al., 1983; Schoenig et al., 1985), and a dose-response when exposure to dietary concentrations of 1 to 7.5% of the sodium salt of saccharin has begun early in life (beginning at birth or immediately at weaning) and is continued for approximately two years (Schoenig et al., 1985). The results of mechanistic studies have shown that certain physiological conditions must be simultaneously or sequentially present for induction of urinary bladder tumorigenesis. These conditions include a urinary pH greater than 6.5, increased urinary sodium concentration, increased urine volume, decreased urine osmolality, presence of urinary crystals or precipitate, and damage to the urothelium resulting in a proliferative (hyperplastic) response. All of these conditions have been studied extensively in male rats but less so in females. The high levels of urinary protein characteristic of many male rats may partially explain the sex predilection. The high intrinsic rate of urothelial proliferation at about the time of weaning is also believed to contribute to the observed tumorigenic effects. The urinary milieu in rats, especially male rats, is sufficiently different from that in humans or other species to support the contention that these observations are rat-specific. Pharmacokinetic

[2] Morrison and Buring (1980) indicate an increased risk for women. Hoover and Strasser (1980) suggest increased risk among low risk (non-smoking, non-occupationally exposed women) and high risk (male heavy smokers) subgroups.

and metabolism data on sodium saccharin do not explain the male rat sensitivity for induction of urinary bladder neoplasms (Sweatman and Renwick, 1979, 1980).

Conclusion

There is evidence of the carcinogenicity of saccharin in rats but less convincing evidence in mice. Mechanistic studies indicate that the observed urinary bladder cancers in rat studies are related to urinary pH, osmolality, volume, presence of precipitate, and urothelial damage with attendant hyperplasia following dietary concentrations of 3% or higher with inconsistent findings at lower dietary concentrations. The factors thought to contribute to tumor induction by sodium saccharin in rats would not be expected to occur in humans. The mouse data are inconsistent and require verification by additional studies. Results of several epidemiology studies indicate no clear association between saccharin consumption and urinary bladder cancer. Although it is impossible to absolutely conclude that it poses no threat to human health, sodium saccharin is not reasonably anticipated to be a human carcinogen under conditions of general usage as an artificial sweetener.

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Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded; or

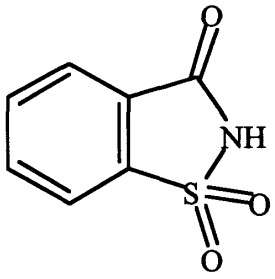
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in previous Reports on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not be reasonably anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

Saccharin
[81-07-2]



1.1 Chemical Identification

Saccharin ($C_7H_5NO_3S$, mol. wt. = 183.19) is also called:

Anhydro-*o*-sulfaminebenzoic acid
3-Benzisothiazolinone 1,1-dioxide
1,2-Benzisothiazol-3(2*H*)-one 1,1-dioxide
o-Benzoic sulfimide
Benzoic sulphimide
o-Benzoic sulphimide
o-Benzosulfimide
Benzosulphimide
o-Benzosulphimide
Benzo-2-sulphimide
o-Benzoyl sulfimide
o-Benzoyl sulphimide
1,2-Dihydro-2-ketobenzisosulfonazole
1,2-Dihydro-2-ketobenzisosulphonazole
2,3-Dihydro-3-oxobenzisosulfonazole
2,3-Dihydro-3-oxobenzisosulphonazole
Garantose
Glucid
Gluside
Hermesetas
3-Hydroxybenzisothiazole-*S*,*S*-dioxide
Insoluble saccharin
Kandiset
Sacarina

Saccharimide
Saccharina
Saccharin acid
Saccharine
Saccharin insoluble
Saccharinol
Saccharinose
Saccharol
Sacharin (Czech)
Sucre edulcor
Sucrette
o-Sulfobenzimide
o-Sulfobenzoic acid imide
2-Sulphobenzoic imide
Zaharina

Saccharin has the RCRA waste number U202.

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	White	HSDB (1996)
Physical State	Monoclinic crystals	Budavari (1996)
Melting Point, °C	228.9-229.7	Budavari (1996)
Density, g/mL	0.828	Budavari (1996)
Odor	Odorless or has a faint aromatic odor	HSDB (1996)
Solubility:		
Water	Soluble in water	Weast and Astle (1980)
Organic Solvents	Soluble in acetone Slightly soluble in chloroform, ethyl ether, and benzene	Weast and Astle (1980); HSDB (1996)
Partition Coefficient:		
Log octanol/water	0.91	HSDB (1996)
Vapor pressure at 25 °C, mm Hg	9.11×10^{-7}	HSDB (1996)

2.0 HUMAN EXPOSURE

Summary: The original uses of saccharin were numerous. Today, it is primarily used as a nonnutritive sweetening agent. From the 1950's to the 1970's, the U.S. consumption of saccharin increased dramatically. Following the ban on saccharin in Canada, stricter legislation on the marketing of saccharin, and the introduction of other artificial sweeteners into the U.S. market, consumption steadily declined. Recently, however, it appears that U.S. saccharin consumption is steady, if not slightly increasing.

Saccharin and sodium saccharin have been produced commercially in the United States for over 80 years. The compounds are produced commercially only by the Maumee process. Calcium saccharin was first produced in the United States in 1953. U.S. imports and production of saccharin has steadily declined. Currently, PMC Specialties Group, Inc. is the only commercial producer of saccharin.

Potential exposure to saccharin occurs through the consumption of dietetic foods and drinks and by use of some personal hygiene products. The concentration of saccharin allowed in these products is regulated by the FDA. Potential exposure to saccharin also occurs in the workplace, specifically in occupations, industries, or facilities that produce and deal with saccharin and its salts.

Regulation of saccharin and its salts is accomplished through many agencies and legislation. The EPA regulates saccharin and its salts under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), and Superfund Amendments and Reauthorization Act (SARA). The FDA regulates saccharin under the Food, Drug, and Cosmetic Act (FD&CA). Saccharin is regulated by OSHA under the Hazard Communication Standard.

2.1 Production

The 1979 Toxic Substances Control Act (TSCA) Inventory identified three U.S. companies producing 1.1 million lb (499 metric tons [Mg]) of saccharin in 1977, while 6.3 million lb (2,860 Mg) were imported. Two U.S. companies produced 1.6 million lb (726 Mg) of sodium saccharin, and 281,000 lb (128 Mg) were imported in 1977. Imports of calcium saccharin, which was first produced commercially in the United States in 1953, amounted to 5,500 lb (2.5 Mg) in 1977. One U.S. company produced 550,000 lb (250 Mg) of the ammonium salt in 1977 (NTP, 1994).

Production of all forms of saccharin increased gradually from 180 Mg in 1957 to an estimated 2,040 Mg in 1970 to an estimated total of 2,177 Mg in 1977 (IARC, 1980). The USITC (1981-1991, 1993-1995) identified one U.S. producer of saccharin and its sodium salt from 1980 to 1994, but no production data were provided for these years. The USITC (1983-1985) also reported that one U.S. company produced saccharin, calcium salt, from 1982 to 1984, but no production data were provided. SRI International (1996) identified one U.S. producer of sodium saccharin, most likely PMC Specialties Group, Inc. which produces saccharin under the trade name SYNCAL® in the United States and worldwide (PMC Specialties Group, 1996).

PMC Specialties Group produces sodium saccharin in crystalline and powder forms, and calcium saccharin and insoluble (acid) saccharin in powder form (PMC Specialties Group, 1996). Production volumes were not available.

The forms of saccharin produced by PMC Specialties Group are listed below, in **Table 2-1**.

Table 2-1. Forms of Saccharin Produced by PMC Specialties Group

Trade Name	Synonym	Chemical Formula	CAS No.	Reference
SYNCAL® GS & GSD	soluble saccharin	$(C_6H_4SO_2NCO)Na \cdot 2H_2O$	128-44-9	PMC Specialties Group (1997a)
SYNCAL® S & SD	soluble saccharin	$(C_6H_4SO_2NCO)Na$	128-44-9	PMC Specialties Group (1997b)
SYNCAL® CAS	calcium saccharin	$(C_6H_4SO_2NCO)_2Ca$	6485-34-3	PMC Specialties Group (1997c)
SYNCAL® SDI	insoluble (acid) saccharin	$C_7H_5NO_3S$	81-07-2	PMC Specialties Group (1997d)

PMC Specialties Group also produces and markets the SYNCAL® saccharin products SWEET-CHEW® (for animal feed) and SHERBRITE® (for the plating industry) (PMC Specialties Group, 1996).

U.S. imports of saccharin have steadily declined from 5.9 million lb (2,700 Mg) in 1983 to 3.7 million lb (1,700 Mg) in 1984, about 1.8 million lb (817 Mg) in 1985, and 1.6 million lb (726 Mg) in 1987 (NTP, 1994). Calcium saccharin was first produced commercially in the United States in 1953.

Saccharin is manufactured commercially by both the Maumee process and the Remsen-Fahlberg method. In the United States, saccharin and sodium saccharin are produced commercially only by the Maumee process (HSDB, 1996), and have been produced for over 80 years (Crammer and Ikan, 1977; cited by IARC, 1980). In the Maumee process, diazotization of methyl anthranilate by treatment with sodium nitrate and hydrochloric acid gives 2-carbomethoxy-benzenediazonium chloride. Sulfonation of this intermediate gives 2-carbomethyoxy-benzenesulfonic acid, which is treated with chlorine to give 2-carbomethoxy-benzenesulfonyl chloride with chlorine. Treatment of this sulfonyl chloride with ammonia, followed by acidification, gives saccharin (IARC, 1980). Saccharin is converted to the sodium salt by treating with sodium hydroxide or sodium bicarbonate. Twenty-three impurities have been reported in this process (Arnold et al., 1983).

In the Remsen-Fahlberg method of producing saccharin, toluene is reacted with chlorosulfonic acid to produce *o*- and *p*-toluenesulfonyl chlorides. The *o*-isomer is isolated and treated with ammonia to form *o*-toluenesulfonamide. Oxidation gives *o*-sulfamoylbenzoic acid, and when this intermediate is heated, saccharin forms (IARC, 1980). Thirty-one impurities have been reported when saccharin is synthesized by this method (Arnold et al., 1983).

2.2 Use

The primary use of saccharin is as a nonnutritive sweetening agent. Its use increased substantially after cyclamates (synthetic chemicals having a sweet taste) were banned in food in 1969 (FESA database). In 1976, the estimated U.S. consumption for all forms of saccharin was 77% in food uses (45% in soft drinks; 18% in tabletop sweeteners; 14% in fruit juices, sweets, chewing gum, and jellies), and 23% in non-food uses (10% in cosmetics and oral hygiene products, such as toothpastes, mouthwash, and lipstick; 7% in drugs, such as coatings on pills; 2% in smokeless tobacco products, such as chewing tobacco and snuff; 2% in electroplating, e.g., a brightener in nickel-plating baths used in the coating of automobile bumpers; 1% for cattle feed; and 1% in miscellaneous uses (IARC, 1980; HSDB, 1996).

The original uses of saccharin were numerous. A few of the original uses were as an antiseptic and preservative to retard fermentation in food, in estimating the circulation time of blood from an antecubital vein to the lingual capillaries, as an antistatic agent in plastics and textiles, as a polymer modifier and accelerator in photosensitive dispersions, as a light-fastness aid in nylon dyes, and as a chemical intermediate for the fungicide probenazole used in controlling rice blast in Japan (Arnold et al., 1983).

Based upon government legislation and market competition, the consumption of saccharin in the United States has varied. Saccharin and saccharin salts were approved under the 1958 Food Additives Amendment to the Food, Drug, and Cosmetics Act. Under the provisions of this act, saccharin was included in those substances that had been in use prior to 1958 and had been accorded GRAS (Generally Recognized As Safe) status. Saccharin was removed from the GRAS list in 1972, however, when questions by the Food and Drug Administration (FDA) about its safety arose (IARC, 1980). During the period when saccharin was recognized as having GRAS status, its consumption increased dramatically. For example, the consumption of saccharin in the United States in 1953 was 21,000 lb (9.5 Mg); in 1962, 2.5 million lb (1,100 Mg); and following the ban on cyclamates in 1969, consumption rose to 4.0 million lb (1,800 Mg) (Arnold et al., 1983). The approval and introduction of other artificial sweeteners such as aspartame and acesulfame-K into the U. S. market lowered the annual per capita consumer consumption of saccharin from 3.5 kg (9.6 mg/day) in 1980 to 2.7 kg (7.4 mg/day) in both 1985 and 1988 (Irving-Monshaw, 1989). The total U.S. consumption of saccharin in 1992 was 700,000 sugar sweetness equivalent tons (2,333 Mg) whereas aspartame's consumption was 1,500 sugar sweetness equivalent tons (8,333 Mg) (Research Studies-USDA ERS, 1992). According to SRI International, saccharin accounted for 39% of the world's consumption of high-intensity sweeteners in 1992, while aspartame accounted for 41% (Dawson, 1994b). The 1994 consumer consumption of saccharin was estimated to be 2,200 Mg in the United States and 1,100 Mg in Europe (Dawson, 1994a).

In 1983, the Calorie Control Council estimated that in the United States, 44 million adults consumed saccharin-sweetened products (NTP, 1994). It has been estimated that the average consumption of saccharin by humans in the United States is about 5 mg/kg body weight/day (Vesely and Levey, 1978). Saccharin consumption is greatest among diabetics and others whose medical conditions require the restriction of calories or carbohydrates (NTP, 1994).

2.3 Environmental Exposure

2.3.1 Environmental Releases

The Toxic Chemical Release Inventory (EPA) listed four industrial facilities that produced, processed, or otherwise used saccharin in 1988. In compliance with Community Right-to-Know Program, the facilities reported releases of saccharin to the environment which were estimated to total 750 lb (340.5 kg) (NTP, 1994). Facilities are required to notify the National Response Center (NRC) when release of saccharin equals or exceeds its reportable quantity of 100 lb (45.4 kg). When saccharin becomes a waste, as a commercial chemical product, a manufacturing chemical intermediate, an off-specification commercial chemical product, or a manufacturing chemical intermediate, it must be managed according to Federal and/or State hazardous waste regulations (HSDB, 1996).

Releases of saccharin to the environment as reported by PMC Specialties Group, the only U.S. commercial saccharin producer listed by the USITC and Cumberland-Swan, Inc., the manufacturer of Sweet 'n Low®, are listed below, in **Table 2-2.**

Table 2-2. Releases of Saccharin to the Environment

Company	Release	1989	1990	1991
PMC Specialties Group	Air	75 lb/yr (34.1 kg/yr)	65 lb/yr (29.5 kg/yr)	64 lb/yr (29.1 kg/yr)
	Land	0 lb/yr	0 lb/yr	0 lb/yr
	Water	0 lb/yr	0 lb/yr	0 lb/yr
	Sewer	0 lb/yr	10 lb/yr (4.5 kg/yr)	10 lb/yr (4.5 kg/yr)
	Other	1,700 lb/yr (771.8 kg/yr)	1,100 lb/yr (499 kg/yr)	1,400 lb/yr (635.6 kg/yr)
Cumberland-Swan, Inc.	Air		250 lb/yr (113.5 kg/yr)	250 lb/yr (113.5 kg/yr)
	Land		0 kg/yr	0 kg/yr
	Water		0 kg/yr	0 kg/yr
	Sewer		250 lb/yr (113.5 kg/yr)	250 lb/yr (113.5 kg/yr)
	Other		2,700 lb/yr (1,226 kg/yr)	350 lb/yr (158.9 kg/yr)

Source: Toxic Release Inventory Systems (TRIS, 1996)

2.3.2 Environmental Occurrence

Saccharin and its salts, as well as the impurity *o*-toluenesulfonamide, do not occur as natural products (IARC, 1980).

2.3.3 Drinking Water and Food

Refer to section 2.3.4 for any information regarding exposure to saccharin from food.

2.3.4 Consumer Products

Potential exposure to saccharin also occurs through the consumption of dietetic foods and drinks and some personal hygiene products, such as certain toothpastes and mouthwashes that use saccharin as a sweetening agent (NTP, 1994). The FDA has authorized the use of saccharin and its salts in beverages in concentrations not to exceed 12 mg/oz (413 mg/L), as a sugar substitute not to exceed 20 mg for each expressed teaspoonful of sugar sweetening equivalency, and in processed food not to exceed 30 mg per serving. Data from the Nationwide Food Consumption Survey, conducted by the USDA from 1977-1978, on calculated daily saccharin intake levels is presented in **Table 2-3**. The survey included responses from 30,770 U.S. residents from the 48 contiguous states. Respondents reported foods eaten and quantities consumed.

Table 2-3. USDA Nationwide Food Consumption Survey (1977-1978): Total Calculated Saccharin Intake Levels, mg/kg bw/day

Age Group (years); Sex	1-2; M & F	3-5; M & F	6-8; M & F	9-14; M & F	15-18; M	19-34; M	19-34; F	35-64; M
Total Average Daily Intake	11.46	9.62	6.76	5.6	5.23	4.98	5.26	4.96
90th Percentile	15.76	19.67	14.12	11.98	7.4	10.19	10.48	10.48

Source: Calorie Control Council (1996)

The amount of saccharin consumed by diabetics in Great Britain was estimated in a study conducted by researchers at the University of Southampton (MAFF, 1994). The highest level consumed (as measured by the 97.5th percentile) was 3.1 mg saccharin per kilogram body weight per day. The study included 761 participants, age 2 years and over. The average consumption of saccharin by diabetics was not provided.

Consumer exposure to saccharin has possibly decreased in recent years due to the introduction of Nutra-Sweet® (aspartame). According to SRI International, saccharin, packaged as an artificial sweetener under the product name Sweet 'n Low®, commands 31.8% of the U.S. market share in artificial tabletop sweeteners. Saccharin is second to aspartame, which commands 67.8% of the market share (Tomasula, 1994).

2.3.5 Biomarkers of Exposure

Saccharin has not been found to be mutagenic, and evidence shows it does not undergo covalent binding to the DNA of a rat's liver or bladder (Lutz and Schlatter, 1977).

2.3.6 Occupational Exposure

Occupational exposure occurs through dermal contact or inhalation of dust at places where saccharin is produced or used. The risk of potential occupational exposure exists for workers involved in the production of saccharin or its salts, in the manufacture and formulation of saccharin-containing products, and during the packaging of the consumer products. A National Occupational Exposure Survey (1981-1983) estimated that 225,095 total workers, including 97,729 women, representing 73 occupations in 107 industries at 7,347 facilities, potentially were exposed to saccharin (NIOSH, 1990). This survey also found 1,150 employees, including 591 women, representing 5 occupations in 1 industry at 11 facilities were potentially exposed to its sodium salt. This same survey found 10,053 employees, including 4,418 females, representing 16 occupations in 19 industries at 454 facilities that either were involved with the production of, dealt with, or were potentially exposed to sodium saccharin dihydrate (RTECS, 1996).

Table 2-4. NIOSH National Occupational Exposure Survey (NOES, 1981-83)[a]: By Industry

Industry	No. of Plants	No. of Employees	No. of Female Employees
Agricultural Services	230	1838	1608
Heavy Construction Contractors	19	3129	75
Special Trade Contractors	20	1683	
Food and Kindred Products	149	497	
Textile Mill Products	66	252	
Lumber and Wood Products	166	2331	
Furniture and Fixtures	94	2630	376
Paper and Allied Products	132	6134	2295
Printing and Publishing	64	477	
Chemicals and Allied Products	23	1329	175
Rubber and Misc. Plastics Products	52	633	
Stone, Clay, and Glass Products	230	762	
Primary Metal Industries	9	264	
Fabricated Metal Products	307	11616	6172
Machinery, Except Electrical	2107	57361	16608
Electric and Electronic Equipment	881	26850	13490
Transportation Equipment	143	8947	1029
Instruments and Related Products	315	8910	3966
Miscellaneous Manufacturing Industries	86	839	116
Railroad Transportation	22	22	
Trucking and Warehousing	37	75	
Water transportation	39	774	
Transportation by Air	75	10086	57
Communication	152	3748	
Electric, Gas, and Sanitary Services	203	9025	
Business Services	24	1106	24
Auto Repair, Services, and Garages	299	1796	
Miscellaneous Repair Services	704	1110	
Health Services	699	60871	51738
Total	7347	225095	97729

[a]National Institute of Occupational Safety and Health (unpublished provisional data as of July 1, 1990).

2.4 Regulations

2.4.1 Occupational Exposure Limits

No occupational standards or criteria have been promulgated (OSHA) or recommended (NIOSH, ACGIH) in the United States for exposure to saccharin in workroom air.

2.4.2 Other Standards and Criteria

The EPA regulates saccharin and its salts under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), and Superfund Amendments and Reauthorization Act (SARA). Saccharin is subject to reporting and record keeping rules under CERCLA, RCRA, and SARA. The EPA proposed raising the statutory reportable quantity (RQ) of 1 lb, established under CERCLA, to 100 lb for saccharin and its salts. The final rule adjusts the RQ from 1 lb to 100 lb. Saccharin is regulated as a hazardous constituent of waste under RCRA, and threshold amounts for facilities which may release saccharin have been established under SARA. OSHA regulates saccharin under the Hazard Communication Standard and as a chemical hazard in laboratories. The FDA regulates saccharin under the Food, Drug, and Cosmetic Act (FD&CA) as a food ingredient not to exceed specific concentrations (NTP, 1994). In compliance with the Delaney Clause, the FDA proposed to ban saccharin as a food additive in 1977 because of the available evidence of its carcinogenicity in animals. Due to conflicting scientific study results as well as the potential benefits of saccharin, a compromise solution was enacted instead of an outright ban. In November, 1977, Congress passed the Saccharin Study and Labeling Act which placed an 18-month moratorium on any action by the FDA against saccharin, and mandated that all products containing saccharin bear the following warning label: "Use of this product may be hazardous to your health. This product contains saccharin, which has been determined to cause cancer in laboratory animals" (Viscusi, 1994). In 1991, the FDA withdrew its call for an outright ban on saccharin in the United States, but warning labels are still required on all packaging (Tomasula, 1994). The moratorium against any further FDA action has been extended to May 1, 1997. FDA regulates, under the Food, Drug, and Cosmetic Act (FD&CA) and the Fair Packaging and Labeling Act, the labeling of various food products containing saccharin and/or saccharin salts. The FDA also regulates how saccharin and certain saccharin salts are used as sweetening agents in food and as a weight control drug under the FD&CA and the Public Health Service Act.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comment
E P A	40 CFR 261—PART 261—IDENTIFICATION AND LISTING OF HAZARDOUS WASTES. Appendix VII—Basis for Listing Hazardous Waste. Promulgated: 46 FR 4619, 1981 with numerous amendments. The hazardous waste number for saccharin and it salts is U202.	App. VIII lists the hazardous constituents of industrial waste streams listed in 40 CFR 261.31.
	40 CFR 261.30 ff.—Subpart D—Lists of Hazardous Wastes.	
	40 CFR 302—PART 302—DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of the substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.
	40 CFR 302.4—Sec. 302.4 Designation of hazardous substances. Limits: Superfund (CERCLA, SARA) final reportable quantity (RQ) is 100 lb (45.4 kg).	EPA designated as hazardous those substances that when released into the environment may present substantial danger to the public health or welfare or the environment.
	40 CFR 302.6—Sec. 302.6 Notification requirements.	Notification of EPA is required if the RQ is released to the environment.
	40 CFR 372-- PART 372--TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Code: 42 U.S.C. 11013, 11028. This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986).	Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, and aid in the development of regulations, guidelines, and standards.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comment
EPA	40 CFR 372—Subpart D—Specific Toxic Chemical Listings. 40 CFR 372.65—Sec. 372.65 Chemicals and chemical categories to which this part applies.	
FDA	21 CFR 100—PART 100—GENERAL. Promulgated: 42 FR 14306, 03/15/77. U.S. Code: 21 U.S.C. 321, 331, 337, 342, 343, 348, and 371.	General state and local requirements along with specific administrative rulings and decisions for various food products.
	21 CFR 100.11—Sec. 100.130 Combinations of Nutritive and Nonnutritive Sweeteners in "Diet Beverages".	The label of any "diet beverage" or diet beverage base that contains saccharin must contain the statement "Contains ____ mg saccharin (or saccharin salt, as the case may be) per ounce, a nonnutritive artificial sweetener.
	21 CFR 101—PART 101—FOOD LABELING. Promulgated: 42 FR 14308, 03/15/77. U.S. Code: 15 U.S.C. 1453, 1454, 1455; 21 U.S.C. 321, 331, 342, 343, 348, and 371.	Requirements are given for the principal display panel (the panel most likely to be examined under customary conditions of display for retail sale) of form food.
	21 CFR 101.11—Sec. 101.11 Saccharin and Its Salts; Retail Establishment Notice.	Retail establishments (except restaurants) that sell food containing saccharin shall display a notice informing the consumer that saccharin products are sold at that location.
	21 CFR 150—PART 150—FRUIT BUTTERS, JELLIES, PRESERVES, AND RELATED PRODUCTS. Promulgated: 42 FR 14445, 03/15/77. U.S. Code: 21 U.S.C. 321, 341, 343, 348, 381, and 379e.	Artificially sweetened fruit containing a packing medium sweetened with saccharin and/or sodium saccharin shall have the specified name "artificially sweetened _____", the blank being filled by name of the fruit or fruit product.
	21 CFR 180—PART 180—FOOD ADDITIVES PERMITTED IN FOOD OR IN CONTACT WITH FOOD ON AN INTERIM BASIS PENDING ADDITIONAL STUDY. Promulgated: 61 FR 14482, 04/02/96. U.S. Code: 21 U.S.C. 321, 342, 343, 348, 371; 42 U.S.C. 241.	Regulations govern specific requirements for food additives in food or additives in contact with food. This regulation is pending additional study.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comment
F D A	21 CFR 180.37—Sec. 180.37 Saccharin, Ammonium Saccharin, Calcium Saccharin, and Sodium Saccharin.	Regulates how these saccharin food additives may be safely used as sweetening agents in food.
	21 CFR 310—PART 310—NEW DRUGS. U.S. Code: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e; 42 U.S.C. 216, 241, 242(a), 262, 263b-263n.	Regulations govern the administrative rulings and decisions on new drug status, new drugs exempted from prescription-dispensing requirements, records, reports, and requests for specific new drugs or devices.
	21 CFR 310.545—Sec. 310.545 Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses.	There is inadequate data to establish general recognition of the safety and effectiveness of saccharin as a weight control drug product.

The regulations in this table have been updated through the Federal Register 100 Vol.62, May 23, 1997.

3.0 HUMAN STUDIES

A number of epidemiological studies have been conducted to determine whether the use of artificial sweeteners (AS), including saccharin, has been associated with human cancer. U.S. epidemiological studies of AS may not be as informative as those from Canada, the United Kingdom, Europe, and Japan, where widespread saccharin use first began (1945 [imported primarily from Japan and the United States], 1916, 1894, and 1945, respectively). Artificial sweetener use in the United States was not widespread until the middle of the 1960s, when cyclamate and saccharin were used together. The IARC Working Group reviewed saccharin epidemiology in the original monograph (IARC, 1980) and updated the review in Supplement 7 to the IARC Monographs (IARC, 1987b). In both reviews by the IARC Working Group, it was concluded that the results from epidemiological studies of saccharin are equivocal. In a review of saccharin by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1993), however, it was concluded that "the epidemiological studies on saccharin did not show any evidence that saccharin ingestion increases the incidence of bladder cancer in human populations".

Epidemiologic studies have in general examined associations between urinary bladder cancer and artificial sweeteners in general, rather than saccharin, per se; this could either inflate or disguise a risk due to saccharin alone. Time trend data are essentially uninformative, since information concerning use of artificial sweeteners and confounding factors is presented only for populations and not for individuals. Cohort studies of diabetics are confounded by reduced smoking in this group. Overall, case-control studies demonstrate at best a small risk for the general population (reviewed in IARC, 1980; IARC, 1987a,b; JECFA, 1993). However, some studies have demonstrated increased risk for groups otherwise at low risk, such as female nonsmokers (Howe et al., 1980; Hoover and Strasser, 1980; Cartwright et al., 1981; Morrison et al, 1982; Mommsen et al., 1983). Heavy users of artificial sweeteners may also be at increased

risk regardless of gender or smoking habits (Hoover and Strasser, 1980). While the available epidemiology data show no consistent evidence that saccharin is associated with increased bladder cancer in general, a small increased risk in some subgroups cannot be excluded.

3.1 IARC (1980) Review of Saccharin Epidemiology

IARC (1980, pp. 171-183; see Appendix A) examined time trends in the United States, England, and Wales and found that there was no marked increase in the incidence of bladder cancer following rapid increase in the use of artificial sweeteners (e.g., see Armstrong and Doll, 1974). In addition, the IARC Working Group found that in the United Kingdom diabetics as a group consume higher quantities of artificial sweeteners and experience lower mortality from bladder cancer than the general population (e.g., see Armstrong and Doll, 1975). The IARC Working Group stated that due to metabolic or dietary differences, use of drugs, exposure to tobacco, or occupational factors associated with diabetics, a carcinogenic effect of sweeteners cannot be excluded (IARC, 1980).

The IARC Working Group evaluated 7 case-control studies (Morgan and Jain, 1974; Simon et al., 1975; Howe et al., 1977; Wynder and Goldsmith, 1977; Miller et al., 1978; Connolly et al, 1978; Kessler and Clark, 1978). Five of the seven studies were negative for bladder cancer and were found to be limited by some inadequacies in experimental design. Of the two studies that examined possible confounding factors in detail, one (Howe et al., 1980 [a reanalysis of data from Howe et al., 1977]) suggested that artificial sweetener use was positively associated with bladder cancer in men but not in women. The association was limited to men who consumed an average of more than eight tablets of saccharin per day or men who used nine or more tablets of AS per day. In both instances, the relative risk (RR) was approximately 3. The IARC Working Group noted that in these small groups, the result could have been due to confounding factors that were not included in the analysis, residual confounding effects of those factors that were considered in the analysis, or chance.

The second study reviewed by the IARC Working Group that considered confounding factors (Kessler and Clark, 1978; cited by IARC, 1980) found no association between bladder cancer and use of AS and suggested that a relative risk of about 1.5 or higher was unlikely.

In 6 out of 7 of the case-control studies reviewed by the IARC Working Group, women with bladder cancer consumed less AS than the controls. The IARC Working Group stated that this observation suggests that there is no association between use of artificial sweeteners and bladder cancer in women.

In a case-control study that was in press when reviewed by IARC (1980), Wynder and Stellman (1980) reported that there was no association between use of artificial sweeteners or diet beverages and bladder cancer. The study included 302 male and 65 female bladder cancer patients who were matched by age, sex, hospital, and hospital-room status to an equal number of patients without bladder cancer. More details on this study after publication are given in subsection 3.2.1.

The 1980 IARC Working Group concluded their review of epidemiological data for AS with the following statement: The epidemiological data taken as a whole cannot with confidence exclude a small increase in risk but provide no clear evidence that artificial sweeteners cause bladder cancer in humans (IARC, 1980). In 1987, the IARC Working Group reiterated the

findings from their 1980 review by concluding that the evidence that the risk of cancer is increased among users of artificial sweeteners is inconsistent (IARC, 1987).

3.2 Human Studies Published Post IARC (1980)

Experimental details for the studies described in this section are presented in **Table 3-1**.

3.2.1 U.S. Case-Control Studies

Hoover and Strasser (1980) conducted a large multicenter bladder cancer case-control study that included 3010 newly diagnosed, histologically confirmed bladder cancer cases and 5783 population-based controls chosen at random. Information collected by personal interview included information regarding quantity of AS consumed, either by table-top or diet-drink use. No increase in overall RR for bladder tumors was found when comparing the use of AS with never having used AS (males: RR = 0.99; CI [Confidence Interval] = 0.89-1.10; females: RR = 1.07; CI = 0.89-1.29). There was no trend found for men for either table-top or diet-drink AS use. A statistically significant trend for table-top, but not diet-drink, consumption was observed for females after adjustment for age, race, and cigarette smoking. For men and women who consumed at least 2 diet drinks and 3 table-top servings/day or at least some diet drinks and at least 6 table-top servings/day, there was a borderline statistically significant RR of 1.45 (CI = 1.00-2.10) after adjustment for sex, age, race, smoking, occupational exposures, region, and education (for males the RR was 1.47; for females the RR was 1.41). Two subgroups—females who had never smoked or been occupationally exposed to known bladder carcinogens and men who smoked heavily—showed a statistically significant relative risk estimate with daily AS use (men: table-top $\geq$6 uses, RR = 1.86; diet drinks $\geq$3 servings, RR = 2.62; women: table-top $\geq$2 uses for $\geq$5-9 years, RR = 1.8; $\geq$2 uses >10 years RR = 2.7). Additional control for coffee drinking, history of geographic area, education, obesity, use of hair dyes, and history of urinary infections did not affect the relative risk. [IARC (1982) reviewed this study in Supplement 4 to the monographs.]

Using a different analytic approach, Walker et al. (1982) reevaluated the study conducted by Hoover and Strasser (1980) and found essentially the same overall result for AS use (RR = 1.2; CI = 1.0-1.5). These investigators used a composite variable that included education, bladder infection, job exposure, and coffee consumption to define baseline risk strata. Odds ratio estimates were adjusted for region, race, sex, and age. The authors found no trends in odds ratios associated with increasing AS use for the different risk categories. However, this reanalysis was criticized by Hoover and Hartge (1982; cited by IARC, 1987b), who argued that the use of stratification did not include sex and age, and suggested that the low- or high-risk groups based on the composite risk variable used in the reanalysis were actually of intermediate risk. [IARC (1982) mentioned these two studies in Supplement 4.]

Morrison and Buring (1980) reported an association of artificial sweetener use and increased risk of lower urinary tract cancer in females. The relative risk of lower urinary tract cancer was 1.6 (95% CI = 0.9-2.7; 69 cases/46 controls) among women who never used dietetic beverages, and 1.5 (95% CI = 0.9-2.6; 54 cases/39 controls) among women who reported use of sugar substitutes. There was also an increased lower urinary tract cancer risk among women after five or more years of dietetic beverage use (RR = 3.7; 22 cases/6 controls), but statistical

estimates were not provided. [This study was described by IARC (1980) as a footnote since it was published after the Working Group Meeting.]

A case-control study was conducted by Wynder and Stellman (1980) between 1977 and 1979 using 302 male and 65 female cases with bladder cancer. Controls were hospital admissions matched for sex, age, hospital, and hospital-room status (an indicator of socioeconomic status). The authors found no association between use of saccharin or diet beverages and bladder cancer. The RR for saccharin use was 0.93 (CI = 0.68-1.28) for men and 0.62 (CI = 0.26-1.40) for women. For diet beverage consumption, the RR was 0.85 (CI = 0.55-1.17) for men and 0.60 (CI = 0.27-1.29) for women. [This study was published after the Working Group meeting and was described in IARC (1980) as a footnote.]

Najem et al. (1982) compared 75 male and female bladder cancer cases with 142 hospital-based controls in a study conducted in 1978 in New Jersey. Controls were matched to cases by age, place of birth, sex, race, source of obtaining cases, and place of current residence. The authors found no statistically significant increased risk of bladder cancer from consumption of saccharin (RR = 1.3 [CI = 0.6-2.8]). However, only 12/75 cases (16%) and 19/142 controls (13%), reported having consumed saccharin. The relative risk was not adjusted for any potentially confounding factors.

Silverman et al. (1983) examined the use of population- versus hospital-based controls to estimate the risk of lower urinary tract cancer from AS consumption. The study was conducted in Detroit, MI as an add-on to the multicenter study conducted by Hoover and Strasser (1980). The study included 391 cases diagnosed from December 1977 to December 1978 in Detroit with transitional or squamous cell carcinoma of lower urinary tract, 305 population-based controls matched to cases by age and sex, and 440 hospital-based controls discharged from the same hospital as a case and matched by age, race, sex, discharge date. Population-based controls had a lower reported AS use compared with hospital-based controls. Using population-based controls, the RRs for men and women were 1.1 and 1.8, respectively. Using hospital-based controls, the RRs for men and women were 0.9 and 1.1, respectively. Using hospital controls without obesity-related diseases, RR was 1.1 for both men and women. Adjustment of RR values for age, smoking, education, and body mass index were found to have no effect on risk.

A New York state study reported no increased risk of bladder cancer for young (20 to 49 yr-old) women who reported using AS more than 100 times (Odds Ratio [OR] = 1.1 [CI = 0.7-1.7]). Cases (173) with bladder cancer diagnosed between 1975 and 1980 were matched by sex, age, and residence within an area code to 173 population-based controls (Piper et al., 1986).

In a study conducted by Nomura et al. (1991), men and women of Japanese or Caucasian ancestry, diagnosed with lower urinary tract cancer between 1977 and 1986 in Oahu were matched to population-based controls by sex, ethnic group, age, and residence. Participants were classified into non-users and users of saccharin based on consumption history 1 year prior to interview or diagnosis. There was no increased risk of lower urinary tract cancer in users (OR for men, 1.1 [CI = 0.7-1.8]; OR for women, 0.7 [CI = 0.3-1.5]).

In an analysis of data from the Hoover and Strasser (1980) study conducted by Sturgeon et al. (1994), it was found that heavy use of AS (≥ 1680 mg/day) was associated with higher-grade, poorly differentiated bladder tumors (RR = 2.2; CI = 1.3-3.6). The analysis included 1860 cases from 10 geographic regions with bladder cancer identified between December 1977 and

March 1978, and 3934 population-based controls. The RR was adjusted for age, sex, cigarette use, history of urinary infection or bladder stones, coffee consumption, family history of urinary tract cancer, high-risk occupation, race, and education.

3.2.2 Canadian Case-Control Studies

Risch et al. (1988) conducted a large multicenter Canadian bladder cancer study that matched 826 cases with population-based controls during 1979-1982. No association with any table-top AS consumption, including a subgroup of nonsmoking females (OR = 1.04; CI = 0.4-2.71) was reported. An OR of approximately 2 was associated with females that drank diet soda; the dose-related trend reached borderline statistical significance. The authors noted that none of the diet soda consumption had exceeded 10 years (Risch et al., 1988). Thus, these authors failed to confirm the increased risk for bladder cancer that they previously reported (Howe et al., 1980) for consumers of artificial sweeteners.

3.2.3 Case-Control Studies From Other Countries

Morrison et al. (1982) conducted a case control study including cases of lower urinary tract cancer cases from Nagoya, Japan (293 cases) and Manchester, United Kingdom (555 cases). Controls (589 Japanese, 735 British) were population-based and were matched to cases by age and sex. The study found no increased risk of lower urinary tract cancer related to AS use (British men, RR = 0.9 [CI = 0.7-1.2]; British women, RR = 0.9 [CI = 0.6-1.4]; Japanese men, RR = 0.7 [CI = 0.5-0.9]; Japanese women, RR = 0.5 [CI = 0.3-0.8]). The study populations from Japan and the United Kingdom used saccharin predominantly (97% of British, 94% of Japanese) for 30-40 years prior to the study. The authors found an increased RR of 1.6 among nonsmoking men from the United Kingdom; the RR for nonsmoking British women was 1.2. There was no increased risk in nonsmoking Japanese or in any group of current or former smokers. The United Kingdom analysis for AS in tablets showed an increased RR among the over-10-tablets-a-day female group (RR = 2.3) and a decrease in males (RR = 0.6).

Another study from the United Kingdom, conducted by Cartwright et al. (1981), included 622 prevalent and 219 incident cases of bladder cancer in West Yorkshire, each of which was matched to hospital-based controls (622 for existing cases, 448 for new cases) for age and sex. Saccharin use was described as regular for > 1 year, at least 5 years prior to diagnosis. Risk was significantly elevated for nonsmoking males (RR = 2.2 [CI = 1.3-3.8]), but not for nonsmoking females (RR = 1.6 [CI = 0.8-3.2]), or for smokers of either sex (male RR = 0.9 [CI = 0.6-1.3]; female RR = 1.2 [CI = 0.5-2.6]). The RR values were adjusted for age and type of case (incident or prevalent).

Mommsen et al. (1983) conducted a small case-control study from Denmark comprised of 47 female cases newly diagnosed with bladder cancer and 94 population-based controls matched by sex, age, and geographic area, including degree of urbanization. Cases were interviewed in person at the hospital, whereas controls received a mailed questionnaire which was followed up by a phone interview. Only 6/47 cases and 2/94 controls reported consumption of saccharin. An elevated risk of bladder cancer was found for all women who had consumed saccharin (RR = 6.7 [CI = 1.5-30.2]). When only nonsmokers who used saccharin were included, the risk decreased (RR = 3.3 [CI = 1.4-7.8]).

In another study from Denmark, however, Møller-Jensen et al. (1983) found no increased risk of bladder cancer from consumption of saccharin (RR for men = 0.68 [CI = 0.45-1.02]; RR for women = 1.04 [CI = 0.51-2.09]). The study included 290 male and 98 female bladder cancer patients who were matched by age and sex to 592 male and 195 female controls selected at random from the general population. Participants were classified as users of saccharin only (72.9%), cyclamate only (10.7%), or both substances.

A case-control study using 117 cases with 117 population-based controls and 117 hospital-based controls was prompted following a report of high bladder cancer incidence in La Plata, Argentina. However, no association between saccharin use and bladder cancer was reported. Controls were matched to cases by sex, age, and residence (population-based controls) or hospital (hospital-based controls). Relative risk values were not provided (Iscovich et al., 1987).

No increased risk of bladder cancer from consumption of saccharin (as a food additive only) was found in a case-control study conducted by Momas et al. (1994) (OR = 1.5 [CI = 0.8-3.0]). The study included 219 men living in a region of France for > 5 years and diagnosed with primary bladder carcinoma between January 1987 and May 1989. The 794 controls were men from the same region who were over 50 years old and had lived in the region > 5 years. Saccharin use was defined as consumption of 365 (units were not given).

3.2.4 Descriptive Studies

Jensen and Kamby (1982) found that *in utero* exposure to saccharin did not appear to increase the bladder cancer incidence in the first 3 decades of life, which was the limitation of their follow-up. This Danish study also found no increased incidence in bladder cancer mortality up to an age of 30 years for persons born from 1941 to 1945, which corresponds to a time period when saccharin use was high in Denmark due to war-time shortages of sugar.

3.2.5 Meta-Analysis

In a meta-analysis that included 12 case-control studies on the relationship between AS and bladder cancer incidence, Elcock and Morgan (1993) estimated a summary RR of near unity (males, 0.958; females; 0.961).

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control	cases: men and women from diagnosed with carcinoma of urinary bladder in 10 geographic regions between Dec. 1977 and Dec. 1978; aged 21-84 yr (cases with history of urinary tract cancer were excluded) controls: age and sex stratified random sample of the general population from the same 10 geographic regions	cases: 3010 controls: 5783 75% of cases and controls were males	cases: 87 controls: 85 (aged 21-64 yr); 87 (aged 65-84)	artificial sweetener	lifetime	never used artificial sweetener ever used diet drink ever used tabletop artificial sweetener ever used diet food ever used any form	Relative Risks: M: 1.00 F: 1.00 M: 0.95 (0.84-1.07) F: 1.02 (0.83-1.25) M: 1.04 (0.92-1.18) F: 1.04 (0.84-1.28) M: 1.02 (0.85-1.22) F: 1.13 (0.87-1.47) M: 0.99 (0.89-1.10) F: 1.07 (0.89-1.29)	race, cigarette use, coffee consumption, occupational exposure (additional control for age, sex, history of diabetes, geographic area, and education did not affect RR)	yes, for two subgroups (non-smoking females; heavy smoking males)	personal interview in home of participants	Hoover and Strasser (1980)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)							Relative Risks for Use of Artificial Sweeteners in Subgroups: no. cases/controls:				Hoover and Strasser (1980)
	Low-Risk White Females (never smoked, no occupational exposures)	cases: 130 controls: 402		table-top sweeteners table-top sweeteners table-top sweeteners	5 years 5-9 years > 10 years	≥ 2 uses per day ≥ 2 uses per day ≥ 2 uses per day	1.3; 14/34 1.8; 13/22 2.7; 16/18 all RR had p < 0.01; 95% CI not provided	age			
	High-Risk White Males (smoked more than 40 cigarettes per day)	cases: 104 controls: 167		table-top sweeteners table-top sweeteners table-top sweeteners table-top sweeteners table-top sweeteners diet drinks diet drinks diet drinks		< 1 uses per day 1-1.9 uses per day 2-3.9 uses per day 4-5.9 uses per day ≥ 6 uses per day 1-1.9 servings per day 2-2.9 servings per day ≥ 3 servings per day	1.28; 12/15 2.07; 19/14 1.96; 16/13 1.33; 8/10 1.86; 7/7 1.20; 14/19 3.33; 10/5 2.62; 6/4 all RR had p = 0.01; 95% CI not provided	age			Hoover and Strasser (1980)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases admitted to Boston hospitals for first primary neoplasm of the lower urinary tract from March 1976 through May 1977; controls from general population of study area	592 cases, 94% with bladder tumors and 74% male; 536 controls	cases: 81% controls: 80%	artificial sweetener	years of use: <5, 5-9, more than 10	no. drinks per day; no. sugar substitutes per day; no. dietetic food servings per week	Relative Risks for Lower Urinary Tract Cancer and Ever-Use of Artificial Sweeteners: no. case/controls M and F: 0.9 (0.7-1.2) for dietetic beverages or sugar substitutes **Dietetic Beverage Use History** M: 0.8 (0.6-1.1); 144/155 F: 1.6 (0.9-2.7); 69/46 **Sugar Substitute Use History** M: 0.8 (0.5-1.1); 101/113 F: 1.5 (0.9-2.6); 54/39	age, sex, smoking history	weak because of low numbers of cases and controls and no statistical estimates of confidence for duration of use	interviews with subjects or proxies if subjects too ill, could not be contacted, or deceased	Morrison and Buring (1980)
				diet drinks	< 5 yr		M: 1.1; 62/59				
				diet drinks	> 10 yr		M: 0.7; 17/21				
				diet drinks	< 5 yr		F: 1.0; 27/27				
				diet drinks	≥ 5 yr		F: 3.7; 22/6 CI and p value not provided				

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases: men and women with a first diagnosis of bladder cancer and admitted to hospital; interviewed between Aug. 1977 and June 1979 controls: patients admitted to hospital for other neoplastic and nonneoplastic conditions; matched to cases by age, sex, hospital, and hospital-room status	cases: 367 controls: 367	not specified	saccharin	≥ 10 yr	≥ 40 mg saccharin/ day (as artificial sweet-ener) ≥ 2 cans diet beverage/d ay (≥192-264 mg saccharin/ day)	Relative Risk: M: 0.93 (0.68-1.28) F: 0.62 (0.26-1.40) M: 0.85 (0.55-1.17) F: 0.60 (0.27-1.29)	RR did not vary when adjusted for history of diabetes, obesity, occupation, education, religion, coffee or tea consumption, and cigarette use (data not provided)	no	personal interview in hospital	Wynder and Stellman (1980)
	cases: men and women with bladder cancer, but with no tobacco-related heart disease, admitted to hospitals/clinics in New Jersey during 1978; mean age, 66.8 yr controls: admitted to hospitals/clinics for other conditions, excluding tobacco-related heart disease and any neoplasm; matched to cases by age, place of birth, sex, race, source of obtaining cases, current residence; mean age, 70.9 yr	cases: 75 controls: 142	not specified	saccharin	not specified	regularly consumed vs. never or occasion-ally consumed	Risk Ratio: 1.3 (0.6-2.8) not significant; p > 0.05 (cases consumed an average of 3.6 tablets/day for a mean period of 6.4 yr; controls consumed an average of 2.5 tablets for 6.3 yr)	none	no	all cases and controls interviewed by 1 nurse; responses recorded on precoded form	Najem et al. (1982)
	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	see Hoover & Strasser (1980)	Relative Risk: 1.2 (1.0-1.5)	age, sex, race, religion	no	re-evaluation of Hoover & Strasser (1980) data	Walker et al. (1982)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases: diagnosed from December 1977-December 1978 in Detroit with transitional or squamous cell carcinoma of lower urinary tract; aged 21-84 yr hospital-based controls: residents of Detroit, discharged from same hospital as case; matched by age, race, sex, discharge date population-based controls: matched by age and sex	cases: 391 hospital-based controls: 305 population-based controls: 440	cases: 91 hospital-based controls: 89 population-based controls: 91	artificial sweetener	lifetime	ever or never used	Relative Risk: using population controls: 1.1 (men); 1.8 (women) using hospital controls: 0.9 (men); 1.1 (women) using hospital controls without obesity-related diseases: 1.1 (men); 1.1 (women)	none (adjustment for age, smoking, education, and body mass index had no effect on relative risk)	not applicable	questionnaire given in person, by phone (only if necessary), or by proxy (only if necessary); add-on to Hoover and Strasser (1980) study	Silverman et al. (1983)
	cases: men and women of Japanese or Caucasian ancestry, diagnosed with lower urinary tract cancer between 1977 and 1986 in Oahu; aged 30-93 yr controls: population-based; matched to cases by sex, ethnic group, age, and residence	cases: 261 controls: 522	cases: 86 controls: 89	saccharin	1 yr	non-user user 1-5 serving-yr 6+ serving-yr	Odds Ratio: M: 1.0; F: 1.0 M: 1.1(0.7-1.8) F: 0.7(0.3-1.5) M: 1.2(0.6-2.4) F: 0.5(0.2-1.6) M: 1.1(0.6-1.9) F: 0.9(0.3-2.9)	cigarette use	no	personal interview in home of participant	Nomura et al. (1991)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
U.S. Case-control (cont.)	cases: women diagnosed with bladder cancer in New York state between January 1975 and September 1980; aged 20-49 controls: population-based; matched to cases by sex, age, and residence within an area code	cases: 173 controls: 173	cases: 80.8 controls: 71	artificial sweetener	ever used artificial sweetener ≥ 100 times	not specified	Odds Ratio: 1.1 (0.7-1.7)	none	not specified	telephone interview during 1982	Piper et al. (1986)
	cases: men and women diagnosed with transitional cell bladder cancer between 1977 and 1978 in 10 geographic regions; aged 21-84 years controls: randomly selected from general population	cases: 1860 controls: 3934	cases: 73 controls: 83	artificial sweetener	lifetime	< 1680 mg/day ≥ 1680 mg/day	Relative Risk: noninvasive: 1.0 invasive: 1.0 Grade I: 1.0 Grade II: 1.0 Grade III/IV: 1.0 noninvasive: 1.3 (0.9-2.1) invasive: 1.3 (0.8-2.3) Grade I: 1.1 (0.5-2.3) Grade II: 1.1 (0.6-2.0) Grade III/IV: 2.2 (1.3-3.6)	age, sex, cigarette use, history of urinary infection or bladder stones, coffee consumption, family history of urinary tract cancer, high-risk occupation, race, education	not specified	personal interview in home of participant	Sturgeon et al. (1994)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Canada Case-control	cases: men and women newly diagnosed with urinary bladder cancer between 1979 and 1982 in Alberta or southcentral Ontario; aged 35-79 yr controls: randomly selected, population-based; matched to cases by age, sex, and area of residence	cases: 826 controls: 792	cases: 67 controls: 53	saccharin	lifetime	30 usage-yr (Usage-years represent cumulative exposure, e.g., 3 uses/ day for 10 yr=30 usage yr)	Odds Ratio: M: 1.01 (0.86-1.18) F: 0.96 (0.79-1.16)	lifetime cigarette consumption and history of diabetes	no	interview in home of participant	Risch et al. (1988)
Other Case-control	cases: residents of Manchester, United Kingdom or Nagoya, Japan diagnosed in 1976-1978 with lower urinary tract cancer; aged 21-89 controls: population-based, matched to cases by age and sex	cases: 555 British, 293 Japanese controls: 735 British, 589 Japanese	cases: 96 (British), 84 (Japanese) controls: 90 (British), 80 (Japanese)	artificial sweetener (97% of British and 94% of Japanese used saccharin)	~ 30-40 yr (most reported first use during or shortly after start of World War II)	ever or never used	Relative Risk: British men: 0.9 (0.7-1.2) British women: 0.9 (0.6-1.4) Japanese men: 0.7 (0.5-0.9) Japanese women: 0.5 (0.3-0.8) There was an increased RR of 1.6 among nonsmoking men from the United Kingdom. RR was not increased for any other nonsmoking group or for any current or former smokers group.	stratified by age (< 65 yr, 65-74 yr, or 75+ yr) Preliminary analysis of British men revealed no effect of occupational history on risk	British cases had an increased RR among the over-10-tablets/day female group (RR = 2.3), but not for 10-tablets/day male group (RR = 0.6). Japanese not evaluated for dose-response. There was no association between duration of use and increase in risk for British or Japanese.	interview in home (British cases/ controls, Japanese controls) or interview in hospital (Japanese cases)	Morrison et al. (1982)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Other Case-control (cont.)	cases: men and women newly and previously diagnosed with bladder cancer in West Yorkshire, United Kingdom controls: hospital-based; matched to cases by age and sex	cases: 622 existing cases, 219 new cases controls: 622 for existing cases, 448 for new cases	not specified	saccharin (as food additive only)	> 1 yr, beginning at least 5 yr before cancer diagnosis	user or non-user	Relative Risk: male nonsmokers: 2.2 (1.3-3.8) female nonsmokers: 1.6 (0.8-3.2) male smokers: 0.9 (0.6-1.3) female smokers: 1.2 (0.5-2.6)	age and type of case (new or existing)	not specified	personal interview (site not specified); cases and controls interviewed by same person	Cartwright et al. (1981)
	cases: women from Denmark newly diagnosed with bladder cancer, average age, 66.4 yr controls: population-based; matched to cases by sex, age, and geographic area, including degree of urbanization	cases: 47 (of the 47 cases, only 6 had consumed saccharin) controls: 94 (of the 94 controls, only 2 had consumed saccharin)	cases: 81 controls: 100	saccharin	not specified	not specified	Relative Risk: all women: 6.7 (1.5-30.2) never-smokers only: 3.3 (1.4-7.8)	none	not specified	cases: personal interview in hospital controls: mailed questionnaire followed by phone interview	Mommsen et al. (1983)
	cases: men and women diagnosed with bladder cancer in Copenhagen, Denmark between May 1979 and April 1981 controls: residents of Copenhagen, randomly selected; matched to cases by age and sex	cases: 388 controls: 787	cases: 94.4 controls: 75.1	artificial sweetener (72.9% used saccharin alone; 10.7% used cyclamate alone; 16.4% used both)	≥ 3 mo	never used or ever used	Relative Risk for Users of Saccharin Alone: M: 0.68 (0.45-1.02) F: 1.04 (0.51-2.09)	not specified	no	interview in home of participant	Møller-Jensen et al. (1983)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Other Case-control (cont.)	cases: men and women living in La Plata, Argentina for ≥ 5 yr and diagnosed with bladder cancer population-based controls: matched to cases by sex, age, residence (street block) hospital-based controls: matched to cases by sex, age, hospital	cases: 117 population-based controls: 117 hospital-based controls: 117	not specified	saccharin	not specified	not specified	RR not specified, but labeled as not significant	not specified	no	personal interview in home (population controls) or hospital (cases and hospital controls)	Iscovich et al. (1987)
	cases: men living in the Hérault region of France for ≥ 5 yr and diagnosed with primary bladder carcinoma between Jan. 1987 and May 1989 controls: randomly selected men from Hérault region; only men over 50 yr old who had lived in Hérault region > 5 yr were included	cases: 219 controls: 794	cases: 80.5 controls: 77.8	saccharin (as a food additive only)	lifetime	< 365 or ≥ 365 (units not specified)	Odds Ratio: 1.5 (0.8-3.0)	not specified	not specified	telephone interview or mailed questionnaire (for those not listed in phone book)	Momas et al. (1994)
Descrip-tive	cohorts: residents of Denmark born between 1941 and 1945 (when saccharin use was high); evaluated from 1961-1976 (aged ≤ 34 yr) controls: residents of Denmark born 1931-1940	not specified	not specified	saccharin	≤ 30 yr (exposure beginning *in utero*)	not specified	There was no increase in bladder cancer mortality during the first 3 decades of life in cohorts.	not specified	no	observed cases in cohorts compared to expected cases (i.e., cases among those born 1931-1940)	Jensen and Kamby (1982)

Table 3-1. Summary of Epidemiology Studies Published Post IARC (1980) (Continued)

Study Design	Study Participants			Nature of Exposure							
	Source	Number	Response Rate (%)	Substance	Duration	Level	Risk Ratio (95% Confidence Interval)	Covariates Used to Adjust OR	Evidence of Dose-Response	Data Collection Method	Reference
Meta-analysis	multiple sources	cases: 5499 M, 2082 F controls: not specified	not specified	artificial sweetener	not specified	not specified	Relative Risk: M[b]: 0.958 (0.69-1.33) F[b]: 0.961(0.85-1.08) all studies[c]: 0.979 (0.92-1.04)	RR inversely weighted by the variance from each study	not specified	meta-analysis of 13 case-control studies	Elcock and Morgan (1993)

Abbreviations: F = female; M = male; OR = odds ration; RR = relative risk

[a] Unless otherwise noted, the type of artificial sweetener consumed was not specified
[b] This category included 12 studies
[c] This category included 13 studies

4.0 MAMMALIAN CARCINOGENICITY

Several conventional carcinogenicity studies of dietary sodium saccharin have been conducted in rats. Four of these studies that meet contemporary standards for hazard identification, including absence of urinary bladder parasites, have shown induction of neoplasia in urinary bladder urothelium of male rats. A condition that appears to be necessary for positive results is exposure to high doses of sodium saccharin close to the time of weaning with continued exposure for two years. In four studies of up to 30 months duration, sodium saccharin was carcinogenic in Charles River CD and Sprague-Dawley male rats as evidenced by a dose-related increased incidence of benign or malignant urinary bladder neoplasms at dietary concentrations of 1% or greater (Tisdel et al., 1974; Arnold et al., 1980; Taylor et al., 1980; Schoenig et al., 1985) and statistically significant increased bladder neoplasia at 4% or greater (Schoenig et al., 1985; Squire, 1985). Non-statistically significant increases in urinary bladder cancer have also been seen in saccharin-treated female rats from studies showing a positive effect in males (Arnold et al., 1980; Taylor et al., 1980). Furthermore, several initiation/promotion studies in different rat strains have shown a reduced latency and/or increased incidence of similar urinary bladder cancers in male and female rats fed sodium saccharin subsequent to treatment with different urinary bladder initiators (e.g., Hicks and Chowaniec, 1977; Cohen et al., 1979; Nakanishi et al., 1980b; West et al., 1986; Fukushima et al., 1990). Several additional rat studies in which sodium saccharin was administered either in the diet or in drinking water were negative for tumorigenicity (Fitzhugh et al., 1951; Lessel, 1971; Schmähl, 1973; Chowaniec and Hicks, 1979; Hooson et al., 1980; Schmähl and Habs, 1984).

Conventional carcinogenicity studies of dietary sodium saccharin in mice have been less rigorously carried out, and have been negative for urinary bladder carcinogenesis. On the other hand, two studies in which saccharin-containing cholesterol pellets were surgically implanted into the urinary bladders of mice have yielded urinary bladder cancers. Three mouse studies have reported positive carcinogenicity following exposure to saccharin. Two of these studies involved surgical implantation of saccharin-containing cholesterol pellets into the urinary bladders and resulted in development of malignant urothelial neoplasms (Allen et al., 1957; Bryan et al., 1970). In the third study, dietary sodium saccharin resulted in increased incidences of malignant thyroid neoplasms (Prasad and Rai, 1986). While the mouse data cannot be discounted, some of these studies had methodological flaws, provided limited information, did not show a dose-response, or had unexpected outcomes that may be species or strain-specific and should be verified by additional studies. Four studies in mice were judged negative for tumorigenesis (Roe et al., 1970; Kroes et al., 1977; Homberger, 1978; Frederick et al., 1989) as were studies in nonhuman primates (McChesney et al., 1977 abstr.; Sieber and Adamson, 1978; both cited by IARC, 1980; Thorgiersson et al., 1994; Cohen et al., 1996 abstr.) and a single hamster study (Althoff et al., 1975).

4.1 Mammalian Carcinogenicity of Saccharin

Full experimental details for the studies described in this section are presented in **Table 4-1**.

4.1.1 Hamsters

No urinary tract tumors were observed in Syrian golden hamsters exposed to 0.156-1.25% sodium saccharin in drinking water for life (50-60 weeks). The incidence of tumors in other tissues was within the range of spontaneously occurring tumors (Althoff et al., 1975).

4.1.2 Mice

Twenty-five days after application of saccharin to the skin (8% solution in acetone), "S" strain mice were given 18 weekly applications of 0.17% croton oil in acetone. Following treatment with croton oil, 14 skin tumors were observed in 7/20 mice exposed to saccharin, while 4 skin tumors were observed in 4/19 control mice treated with croton oil only. The difference was not significant (p value not given) (Salaman and Roe, 1956; cited by IARC, 1980).

An increased incidence of bladder cancer ($p = 0.01$; χ^2 test) was observed in "stock" mice that had saccharin/cholesterol pellets (2 mg saccharin/8 mg cholesterol) implanted in their urinary bladder lumina for 40 or 52 weeks (Allen et al., 1957). The authors noted that the presence of the cholesterol pellet in the bladder may have had a promoting action, and that the method of bladder implantation detects incomplete carcinogens. It was not specified whether other tissues were examined. The saccharin used was of unknown purity and the study involved small numbers of animals whose sex was not specified.

As part of a combined carcinogenesis and tumor promotion study (Roe et al., 1970), female Swiss mice were given a 5% saccharin diet for 18 months. Based upon macroscopic examination of all major organs except brain, pituitary, and spinal cord, there were no alterations in gross lesions or tumor incidences in saccharin-treated mice. The necropsy included careful macroscopic examination of urinary bladder.

Stoner et al. (1973; cited by IARC, 1980) found that intraperitoneal (i.p.) saccharin exposure (8 weeks, 0.6 or 3.3 g/kg/day) of A/He mice was not associated with induction of pulmonary tumors. No other organs were examined. In a 6 generation study, Kroes et al. (1977) found that the incidence of urinary bladder carcinoma was not significantly increased in Swiss SPF mice exposed to 0.2 or 0.5% saccharin diet for 21 months. It was not specified whether other tissues were examined.

A second cholesterol:saccharin (4:1) pellet implantation study in female Swiss mice significantly increased the incidence of urinary bladder carcinomas but not in the degree of malignancy in mice living more than 175 days after bladder implantation versus controls (cholesterol pellet implants only) (Bryan et al., 1970). Since all of the saccharin was removed from the implanted pellets within 1.5 days and the cholesterol plus saccharin pellet was porous, having lost 20% of its weight, it has been argued that the cholesterol:saccharin pellet was different and perhaps more irritating than the pellet comprised of only cholesterol and, furthermore, there is some concern regarding how closely pellet implantation resembles chronic oral exposure to saccharin (Cranmer, 1980).

The incidence of transitional-cell bladder cancers, lung tumors, hepatomas, or lymphomas was not significantly increased in Charles River CD mice exposed to a 1 or 5% sodium saccharin diet for up to 2 years (Homburger, 1978). Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically.

Prasad and Rai (1986) orally administered albino mice 0.5, 1.0, or 1.5 g/kg saccharin (purity not specified) dissolved in 1 mL of distilled water for 1 yr, beginning at 6 weeks of age. Papillary adenocarcinoma of the thyroid was found in male (5/10) and female (3/10) mice exposed to the highest dose. The tumors were detected during months 9-12 of the experiment and were malignant in nature; metastases were found in the lungs. No information was provided on gross or microscopic examinations of the bladder. Although a control group was used (10 males, 10 females), the tumor incidence in these mice was not reported. The saccharin used in this study was purchased from Boots Co., Bombay, India.

In female weanling BALB/c mice administered a 0, 0.1, 0.5, 1.0, or 5.0% sodium saccharin diet for 117 weeks, there was a marginally significant dose-response ($p = 0.04$) in the incidence of Harderian neoplasms (27/163, 32/172, 29/160, 22/132, and 22/84, respectively). There was no significant increase, however, for bladder, liver, breast, adrenal, or lung tumors, or for reticulum cell sarcoma or lymphoma in any dose group (Frederick et al., 1989). Neither the authors nor the NTP staff consider the Harderian gland response to be biologically significant.

4.1.3 Rats

Seven of 18, 21-day-old Osborne-Mendel rats exposed to a 5% saccharin diet for up to 2 years developed abdominal lymphosarcomas (Fitzhugh et al., 1951). The authors stated that this was not "out of line with the incidence (of abdominal lymphosarcomas) in a comparable group of rats", but noted an uncommon co-occurrence of thoracic lymphosarcomas with abdominal lymphosarcomas in 4 of the 7 rats treated for 102 or more weeks. Urinary bladders were not evaluated. Although controls were used in this study, the control tumor incidence was not provided. IARC (1980) reviewed Fitzhugh et al. (1951) and noted the small number of animals exposed.

Saccharin was negative for tumorigenesis in male and female Boots-Wistar rats exposed to a 0.005, 0.05, 0.5, or 5% saccharin diet for 2 years. Of 4 rats exposed to the highest dose and examined histologically, 1 female had a bladder papilloma (Lessel, 1971). IARC (1980) noted the small number of bladders examined histologically. It was not specified whether other tissues were examined.

There was no increase in the incidence of benign and malignant mesenchymal tumors or of bladder tumors in 70- to 90-day-old BD rats exposed to 0.2 or 0.5% sodium saccharin in the diet for up to 30 months (Schmähl, 1973; cited by IARC, 1980). It was not specified whether other tissues were examined.

In a two-generation study, the incidence of bladder cancer was not increased in F_1 male or female Charles River CD rats exposed to 0.01, 0.1, 1.0, or 5% sodium saccharin for up to 28 months. However, the incidence of urinary bladder transitional-cell neoplasms in F_1 male rats exposed to a 7.5% sodium saccharin diet for up to 28 months was significantly increased when compared to controls (7/23 vs. 1/29 in controls). In addition, there were 2/31 urinary bladder neoplasms in F_1 females exposed to 7.5% saccharin versus 0/24 in controls. The F_0 parents were fed test diets from weaning, through mating, and through gestation to the weaning of their litters. The occurrence of the bladder neoplasms was not correlated with the presence of bladder stones, and bladders were free of parasites (Taylor et al., 1980).

In a 2-generation study, there was an increased incidence of transitional-cell carcinoma of the bladder in F_1 male Sprague-Dawley rats fed 5% sodium saccharin in the diet for 100 weeks (7 tumors in 20 exposed rats vs. 0 tumors in 20 controls). Carcinomas were not observed in the bladder of female rats exposed similarly. Male and female rats fed a 0.05 or 0.5% sodium saccharin diet for 100 weeks did not show an increased incidence of neoplasms at any site (Tisdel et al., 1974).

Urinary bladder tumors were not observed in Wistar rats exposed to 2.5 g sodium saccharin/kg/day for up to 28 months (Furuya et al., 1975 abstr.). IARC (1980) noted the incomplete reporting of this study.

Sodium saccharin was negative for urinary bladder tumorigenesis in male and female weanling Charles River CD rats exposed in the diet to 90, 270, 810, or 2430 mg sodium saccharin/kg/day for 26 months. Non-invasive bladder tumors were detected in 1/60 males and 1/60 females exposed to 90 mg/kg and in 2/60 males exposed to 810 mg/kg, but none were detected in the rats exposed to 2430 mg/kg. The authors found that the presence of bladder calculi was not associated with exposure or the presence of bladder tumors. The combined incidence of lymphomas and leukemias in males given the highest dose was 7/54 (vs. 2/57 in controls), but the statistical significance of this was not specified. All major tissues were examined (Munro et al., 1975).

The incidences of tumors of the urinary bladder, pituitary, breast, and subcutaneous tissue were not increased in Charles River CD-1 rats exposed to 1 or 5% sodium saccharin for up to 2 years. The authors noted that in 33% of all examined urines, *Trichosomoides crassicauda* ova were found (Homburger, 1978). Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically.

Sodium saccharin had no significant effect on tumor incidence in Wistar SPF rats exposed to 4 g saccharin/kg body weight in the diet for 2 years. Although there was an increase in the total number of exposed males with tumors at any site (10/70 males vs. 1/52 male controls), site-specific tumor incidences were not statistically significant. Sodium saccharin also had no significant effect on tumor incidence in Wistar SPF rats exposed to 2 g saccharin/kg in drinking water for 2 years. There was an increase in the total number of exposed males with tumors at any site (11/71 males vs. 1/52 male controls) in rats exposed to saccharin in drinking water, but site-specific tumor incidences were not statistically significant. All major organs were examined macroscopically. The bladder, kidneys, lungs, liver, spleen, pancreas, ovaries, uterus, and any other organ with an abnormal appearance were examined histopathologically (Chowaniec and Hicks, 1979).

In a two-generation study, the incidence of benign plus malignant bladder tumors was significantly increased ($p < 0.03$) in male Sprague-Dawley rats from both the F_0 and F_1 generations (F_0: 7/38 vs. 1/36 in controls; F_1: 12/45 vs. 0/42 in controls). The F_0 generation was exposed to a 5% sodium saccharin diet for 90 days prior to mating with continued lifetime exposure (up to 142 weeks), while the F_1 pups were exposed for up to 127 weeks. The incidence of benign plus malignant bladder tumors was not statistically increased in F_0 and F_1 females and there was no increase in the incidence of tumors of other tissues in males or females. Two F_1 saccharin-dosed females, however, did have malignant urinary bladder tumors. All organs

and all grossly abnormal areas of dermal, supportive, or skeletal tissues were examined histologically (Arnold et al., 1980).

As part of an initiation/promotion study (Hoosan et al., 1980), female Wistar rats were exposed to 2 g/kg/day of sodium saccharin in drinking water or in diet. There was no increase in urinary bladder neoplasms or other tumors in rats exposed to saccharin for two years.

There was no statistically significant increase in tumor incidence in offspring of pregnant Sprague-Dawley rats administered 0.2, 1, or 5 g saccharin/kg in aqueous solution by gavage on gestation days 14, 17, and 20. Offspring were fed normal diet and observed for life (approximately 2 years) or were killed when moribund. Complete necropsies were performed. All urinary bladders and any organs with macroscopically visible abnormalities were examined histologically (Schmähl and Habs, 1980).

The incidence of urinary bladder transitional cell papilloma was significantly increased in male ACI rats administered 5% sodium saccharin in the diet for 52 weeks beginning at 6 weeks of age (9/32 vs. 0/28 in controls, $p < 0.01$). Calculi were observed in 1 rat with bladder cancer and there was a higher level of urinary $MgNH_4PO_4$ crystals in treated rats than in controls. At least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda*, which could have enhanced cell proliferation in the bladder. The bladder, liver, and kidneys were the only tissues examined histologically. Females were not included in the study (Fukushima et al., 1983).

No tumors were detected in the bladder, liver, or kidneys of male F344, Sprague-Dawley, or Wistar rats administered 5% sodium saccharin in the diet for 52 weeks beginning at 6 weeks of age. Females were not evaluated (Fukushima et al., 1983).

In a two-generation study, administration of a mixture of 2 or 5% sodium saccharin and sodium cyclamate (1:10 ratio) in the diet of Sprague-Dawley rats was not carcinogenic. Full necropsies were performed, including evaluation of the urinary tract (Schmähl and Habs, 1984).

In a large 2-generation study, F_0 rats were started on a test diet at 6 weeks of age; F_1 rats were started on the same test diet between 28 and 38 days of age. There was a clear dose response for urinary bladder tumors in F_1 male Charles River CD rats exposed to 1.0 to 7.5% sodium saccharin in the diet for up to 30 months (1.0%, 5/658; 3.0%, 8/472; 4.0%, 12/189; 5.0%, 15/120; 6.25%, 20/120; 7.5%, 37/118; controls, 0/324) (Schoenig et al., 1985). Females were not evaluated in this study. The authors concluded a no-effect level for bladder tumors at the 1% dietary level based upon lack of statistical significance and historic control incidences at their laboratory. Following independent review of the urinary bladder lesions, Squire also concluded a no-effect level for bladder tumors at 1% (Squire, 1985). The bladder tumor incidence in rats exposed to 5% sodium saccharin only during gestation was 0/122, while that in rats exposed to 5% sodium saccharin from birth for a single generation was 12/120 (Schoenig et al., 1985). The urinary bladder, urethra, ureter, kidneys, and all gross lesions and tissue masses were examined histologically (Schoenig et al., 1985).

Bladder carcinomas and precancerous lesions were not observed in 6-week-old male analbuminemic (low level of albumin in the serum) Sprague-Dawley rats exposed to 5% sodium saccharin in the diet for 80 weeks (Homma et al., 1991). Only the bladder was examined.

4.1.4 Nonhuman Primates

Histopathological examination of urinary bladders, kidneys, and testes of surviving and deceased male and female rhesus monkeys (exposed to 20, 100, or 500 mg saccharin/kg/day in the diet for 79 months) showed no abnormal pathology (McChesney et al., 1977 abstr.; cited by IARC, 1980).

Sieber and Adamson (1978; cited by IARC, 1980) found that sodium saccharin was negative for gross neoplasia in monkeys (4 strains, not specified by IARC) exposed to 25 mg/kg/day in the diet for 9 yr. This study was ongoing in 1980.

Twenty 0 to 1-yr-old monkeys (Cynomolgus, Rhesus, and African Green were used but additional details were not provided) were exposed to 25 mg sodium saccharin/kg/day by mouth in water for at least 20 yr. Five monkeys died from either varicella, pneumonia, or unknown reasons. No tumors were found in the dead monkeys nor were there any indications of tumors in the 15 surviving monkeys. Complete necropsies were performed on all animals that died. Various unspecified hematological and biochemical tests were routinely performed on survivors (Thorgeirsson et al., 1994).

Results from the surviving monkeys from the Thorgeirsson et al. (1994) study were subsequently reported (Cohen et al., 1996 abstr.). There were no calculi, unusual crystals, increased crystalluria, or calcium phosphate precipitate in urine of cynomolgus and rhesus monkeys administered 25 mg sodium saccharin/kg/day for 17 to 23 years. Urine was analyzed during the last year of life. There was no association between ingestion of sodium saccharin and urinary protein content. Urinary bladders were free of hyperplasia and tumors and scanning electron microscopy revealed no difference in the appearance of the urothelium in exposed and age-matched control monkeys (Cohen et al., 1996 abstr.). It was not specified in the abstract whether other tissues were examined.

Table 4-1. Mammalian Carcinogenicity

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.1.1 Hamsters							
8-wk-old Syrian golden hamsters	30M, 30F	none	sodium saccharin made by Maumee process, purity not specified	0.156-1.25% in drinking water	50-60 wk	Negative No urinary tract neoplasms were observed. The incidence of other neoplasms was within the range of spontaneously occurring tumors.	Althoff et al. (1975)
4.1.2 Mice							
'S' strain mice (age not specified)	20 (sex not specified)	19 (sex not specified)	saccharin[a] made by Remsen-Fahlberg method, purity not specified	8% solution in acetone, applied to skin	22 wk	Negative Twenty-five days after application of saccharin, animals were given 18 weekly applications of 0.17% croton oil in acetone. Following treatment with croton oil, 14 skin tumors were observed in 7/20 animals exposed to saccharin, while 4 skin tumors were observed in 4/19 control animals treated with croton oil only. This difference was not significant (p value not given).	Salaman and Roe (1956; cited by IARC, 1980)
"stock' mice (age not specified)	20 (sex not specified)	28 (sex not specified)	saccharin[a], method of production and purity not specified	2 mg saccharin/8 mg cholesterol pellets	40 or 52 wk	Positive Saccharin/cholesterol pellets were implanted in urinary bladder lumina. Controls received cholesterol pellets. Of mice which survived for at least 30 weeks, 4/13 saccharin-treated mice and 1/24 control mice had bladder cancer ($p=0.01$; χ^2 test). The authors noted that the presence of the cholesterol pellet in the bladder may have had a promoting action and that the method of bladder implantation detects incomplete carcinogens. It was not specified whether other tissues were examined.	Allen et al. (1957)
60- to 90-day-oldSwiss mice	100F	100F	sodium saccharin; method of production and purity not specified	20-24 mg pellets with 20% sodium saccharin suspended in cholesterol	13 mo	Positive Saccharin/cholesterol pellets were implanted into the urinary bladder lumina. Controls received cholesterol pellets. Incidences of mouse bladder carcinomas in exposed animals were 47 and 52% as compared with incidences of 13 and 12% in controls. The time required for 50% of the compound to be eluted was about 5.5 hours, so the exposure of the mouse bladder to saccharin was very brief.	Bryan et al. (1970)
Swiss mice (age not specified)	50F	50F	saccharin[a], method of production and purity not specified	5% in diet	18 mo	Negative Saccharin did not alter incidence of tumors (type not specified) and did not affect urinary bladder pathology when bladder was observed macroscopically. It was not specified which other tissues were examined.	Roe et al. (1970)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
A/He mice (age not specified)	20F per dose	30F	saccharin[a] method of production and purity not specified	0.6 or 3.3 g/kg/day i.p.	8 wk	Negative Exposed animals were killed after 21 weeks, controls were killed after 24 weeks. Exposure to saccharin was not associated with induction of pulmonary tumors. The lungs were the only tissue examined.	Stoner et al. (1973; cited by IARC, 1980)
Swiss SPF mice (age not specified)	50M, 50F per dose	50M, 50F	saccharin[a], made by Remsen-Fahlberg method, 0.5% *o*-toluenesulfonamide, impurity	0.2 or 0.5% in diet (6-generation study)	21 mo	Negative Exposure to saccharin did not significantly alter the incidence of urinary bladder carcinoma. It was not specified whether other tissues were examined.	Kroes et al. (1977)
Charles River CD mice (age not specified)	25M, 25F per dose	25M, 25F	sodium saccharin, method of production not specified, 345 mg/kg *o*-toluenesulfonamide	1 or 5%	≤ 2 yr	Negative Animals were sacrificed when obvious tumors were seen or when they were moribund. Survivors were killed at 2 years. Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically. The incidence of transitional-cell bladder cancers in treated animals was not significantly different from that in controls. Lung tumors, hepatomas, and lymphomas occurred with similar frequency in exposed and control animals. This study was complicated by the presence of the worm *Trichosomoides crassicauda* in treated and control animals. The author stated that this parasite is known to cause extensive papillomatosis of the bladder.	Homburger (1978)
6-wk-old albino mice	10M, 10F per dose	10M, 10F	saccharin[a], method of production and purity not specified (purchased from Boots Co., Bombay, India)	0.5, 1.0, or 1.5 g/kg/day in 1 mL distilled water, by gavage (times/wk not specified)	1 yr	Positive Papillary adenocarcinoma of the thyroid was found in male (5/10) and female (3/10) mice exposed to the highest dose. The tumors were detected during months 9-12 of the experiment and were malignant in nature; metastatic deposits were found in the lungs. No information was provided on gross or microscopic examinations of the bladder. The tumor incidence in controls was not reported.	Prasad and Rai (1986)
18- to 19-wk-old BALB/c mice	192F (0.1%) 192F (0.5%) 144F (1.0%) 96F (5.0%)	192F (basal diet alone)	sodium saccharin, >98% pure, method of production not specified	0, 0.1, 0.5, 1.0, or 5.0% diet	117 wk	Negative There was a marginally significant trend (p=0.04) in the incidence of Harderian neoplasms (27/163, 32/172, 29/160, 22/132, 22/84). There was no significant dose-response for bladder, liver, breast, adrenal, or lung tumors, or for reticulum cell sarcoma or lymphoma in any dose group. The Harderian gland response was not considered to be biologically significant.	Frederick et al. (1989)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.1.3 Rats							
21-day-old Osborne-Mendel rats	10M, 10F per dose	10M, 10F	saccharin[b], method of production and purity not specified	0.01, 0.1, 0.5, 1, or 5% in diet	≤ 2 yr	Negative Seven of 18 animals (sex not specified) receiving 5% dose developed abdominal lymphosarcomas. The authors stated that this was not "out of line with the incidence (of abdominal lymphosarcomas) in a comparable group of rats", but noted the uncommon co-occurrence of thoracic lymphosarcomas with abdominal lymphosarcomas in 4 of the 7 rats treated for 102 or more weeks. Tumor incidence in controls was not provided. Urinary bladders were not evaluated. IARC (1980) noted the small number of animals used in this study.	Fitzhugh et al. (1951)
Boots-Wistar rats	20M, 20F per dose	20M, 20F	saccharin[a], made by Remsen-Fahlberg method, purity not specified	0.005, 0.05, or 5% in diet	2 yr	Negative Tumor incidence was similar in control and exposed animals. Of 5 bladders from animals exposed to the highest dose, 1 female had a bladder papilloma. IARC (1980) noted the small number of bladders examined histologically. It was not specified whether other tissues were examined.	Lessel (1971)
70- to 90-day-old BD rats	52M, 52F per dose	52M, 52F	sodium saccharin, made by Remsen-Fahlberg method, purity not specified	0.2 or 0.5% in diet	≤ 30 mo	Negative The incidence of benign and malignant tumors was similar in control and exposed animals. No bladder tumors were observed. *Strongyloides capillaria* was found in the urinary tract of 16% of all animals. [original paper in German]	Schmähl (1973)
weanling SD rats	20M, 20F per dose	20M, 20F	sodium saccharin, made by Remsen-Fahlberg method, purity not specified	0.05, 0.5, or 5% in diet	100 wk	Positive (males only, at highest dose) F_0 generation was fed same dose as offspring. There were seven transitional-cell carcinomas of the urinary bladder, but only in males fed the highest dose. A review by IARC (1980) noted that this incidence was significant (p=0.001).	Tisdel et al. (1974)
Wistar rats (age not specified)	54-56M	54-56M	sodium saccharin, method of production and purity not specified	2.5 g/kg body weight/day	≤ 28 mo	Negative No urinary bladder tumors were observed. It was not specified whether other tissues were examined. IARC noted the incomplete reporting of this study.	Furuya et al. (1975 abstr.)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
Weanling Charles River CD rats	240M, 240F	60M, 60F	sodium saccharin, method of production and purity not specified	90, 270, 810 or 2430 mg/kg/day in diet	26 mo	Negative Non-invasive bladder tumors were detected in 1/60 males and 1/60 females exposed to 90 mg/kg and in 2/60 males exposed to 810 mg/kg, but not in any rats exposed to 2430 mg/kg. The presence of bladder calculus was not associated with exposure or with the presence of bladder tumors. Saccharin administration was not accompanied by an increase in tumor incidence. The combined incidence of lymphomas and leukemias in males given the highest dose was 7/54 (vs. 2/57 controls), but the statistical significance of this was not specified. All major tissues were examined.	Munro et al. (1975)
Charles River CD-1 rats (age not specified)	25M, 25F per dose	25M, 25F	sodium saccharin, method of production not specified, 345 mg/kg *o*-toluenesulfonamide	1 or 5% in diet	≤ 2 yr	Negative Animals were sacrificed when obvious tumors were seen or when they were moribund. Survivors were killed at 2 yr. Any tissue with an abnormal appearance and all vital organs from at least half of the animals were examined histologically. The incidences of tumors of the urinary bladder, pituitary, breast, and subcutaneous tissue were similar in control and exposed animals. The author stated that one third of all examined urines were thought to contain *Trichosomoides crassicauda* ova.	Homburger (1978)
Wistar SPF rats (age not specified)	75M, 75F	55M, 50F (these controls also used for drinking water study; see below)	sodium saccharin, made by Remsen-Fahlberg method, 698 mg/kg *o*-toluenesulfonamide	4 g/kg body weight; in diet	2 yr	Negative Although there was an increase in the total number of exposed males with tumors at any site (10/70 males vs. 1/52 male controls), site-specific tumor incidences were not statistically significant. All major organs were examined macroscopically. The bladder, kidneys, lungs, liver, spleen, pancreas, ovaries, uterus, and any other organ with an abnormal appearance were examined histopathologically.	Chowaniec and Hicks (1979)
Wistar SPF rats (age not specified)	75M, 50F	55M, 50F	sodium saccharin, made by Remsen-Fahlberg method, 698 mg/kg *o*-toluenesulfonamide	2 g/kg body weight; in drinking water	2 yr	Negative Although there was an increase in the total number of exposed males with tumors at any site (11/71 males vs. 1/52 male controls), site-specific tumor incidences were not statistically significant. All major organs were examined macroscopically. The bladder, kidneys, lungs, liver, spleen, pancreas, ovaries, uterus, and any other organ with an abnormal appearance were examined histopathologically.	Chowaniec and Hicks (1979)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
32-day-old SD rats	50M, 50F	50M, 50F	sodium saccharin, made by Maumee process, < 0.05 ppm *o*-toluenesulfonamide	5% in diet	90 days (adults), ~700 days (pups)	Positive (only males) Incidence of benign plus malignant bladder tumors was significantly increased ($p < 0.03$) in exposed male rats from both the F_0 and F_1 generations (F_0: 7/38 vs. 1/36 controls; F_1: 12/45 vs. 0/42 controls). The incidence of benign plus malignant bladder tumors was not statistically increased in F_0 or F_1 females and there was no increase in the incidence of tumors of other tissues in males or females. All organs and all grossly abnormal areas of dermal, supportive, or skeletal tissues were examined histologically. There were no effects on reproduction, longevity, or hematological parameters.	Arnold et al. (1980)
Wistar rats (age not specified)	50F	63F	sodium saccharin, made by Maumee process, purity not specified	2 g/kg body weight/day	2 yr	Negative No bladder neoplasms occurred in control or exposed rats. Overall tumor incidence did not differ between control and exposed rats. It was not specified in the review which tissues besides bladders were examined. IARC noted that animals were started on the test diet not at weaning, but after several weeks on a normal diet.	Hooson et al. (1980; cited by IARC, 1980)
pregnant SD rat	5F (low dose) 6F (mid dose) 7F (high dose)	5F	saccharin[b] [< 10 ppm *o*-toluene sulfonamide], method of production not specified	0.2, 1, or 5 g/kg by gavage in aqueous solution, administered on gestation days 14, 17, and 20	3 days	Negative There was no statistically significant increase in tumor incidence in offspring that were fed normal diet and observed for life (~ 2 yr) or were killed when moribund, as compared to offspring of controls. Complete necropsies were performed. All urinary bladders and any organs with macroscopically visible abnormalities were examined histologically.	Schmähl and Habs (1980)
in utero Charles River CD rats	240M, 240F	48M, 48F	sodium saccharin, made by Remsen-Fahlberg method, 350 ppm *o*-toluenesulfonamide	0.01, 0.1, 1, 5, or 7.5% in diet	≤ 2 yr	Positive in males at highest dose. There was an increased incidence of urinary bladder tumors in F_1 males fed 7.5% sodium saccharin (7/23 vs. 1/29 controls). F_0 rats were fed test diet continuously from weaning through mating, and through gestation to weaning of their litters. Complete necropsies were performed. The urinary bladder, all tumors, and any grossly abnormal tissues were examined histologically.	Taylor et al. (1980)
6-wk-old ACI rats	48M	45M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Positive The incidence of urinary bladder transitional cell papilloma was significantly increased at 52 wk (9/32 vs. 0/28 controls, $p < 0.01$). Calculi were observed in 1 rat with bladder cancer and there was a higher level of urinary "crystals" in treated rats than in controls. The bladder, liver, and kidneys were the only tissues examined histologically. At least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda*.	Fukushima et al. (1983)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old F344 rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Negative No tumors were detected in bladder, liver, or kidneys.	Fukushima et al. (1983)
6-wk-old SD rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Negative No tumors were detected in bladder, liver, or kidneys.	Fukushima et al. (1983)
6-wk-old Wistar rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene sulfonamide] method of production not specified	5% in diet	52 wk	Negative No tumors were detected in bladder, liver, or kidneys.	Fukushima et al. (1983)
newborn SD rats	33M, 39F (low dose) 34M, 37F (high dose)	36M, 34F	sodium saccharin [0.0005% *o*-toluene sulfonimide] and sodium cyclamate, method of production not specified	2 or 5% sodium saccharin and sodium cyclamate in the diet (1:10 ratio)	lifetime (parents were also fed same dose)	Negative The mixture of sodium saccharin and sodium cyclamate was not carcinogenic at either dose. Detailed necropsies were performed, including evaluation of the urinary tract.	Schmähl and Habs (1984)
28- to 38-day-old Charles River CD rats	1%, 700M; 3%, 500M; 4%, 200M; 5%, 125M; 6.25%, 125M; 7.5%, 125M; 5% (through gestation), 125M; 5% (following gestation), 125M	350M	sodium saccharin, made by Maumee process, >99% pure	1.0, 3.0, 4.0, 5.0, 6.25, or 7.5% in diet (same dose used for parent and offspring)	30 mo	Positive Parents were exposed to same dose from 6 weeks of age. A clear dose response for urinary bladder tumors was observed in F_1 male rats (1.0%, 5/658; 3.0%, 8/472; 4.0%, 12/189; 5.0%, 15/120; 6.25%, 20/120; 7.5%, 37/118; all vs. 0/324 in controls). Female F_1 rats were not evaluated. Tumor incidence in rats exposed only to 5% sodium saccharin during gestation was similar to controls. 12/120 rats exposed to 5% sodium saccharin from birth for a single generation had bladder tumors. The urinary bladder, urethra, ureter, kidneys, and all gross lesions and tissue masses were examined histologically.	Schoenig et al. (1985)
6-wk-old analbuminemic and SD rats	35M analbuminemi 36M SD	12M analbuminemic, 14M SD	sodium saccharin, method of production and purity not specified	5% in diet	80 wk	Negative No bladder carcinomas or precancerous lesions were observed in any of the rats. Only the bladder was examined.	Homma et al. (1991)

Table 4-1. Mammalian Carcinogenicity (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.1.4 Nonhuman Primates							
rhesus monkeys (age not specified)	7M, 7F	3M, 3F	sodium saccharin, method of production not specified, 'purified'	20, 100, or 500 mg/kg/day in diet	79 mo	Negative Histopathological examination of urinary bladders, kidneys, and testis of surviving and deceased animals showed no abnormal pathology.	McChesney et al. (1977 abstr.; cited by IARC, 1980)
monkeys (4 unspecified strains)	10 total	0	sodium saccharin, method of production not specified, 'purified'	25 mg/kg/day in diet	9 yr	Negative Clinical observation revealed no gross neoplasia. This study was ongoing in 1980.	Sieber and Adamson (1978; cited by IARC, 1980)
0 to 1-yr-old monkeys (Cynomolgu sRhesus, and African Green)	20 total	0	sodium saccharin, method of production and purity not specified	25 mg/kg/day in diet	>20 yr	Negative Dose corresponds to 5 cans diet soda per day by 70 kg human. Five monkeys died from either varicella, pneumonia, or unknown reasons. No tumors were found in the dead or in any of the 15 surviving monkeys. Complete necropsies were performed on all animals that died. Various unspecified hematological and biochemical tests were routinely performed on survivors.	Thorgeirsson et al. (1994)
Cynomolgus and Rhesus monkeys (age not specified)	not specified	not specified	sodium saccharin, method of production and purity not specified	25 mg/kg/day in diet	17-23 yr	Negative Urine was analyzed during last year of life. There were no calculi, unusual crystals, increased crystalluria, or calcium phosphate precipitate in urine. There was no association between ingestion of sodium saccharin and urinary protein content. Urinary bladders were free of hyperplasia and tumors. There was no difference in appearance of urothelium in exposed and age-matched control monkeys. It was not specified whether other tissues were examined.	Cohen et al. (1996 abstr.)

Abbreviations: F = females; i.p. = intraperitoneally; M = males;

[a] No distinction was made between saccharin and its sodium salt in the IARC discussion

[b] No distinction was made between saccharin and its sodium salt

4.2 Initiation/Promotion and Co-Carcinogenicity Studies

Experimental details for the studies described in this section are presented in **Table 4-2.**

4.2.1 Benzo[*a*]pyrene (BP)

Saccharin did not enhance the incidence of tumors in the forestomach of mice exposed to a test diet containing 5% saccharin (for 72 wk) starting 7 days after an initial single gastric instillation of 0.2 mL polyethylene glycol containing 50 µg BP. No pathological changes were observed macroscopically in urinary bladders of saccharin-exposed mice (Roe et al., 1970). It was noted that BP is not organotropic for the bladder and that histological examination of the urinary bladders was not conducted (IARC, 1980).

4.2.2 *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)

Sodium saccharin, administered in the diet at 0.04, 0.2, 1, or 5% for 32 wk, did not produce any effects in 6-wk-old Charles River F344 rats that were not pretreated with BBN (0.01% in water for 4 wk). A sodium saccharin dose-dependent increase in papillary or nodular hyperplasia of the urinary bladder was statistically significant in females (1% and 5% sodium saccharin groups) and males (5% sodium saccharin group) in the BBN-exposed groups (Nakanishi et al., 1980a).

The effects of sequential administration (initiation/promotion protocol) of 0.01% BBN in drinking water and 5.0% sodium saccharin in feed and co-administration of 0.001% BBN in drinking water and 5.0% sodium saccharin in feed, were studied in 8-wk-old male Wistar rats by Nakanishi et al. (1980b). In the first experiment (sequential administration), rats received BBN for 4 wk and then sodium saccharin for an additional 32 wk. In the second experiment (co-administration), rats were fed BBN and sodium saccharin for 40 wk. When rats were administered BBN and sodium saccharin concurrently, there was an increased incidence of urinary bladder papilloma (10/24 vs. 0/12 in controls). Sequential administration produced a non-statistically significant increase (9/31 vs. 0/12 in controls) in the incidence of bladder papilloma. In addition, there was one transitional cell carcinoma among the 31 rats that received saccharin sequentially and two transitional cell carcinomas among the 24 rats receiving saccharin concurrently with BBN. Transitional cell hyperplasia was noted in rats receiving sodium saccharin alone as well as in groups receiving BBN before and during saccharin administration.

Nakanishi et al. (1982) reported that there was no statistically significant increase in the incidence of hepatocellular carcinoma or urinary bladder papilloma in male F344 rats (age not specified) initiated with 0.01% BBN in drinking water for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered BBN alone. Sodium saccharin significantly enhanced urothelial hyperplasia after BBN pretreatment and produced a non-statistically significant increase in urinary bladder papillomas (6/29 vs. 0/29 in controls).

The comparative tumor-promoting effects of 5% sodium saccharin, 5% sodium L-ascorbate, 5% L-ascorbic acid, 5% sodium saccharin plus sodium L-ascorbate, or 5% sodium saccharin plus L-ascorbic acid were studied in 6-wk-old male F344 rats. Rats were initiated with 0.05% BBN in drinking water for 4 wk and were then fed the test diets for an additional 32 wk. The authors found that bladder-cancer promotion by sodium saccharin was inhibited by L-

ascorbic acid and enhanced by sodium L-ascorbate, apparently as a function of urinary pH. Sodium saccharin alone, sodium L-ascorbate alone, and these two compounds in combination caused increased incidences of urothelial hyperplasia, papilloma, and carcinoma in the urinary bladder (Fukushima et al., 1990).

Yu et al. (1992) studied the tumor-promoting effects of sodium saccharin alone and in combination with nordihydroguaiaretic acid (an antioxidant and inhibitor of arachidonic acid metabolism) in BBN-initiated male F344 rats. BBN (0.05%) was administered to 6-wk-old rats in the drinking water for 4 wk. The rats were then fed 5% sodium saccharin with or without the antioxidant for an additional 36 wk. Nordihydroguaiaretic acid was coadministered at a concentration of 0.1% in the diet. The authors found that sodium saccharin promoted BBN tumorigenicity, while nordihydroguaiaretic acid plus sodium saccharin decreased the incidences of papilloma. Both groups receiving sodium saccharin had urothelial hyperplasia.

4.2.3 2-Acetylaminofluorene (AAF)

Nakanishi et al. (1982) reported that there was no significant increase in the incidence of hepatocellular carcinoma or urinary bladder carcinoma in male F344 rats (age not specified) initiated with 0.02% AAF in the diet for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered AAF alone. There was a statistically significant increase in urothelial hyperplasia in the sodium saccharin-promoted rats.

The effect of lifetime sodium saccharin dosing (0.1, 0.5, 1.0, or 5.0% diet for 117 wk), administered 2 wk after initiation with AAF (200 ppm diet for 13 weeks), on female weanling BALB/c mice was studied by Frederick et al. (1989). No dose-related increase in tumor incidence was found in initiated mice exposed to 0.1-5% sodium saccharin diet.

In female Horton SD rats (age not specified) co-administered 300 mg AAF/kg diet and 5% sodium saccharin in the diet for 40 wk, no animals developed malignant lesions of the urinary bladder. Eleven of the 12 AAF-treated rats (no sodium saccharin in diet) developed palpable mammary and ear-duct tumors, while 6/12 animals exposed to AAF and sodium saccharin developed these tumors. Liver tumors occurred in control and exposed animals (Ershoff and Bajwa, 1974; cited by IARC, 1980). IARC (1980) noted that the small number of animals used and the fact that food consumption was not measured prevented the evaluation of AAF and sodium saccharin exposure.

4.2.4 *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT)

The effects of sodium saccharin in FANFT-initiated (0.2% diet for 6 wk) 4-wk-old male F344 rats were studied by Cohen et al. (1979). Subsequent to initiation with FANFT, rats were exposed to a 5% sodium saccharin diet (*o*-toluenesulfonamide free) for up to 83 wk. Two other groups received *o*-toluenesulfonamide-free sodium saccharin either with or without FANFT initiation following a 6-wk no-exposure period. The incidence of bladder cancer was not increased in the sodium saccharin-only group (0/20) when compared to the no-exposure control group (0/42). In the FANFT-initiated control group, 4/20 rats developed bladder cancer and 1/20 rats developed bladder papilloma. In the FANFT plus sodium saccharin groups with or without a 6-wk no-exposure period, the incidences of bladder cancer were 13/18 and 18/19, respectively.

Fukushima et al. (1981) fed 5-wk-old male F344 rats 0.2% FANFT diet for only a 4-wk initiation period in order to decrease the production of bladder cancer in the FANFT-only group. Rats were subsequently fed a 5% sodium saccharin or control diet for 100 wk. There was a significant increase ($p < 0.03$) in the incidence of carcinoma of the bladder as compared to FANFT-only controls (5/26 vs. 0/25).

Murasaki and Cohen (1983a) evaluated the co-carcinogenicity of FANFT (0.005% diet) and sodium saccharin (5% diet) administered to 5-wk-old male Fischer rats for 2 yr. The authors reported that the incidence of bladder lesions was marginally significant ($p < 0.06$), when compared to the incidence in FANFT-only controls (5/16 vs. 0/11). There were no statistically significant increases in tumor incidences for other tissues.

Imaida and Wang (1986) studied sodium saccharin as a promoter in a two-stage carcinogenesis model. Groups of 42 or 43 male weanling F344 rats were exposed to 5% sodium saccharin in AIN-76A diet for 100 wk subsequent to a 4-wk regimen of exposure to either 0.2% FANFT in Wayne diet or 0.005% *N,N*-dibutylnitrosamine (DBN) in drinking water and control Wayne diet. None of the control rats fed sodium saccharin alone developed bladder, liver, esophageal, or forestomach tumors. There was no statistically significant increase in the incidence of tumors of the bladder, liver, or esophagus in rats initiated with FANFT or DBN, with a subsequent dietary administration of sodium saccharin, as compared to FANFT-only and DBN-only controls, respectively. However, the group receiving FANFT initiation followed by sodium saccharin promotion did have an increased incidence of urinary bladder carcinomas ($p = 0.059$).

The comparative effects of different chemical forms of saccharin and ascorbate in conjunction with other chemicals that would affect the urinary ionic composition and pH were studied by Cohen et al. (1991b). Rats (5-wk-old male F344) were exposed to 0.02% FANFT or control diet for 6 wk. Subsequent to administration of FANFT, rats were exposed to 3 or 5% sodium saccharin, 3.12 or 5.2% calcium saccharin, 2.53 or 4.21% acid saccharin, 4.44% ascorbic acid, or 5% sodium ascorbate diet for 72 wk. Carcinomas and papillomas developed in 12/39 (31%) and 5/39 (13%) rats, respectively, in the FANFT-only group. A statistically significant increased incidence of tumorigenesis occurred in all of the other groups, with the exception of acid saccharin, ascorbic acid, and low-dose calcium saccharin. Sodium saccharin > sodium ascorbate > calcium saccharin for enhancement of bladder tumorigenesis; none of the forms of saccharin were tumorigenic without FANFT initiation. The authors found that an elevated urinary pH increased tumorigenicity. However, elevated urinary sodium concentrations are sufficient, as shown by the enhancement of bladder tumor promotion by sodium saccharin and sodium ascorbate, and by the enhanced bladder tumorigenicity of calcium saccharin when sodium chloride was added to the calcium saccharin exposure. Masui et al. (1991) analyzed the tumors in this study for H-*ras* mutations by Western blotting using a monoclonal antibody against p21. H-*ras* mutations were found in 2/3 and 3/6 bladder tumors from rats exposed to FANFT alone, and 4/20 and 1/10 H-*ras* mutations were found in tumors from rats exposed to FANFT initiation with 3 or 5% sodium saccharin promotion, respectively.

Okamura et al. (1991) compared the Prolab 3200 with the AIN-76A diet for the promoting effects of sodium saccharin and found that male F344 rats on Prolab 3200 diet exhibited sodium saccharin (5% diet for 100 wk) enhancement of bladder tumors when initiated

for 4 wk with 0.2% FANFT. This effect was not found in the AIN-76A-fed rats initiated with FANFT and fed 5% sodium saccharin for 100 wk.

4.2.5 *N*-Methyl-*N*-nitrosourea (MNU)

A series of reports on studies conducted by Hicks et al. (1973, 1975) and Hicks and Chowaniec (1977) evaluated sodium saccharin (2-yr exposure) following intravesicular instillation of MNU (single dose of 1.5 or 2 mg) in 6- to 8-wk-old Wistar rats. In 138 rats in the male and female 4 g/kg/day dietary sodium saccharin-only group, 3 bladder tumors were found. Administration of 2 g/kg/day of sodium saccharin in drinking water for two yr did not produce any bladder tumors in male and female Wistar rats. Bladder tumors were found in 23/49 (47%) female rats in an MNU plus 2 g/kg/day dietary sodium saccharin group. Bladder tumor incidence was increased in the MNU plus 4 g/kg/day sodium saccharin female group (27/47; 57%).

In an effort to reproduce the experiments of Hicks et al. (1973, 1975) and Hicks and Chowaniec (1977), Mohr et al. (1978) instilled 2 mg MNU in the bladders of female Wistar/AF-Han rats which were subsequently fed 2% sodium saccharin for the first 10 wk and 4% afterwards [up to 2 yr] (specific dosing regimen not specified). In the MNU-only group, bladder tumors were found in 19/49 (39%) rats; and ureter tumors were found in 8/49 (17%) rats, while 14/49 (28%) rats developed renal pelvis tumors. In the MNU plus sodium saccharin group, incidences of renal pelvis, ureter, and bladder tumors were 43, 11, and 39%, respectively. The high incidence of tumors in the MNU-only group was explained by the original authors as a result of the use of MNU within 15 min of dissolution and the assumption that in their experiment the dose of MNU was not subcarcinogenic.

Hooson et al. (1980) studied the contribution of the sodium saccharin contaminant *o*-toluenesulfonamide in the promotion of MNU-initiated bladder carcinogenesis in female Wistar rats (age not specified). No statistically significant differences were found in bladder tumor incidence with administration of a single 0.15 mL-dose of MNU, followed 2 wk later by daily administration in drinking water or diet of either 2 g/kg *o*-toluenesulfonamide-free sodium saccharin or 2 g/kg sodium saccharin containing 40 mg/kg *o*-toluenesulfonamide for 2 yr, as compared to a control group given MNU alone. There was, however, a decrease in the latency period in the MNU+sodium saccharin treated groups (55 and 52 wk vs. 87 wk for MNU-only controls).

West et al. (1986) exposed 8-wk-old female Sprague-Dawley rats, which had previously been dosed with a single dose of MNU or by saline transurethral instillation into the bladder, to 0.1, 0.5, 1.0, 2.5, or 5% sodium saccharin in the diet. Other groups received MNU followed by 2% sodium saccharin in water or 5% acid saccharin diet. Sodium saccharin dosing was initiated 2 days after rats were dosed with MNU and continued until the termination at 102 wk. In MNU-exposed rats, histopathological examination revealed papillomas and carcinomas of the urinary bladder. A mortality-adjusted increase in tumor incidence and a decrease in time-to-tumor with increasing sodium saccharin dose for the 0-2.5% doses in dead and moribund rats was reported. A statistically significant increase in bladder tumor prevalence ($p < 0.0012$) was found for the group of rats exposed to 2.5% sodium saccharin plus MNU vs. the MNU-only control group. The greatest number of tumors developed in rats that received four doses of MNU alone throughout the experiment. Rats not exposed to MNU that were dosed with a 0.1-5% sodium

saccharin or 5% acid saccharin diet or 2% sodium saccharin in the drinking water developed a small number of tumors that were not significantly different from controls.

In the bladders of female Sprague-Dawley rats exposed to 1.0, 2.5, or 5.0% dietary sodium saccharin (given 4 wk immediately preceding, following, or centered on the day of bladder instillation of 0.5mg MNU), MNU-induced tumorigenesis was not enhanced (West et al., 1994). After the 4-wk administration of dietary sodium saccharin, rats were maintained on control diet. Additional groups of rats were dosed neonatally in the milk by administration of the same three levels of dietary saccharin to the dams during three wk of lactation. These latter groups then received MNU by bladder instillation at 8 wk of age and remained on the control diet for up to 106 wk of age. This neonatal exposure to saccharin did not enhance MNU-induced bladder tumors.

4.2.6 Freeze Ulceration

Five-wk-old male F344 rats had their bladder cells initiated with application of a steel rod frozen in dry ice and acetone (freeze ulceration). Rats were subsequently fed a control diet for two wk and then a sodium saccharin diet for 102 wk. This treatment resulted in 5/20 (25%) carcinomas and 1/20 (5%) papillomas compared with none when rats were exposed to either dosage regimen (freeze ulceration or sodium saccharin diet) alone. When 0.2% FANFT was administered in the diet for 2 wk after freeze ulceration followed by 5% sodium saccharin diet for 102 wk, 4/23 (17%) of the bladders had tumors. Reversing the order of FANFT and freeze ulceration exposure resulted in an 8/22 (36%) incidence of bladder tumors. Tumors were not found in rats that had received FANFT or sodium saccharin alone (Cohen et al., 1982).

Hasegawa et al. (1985) fed 5-wk-old male F344 rats 5% sodium saccharin diet either immediately or 2, 4, 6, or 18 wk following freeze ulceration of the bladder. There was a significant increase in the incidence of transitional cell carcinoma of the bladder in all of these groups as compared to a freeze ulceration-only control group, except in the group fed sodium saccharin 2 wk after freeze ulceration (11/36, 6/36, 12/40, 7/36, 9/39 vs. 1/39 in controls). No bladder carcinoma was detected in control rats administered saccharin alone.

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.1 Benzo[*a*]pyrene (BP)							
Swiss mice (age not specified)	50F	50F	saccharin[a], method of production and purity not specified	5% diet	18 mo	Negative Animals were gavaged with a single 0.2 mL dose of polyethylene glycol, either alone or containing 50 µg BP. Seven days after BP treatment, exposure to saccharin was begun. BP induced an increased incidence of tumors of the forestomach but saccharin did not enhance this increase. No pathological changes were observed macroscopically in urinary bladders of saccharin-exposed mice. IARC (1980) noted that BP is not organotropic for the bladder and that a histological examination of the urinary bladders was not done.	Roe et al. (1970)
4.2.2 *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)							
6-wk-old Charles River F344 rats	30M, 31F	30M, 31F	sodium saccharin	0.04, 0.2, 1, or 5% diet	32 wk	Positive Rats were preexposed to 0.01% BBN in water for 4 wk. Sodium saccharin did not produce any effects in rats that were not preexposed to BBN. In the BBN exposed groups, a sodium saccharin dose-dependent increase in papillary or nodular hyperplasia of the urinary bladder achieved statistical significance in females (1% and 5% sodium saccharin) and males (5% sodium saccharin).	Nakanishi et al. (1980a)
8-wk-old Wistar rats	40M (BBN/ sodium saccharin)	24M (BBN alone) 24M (sodium saccharin alone) 18M (no chemicals)	sodium saccharin, >99.5% pure, 7 ppm *o*-toluenesulfon-amide	sodium saccharin: 5% diet 0.01% in drinking water	Rats pretreated with BBN for 4 wk and then given sodium saccharin for 32 wk	Negative for urinary bladder cancer There was no statistically significant increase in the incidence of urinary bladder cancer. There was an increased incidence of urinary bladder papillomas (10/24 vs. 0/12 in controls).	Nakanishi et al. (1980b)
F344 rats (age not specified)	31M	30M (BBN alone)	sodium saccharin, 7 ppm *o*-toluenesulfonamide; method of production and purity not specified	0.01% BBN in drinking water for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Negative There was no significant increase in the incidence of hepatocellular carcinoma or urinary bladder papilloma.	Nakanishi et al. (1982)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old F344 rat	15-16M/ dose group	15-16M (BBN alone)	sodium saccharin sodium L-ascorbate L-ascorbic acid sodium saccharin plus sodium L-ascorbate sodium saccharin plus L-ascorbic acid Methods of production and purities not specified	5% diet 5% diet 5% diet 5% diet 5% diet	32 wk (All rats were administered drinking water containing 0.05% BBN for 4 wk and were then given test diet or control diet for an additional 32 wk)	Positive with BBN pretreatment. When administered individually following BBN initiation, sodium saccharin and sodium L-ascorbate significantly increased the incidence of urinary bladder hyperplasia (14/15 and 15/16), papilloma (9/15 and 13/16), and carcinoma (5/15 and 11/16) versus BBN controls (4/15, 4/15, and 0/15). Co-administration of sodium saccharin and sodium L-ascorbate also significantly increased the incidences of bladder hyperplasia, bladder papilloma, and bladder carcinoma. These increased incidences were accompanied by increases in urinary sodium ion concentration and pH. Co-administration of sodium saccharin and L-ascorbic acid caused a decrease in urinary pH and no change in urinary sodium ion levels, and did not increase the incidence of hyperplasia, papilloma, or carcinoma.	Fukushima et al. (1990)
6-wk-old F344 rats	23M (BBN + sodium saccharin) 23M (BBN + sodium saccharin + nordihydro-guaiaretic acid	11M (sodium saccharin alone) 11M (sodium saccharin + nordihydro-guaiaretic acid) 11M (nordihydro-guaiaretic acid alone) 20M (BBN alone) 20M (BBN + nordihydro-guaiaretic acid	sodium saccharin nordihydroguaiaretic acid BBN Methods of production and purity not specified	5% diet 0.1% diet 0.05% in drinking water	36 wk	Positive for tumor promotion with 4-wk BBN pretreatment. Incidences of urinary bladder urothelial hyperplasia and papilloma were increased in sodium saccharin-treated rats versus controls. Incidences of papillary or nodular hyperplasia and papilloma were decreased by nordihydroguaiaretic acid (antioxidant; inhibitor or arachidonic acid metabolism) alone or in combination with sodium saccharin compared with sodium saccharin alone.	Yu et al. (1992)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.3 2-Acetylaminofluorene (AAF)							
21- to 26-day-old BALB/c mice	saccharin dose in parentheses 192F (0.1%) 192F (0.5%) 144F (1.0%) 96F (5.0%)	192F (AAF alone)	sodium saccharin, >98% pure, method of production not specified.	sodium saccharin: 0, 0.1, 0.5, 1.0 or 5.0% diet AAF: 200 ppm diet	13 wk initiation with AAF 2 wk control diet; 117 wk sodium saccharin diet (132 wk total)	Negative Sodium saccharin had no effect on the urinary bladder tumorigenic response of initiated mice. An increased trend (p=0.04) of Harderian gland neoplasms was not considered to represent a positive tumorigenic response.	Frederick et al. (1989)
Horton SD rats (age not specified)	62F	62F	sodium saccharin, method of production and purity not specified	5% diet	40 wk	Negative for tumorigenesis with co-administration of AAF All animals were fed 300 mg AAF/kg diet for the duration of the study. Eleven of the 12 controls (no sodium saccharin in diet) developed palpable mammary and ear-duct tumors, while 6/12 animals exposed to AAF and sodium saccharin developed these tumors. Liver tumors occurred in control and exposed animals. No animals had malignant lesions of the urinary bladder. IARC noted the small number of animals used and the fact that food consumption was not measured, preventing the evaluation of AAF and sodium saccharin exposure.	Ershoff and Bajwa (1974; cited by IARC, 1980)
F344 rats (age not specified)	31M	30M (AAF alone)	sodium saccharin, 7 ppm *o*-toluenesulfonamide; method of production and purity not specified	0.02% AAF in diet for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Negative There was no significant increase in the incidence of hepatocellular carcinoma or urinary bladder papilloma. There was a statistically significant increase in urothelial hyperplasia in the sodium saccharin-promoted rats.	Nakanishi et al. (1982)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.4 *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT)							
4-wk-old Fischer rats	1) 20M 2) 20M	3) 20M (sodium saccharin alone) 4) 20M (FANFT alone) 5) 42M (no exposure)	sodium saccharin (*o*-toluenesulfonamide free) Method of production and purity not specified	1) 0.2% FANFT for 6 wk followed by 5% sodium saccharin diet 2) 0.2% FANFT for 6 wk followed by 6-wk no-exposure period, followed by 5% sodium saccharin diet 3) 5% sodium saccharin diet alone 4) FANFT initiation alone 5) no exposure	83 wk	Positive with FANFT initiation Sodium saccharin was negative for bladder tumorigenesis when administered alone. Incidence of bladder cancer in groups 1, 2, 3, 4, and 5 were as follows: 18/19, 13/18, 0/20, 4/20, and 0/42, respectively.	Cohen et al. (1979)
5-wk-old F344 rats	26M	25M	sodium saccharin, method of production and purity not specified.	FANFT: 0.2% diet sodium saccharin: 5% diet	FANFT for 4 wk; sodium saccharin for 100 wk	Positive with FANFT pretreatment There was a significant increase ($p < 0.03$) in the incidence of carcinoma of the bladder as compared to FANFT-only controls (5/26 vs. 0/25).	Fukushima et al. (1981)
5-wk-old Fischer rats	20M	20M	sodium saccharin FANFT Method of production and purity not specified	5% diet 0.005% diet	2 yr	Equivocal The incidence of bladder lesions was marginally significant ($p < 0.06$), when compared to the incidence in FANFT-only controls (5/16 vs. 0/11). There were no statistically significant increases in tumor incidences for other tissues.	Murasaki and Cohen (1983a)
weanling F344 rats (age not specified)	42M (sodium saccharin and FANFT) 42M (sodium saccharin and DBN)	42M (FANFT alone) 43M (DBN alone) 42M (sodium saccharin alone)	sodium saccharin Method of production and purity not specified	4-wk exposure to either 0.2% FANFT in Wayne diet or 0.005% *N,N*-dibutylnitrosamine (DBN) in drinking water and control Wayne diet, followed by 5% sodium saccharin in AIN-76A diet for 100 wk	see dose	Negative None of the control rats fed sodium saccharin alone developed bladder, liver, or esophageal tumors. There was no statistically significant increase in the incidence of tumors of the bladder, liver, or esophagus in rats initiated with FANFT or DBN and subsequently promoted with sodium saccharin, as compared to FANFT-only and DBN-only controls, respectively. There was a non-statistically significant increase ($p=0.059$) in urinary bladder carcinomas in FANFT-initiated, sodium saccharin-promoted rats.	Imaida and Wang (1986)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old F344 rats	240M 160M 120M	40M[b] 40M[b] 40M[b] [b]shared controls	sodium saccharin calcium saccharin acid saccharin ascorbic acid sodium ascorbate	3 or 5% diet 3.12 or 5.2% diet 2.53% diet 4.44 % diet 5 % diet	72 wk	Positive with FANFT pretreatment. Without FANFT initiation, sodium saccharin, calcium saccharin, and acid saccharin were non-tumorigenic. With a 6 wk period of FANFT initiation, sodium saccharin was tumorigenic at the 5 and 3% dose levels. Calcium saccharin was slightly tumorigenic but not in a dose response manner. Acid saccharin was not tumorigenic. One of 2 diets was fed: Prolab 3200 or NIH-07. Urinary pH of rats fed Prolab was higher and sodium saccharin promoted more bladder tumors in these rats.	Cohen et al. (1991b)
5-wk-old F344 rats	240M 160M 120M	40M[b] 40M[b] 40M[b] [b]shared controls	sodium saccharin calcium saccharin acid saccharin ascorbic acid sodium ascorbate	3 or 5% diet 3.12 or 5.2% diet 2.53% diet 4.44 % diet 5 % diet	72 wk	Tumors were analyzed for H-*ras* mutations by Western blotting using a monoclonal antibody against p21. H-*ras* mutations were found in 2/3 and 3/6 bladder tumors from rats exposed to FANFT alone, and 4/20 and 1/10 H-*ras* mutations were found in tumors from rats exposed to FANFT initiation with 3 or 5 % sodium saccharin promotion, respectively.	Masui et al. (1991)
5-wk-old F344 rats	30M (FANFT + sodium saccharin)	30M (FANFT alone) 25M (sodium saccharin alone)	sodium saccharin, method of production and purity not specified	5% AIN-76A diet	100 wk	Negative Sodium saccharin did not promote bladder cancer in rats initiated for 4 wk with 0.2% FANFT and fed AIN-76A diet. This was probably due to the low urinary pH of rats fed AIN-76A diet.	Okamura et al. (1991)
5-wk-old F344 rats	30M (FANFT + sodium saccharin)	30M (FANFT alone)	sodium saccharin, method of production and purity not specified	5% Prolab diet	100 wk	Positive with FANFT pretreatment. Rats initiated with 0.2% FANFT for 4 wk and fed Prolab diet containing sodium saccharin had an increased incidence of bladder tumors, as compared to FANFT-controls (40% vs. 17% incidence of bladder tumors, respectively). Sodium saccharin was not administered alone in Prolab diet.	Okamura et al. (1991)
4.2.5 *N*-Methyl-*N*-nitrosourea (MNU)							
6- to 8-wk old Wistar rats	M and F (number used varied between reports)	M and F (number used varied between reports)	sodium saccharin, method of production and purity not specified	2 or 4 g/kg bw/day in diet or 2 g/kg bw/day in drinking water	2 yr	Positive with MNU pretreatment and dietary administration of sodium saccharin. Bladder tumor incidences were as follows: 3/138 in male and female 4 g/kg bw/day sodium saccharin-only group, 23/49 (47%) rats in the MNU plus 2 g/kg bw/day sodium saccharin female group, and 27/47 (57%) in the MNU plus 4 g/kg bw/day sodium saccharin female group. Administration of 2 g/kg bw/day of sodium saccharin in drinking water did not produce bladder tumors in either sex.	Hicks et al. (1973) preliminary report; Hicks et al., 1975); Hicks and Chowaniec (1977; cited by Whysner and Williams, 1996)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
Wistar/AF-Han rats (age not specified)	F (number not specified), instilled with 2 g MNU in the bladder	F (number not specified)	sodium saccharin, method of production and purity not specified	2% sodium saccharin in the diet for the first 10 wk and 4% afterwards	≤ 2 yr	Negative for increase following MNU pretreatment In the MNU-only group, bladder tumors were found in 19/49 (39%) rats; and ureter tumors were found in 8/49 (17%) rats, while 14/49 (28%) rats developed renal pelvis tumors. In the MNU plus sodium saccharin group, incidences of renal pelvis, ureteran and bladder tumors were 43, 11 and 39% respectively. The high incidence of tumors in the MNU-only group was explained by the original authors as a result of the use of MNU within 15 min of dissolution.	Mohr et al. (1978)
Wistar rats (age not specified)	63F (MNU + sodium saccharin containing 40 mg/kg *o*-toluene-sulfonamid) 63F (MNU + sodium saccharin free of *o*-toluene-sulfonamid)	63F (MNU alone)	MNU sodium saccharin prepared by the Remsen-Fahlberg method, containing 40 mg/kg *o*-toluene-sulfonamide sodium saccharin prepared by the Maumee process, no *o*-toluenesulfonamide	0.15 mL instilled into bladder 2 g/kg/day in drinking water 2 g/kg/day in drinking water 2 g/kg/day in diet	single dose 2 yr (started 2 wk after MNU) 2 yr (started 2 wk after MNU) 2 yr (started 8 days after MNU)	Negative There was no increase in tumor incidence in rats administered *o*-toluene-sulfonamide-free sodium saccharin or in rats administered *o*-toluene-sulfonamide-free sodium saccharin, as compared to the MNU-only control group, but the latency period was shorter (55 and 52 wk vs. 87 wk for controls).	Hooson et al. (1980)
8-wk-old SD rats	960F 120F 120F	240F[b] 240F[b] 240F[b] [b] shared controls	sodium saccharin sodium saccharin acid saccharin Methods of production and purities not specified	0.1, 0.5, 1, 2.5, or 5% diet 2% drinking water 5% diet	102 wk	Positive with MNU pretreatment with dietary administration of sodium saccharin. Rats were given either a single dose (300 µL) of saline or an initiating dose (0.5 mg/300 µL saline) of MNU, a potent direct acting carcinogen, via trans-urethral instillation. A significant increase in the incidence of benign papillomas was seen in MNU-pretreated rats when fed 0.1-2.5% sodium saccharin. Rats which received 5% sodium saccharin had a benign papilloma incidence similar to controls. Acid saccharin also required MNU initiation for production of tumors. Sodium saccharin administered in drinking water was not as effective in producing tumors as was sodium saccharin administered in the diet.	West et al. (1986)
8-wk-old Sprague-Dawley rats	30F (saccharin alone) 60F (saccharin plus MNU)	78F	sodium saccharin, method of production and purity not specified	up to 5% given 4 wk before, during or after MNU initiation; rats then fed control diet	112 wk	Negative MNU-induced tumorigenesis was not enhanced by the 4-wk sodium saccharin exposure	West et al. (1994)

Table 4-2. Initiation-Promotion and Co-Carcinogenicity Studies (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
4.2.6 Freeze Ulceration							
5-wk-old F344 rats	M (number not specified)	not specified	sodium saccharin, method of production and purity not specified	5%	102 wk	Positive with freeze ulceration pretreatment. In rats with freeze ulceration initiation followed by saccharin diet, 5/20 (25%) had carcinomas or papillomas. FANFT pretreatment for 2 wk after freeze ulceration and subsequent sodium saccharin exposure resulted in 4/23 (17%) incidences of bladder tumors. Reversing order of FANFT and freeze ulceration exposure resulted in an 8/22 (36%) incidence of bladder tumors. Tumors were not found in rats that received FANFT, sodium saccharin, or freeze ulceration alone.	Cohen et al. (1982)
5-wk-old F344 rats	40M per group (freeze ulceration followed 0, 2, 4, 6, or 18 wk later with sodium saccharin)	40M (sodium saccharin alone) 40M (freeze ulceration alone) 40M (no treatment)	sodium saccharin, method of production and purity not specified	5% diet	104 wk total (saccharin was administered either immediately after freeze ulceration or after 2, 4, 6, or 18 wk)	Positive with freeze ulceration pretreatment. There was a significant increase in the incidence of transitional cell carcinoma of the bladder in rats subjected to freeze ulceration and then fed sodium saccharin either immediately or 4, 6, or 18 wk later, as compared to freeze ulceration-only control (11/36, 6/36, 12/40, 7/36, 9/39 vs. 1/39 in controls). The increase was not significant in rats fed sodium saccharin 2 wks after freeze ulceration. None of the saccharin-only or no-treatment control rats developed bladder carcinoma.	Hasegawa et al. (1985)

Abbreviations: F = females; M = males
[a] No distinction was made between saccharin and its sodium salt in the IARC discussion
[b] No distinction was made between saccharin and its sodium salt

5.0 GENOTOXICITY

Extensive reviews of the genotoxicity of saccharin have been conducted by Ashby (1985), IARC (1980, pp. 148-150, see Appendix A; 1982, see Appendix B; 1987b, see Appendix C), and, most recently, by Whysner and Williams (1996). The studies summarized below are largely based on these reviews; additional, relevant studies are presented in **Table 5-1**, while the Genetic Activity Profiles published by IARC (1987a) for saccharin are provided in **Figures 5-1** and **5-2.**

Most of the numerous *in vitro* and *in vivo* genotoxicity studies on sodium saccharin have been negative with occasional inconsistent or conflicting results and false positive results attributed to factors such as mutagenic impurities, inhibition of DNA synthesis, and osmotic effects.

5.1 Noneukaryotic Systems

5.1.1 Gene Mutations

Both sodium saccharin and saccharin (form unspecified) have been reported as negative in 15 studies for the induction of reverse mutations in *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535, TA1537, and TA1538 (not all strains were tested in all studies), with and without S9 activation, and using either the plate incorporation or pre-incubation forms of the assay.

In a study that evaluated the induction of reverse mutations in *S. typhimurium* by 4 commercially available saccharin samples and by 1 highly purified saccharin sample in the presence and absence of S9, the commercially produced samples were positive for mutation induction, whereas the highly purified sample was negative (Batzinger et al., 1977). The author concluded that commercial saccharin samples contain mutagenic impurities.

5.1.2 DNA Damage

Saccharin (form unspecified) was reported as negative for the induction of prophage (Rossman et al., 1991) and DNA damage/SOS repair in *Escherichia coli* (DeFlora et al., 1984).

5.1.3 DNA Synthesis

Saccharin did not alter DNA synthesis, as measured by the incorporation of [^{3}H]thymidine, in *S. typhimurium* (Beljanski et al., 1982).

5.2 Lower Eukaryotic Systems

5.2.1 *Saccharomyces cerevisiae*

Sodium saccharin without metabolic activation was reported to be positive in the yeast, *Saccharomyces cerevisiae*, for the induction of aneuploidy, gene conversion, and reverse mutations. However, in two other yeast studies (including one conducted using a 9-fold higher dose), saccharin (form unspecified) was negative for gene conversion and mutation induction but positive again for the induction of aneuploidy.

5.2.2 *Drosophila melanogaster*

Sodium saccharin was initially found in a 1971 study to be positive for the induction of sex-linked recessive mutants and negative for heritable translocations in *Drosophila*

melanogaster. However, two subsequent studies conducted using equal or higher doses reported weak positive or negative results for the induction of sex-linked recessive mutations.

5.2.3 Higher Plants

Ma et al. (1995) concluded that saccharin induced micronuclei in the root-tips of *Allium cepa* (onions) and *Vicia fava* (beans) following a 6-hour exposure at 120 mM.

5.3 Mammalian Systems *In Vitro*

5.3.1 Gene Mutations

In two studies, sodium saccharin was reported to be weakly mutagenic in mouse lymphoma L5178Y cells at very high doses (>10 mg/mL) and only in the presence of metabolic activation. A third study using doses as high as 20 mg/mL up was negative. Sodium saccharin, at doses above 10 mg/mL, was also reported to induce a highly significant increase in the number of ouabain-resistant mutants in human RSa embryo cells (Suzuki and Suzuki, 1988), and to increase the number of mutations at the k-*ras* gene, codon 12 in SW480 human colon adenocarcinoma cells (Suzuki and Suzuki, 1993). However, based on either the weakness of the response and/or the magnitude of the doses required to elicit a positive response, these data would be considered to be of questionable value using current testing practices.

5.3.2 DNA Damage

Sodium saccharin (without metabolic activation) was weakly positive or positive for the induction of sister chromatid exchanges (SCE) in three studies using Chinese hamster cells and in two studies using human lymphocytes. Sodium saccharin and saccharin, in the absence of metabolic activation, were reported to be negative for SCE induction in one study using mouse embryo fibroblasts and in two studies using human lymphocytes. Studies with metabolic activation were either not conducted or were negative for SCE induction. In the positive studies, the doses capable of inducing a significant increase in SCE ranged from 1 to 12 mg/mL and the maximum increase in SCE was generally less than two-fold. As discussed by Ashby (1985) and based on our current appreciation of the various processes involved in SCE induction, this increase in SCE more likely reflects the ability of saccharin at high doses to inhibit DNA synthesis rather than an ability to cause DNA damage.

5.3.3 Inhibition of DNA Repair

Skare and Wong (1985) reported that sodium saccharin did not inhibit the repair of UV-induced DNA damage in WI-38 human fetal lung fibroblasts.

5.3.4 DNA Synthesis

Yanagisawa et al. (1987) and Heil and Reifferscheid (1992) both concluded that sodium saccharin at relatively high doses inhibited the rate of DNA synthesis, as measured by incorporation of [^{3}H]thymidine after treatment, in human B-32 fibroblasts or HeLa S3 cells.

5.3.5 Chromosomal Damage

Sodium saccharin was found to be positive without S9 activation in ten studies and negative in two studies for the induction of chromosome aberrations using Chinese hamster cells and human lymphocytes. Ashby (1985) and Whysner and Williams (1996) concluded that the high dose levels used (up to 48 mg/mL) may have caused osmotic changes leading to false positive results.

5.3.6 Cell Transformation

Saccharin (form unspecified) was found to give negative results for cell transformation in BALB/c3T3 and C3H 10T1/2 mouse and RLV Fischer rat embryo cells. Sakai and Sato (1989) also reported that sodium saccharin did not increase the number of transformed foci in BALB/3T3 cells with or without a 2-week promotion period with TPA.

5.4 Mammalian Systems *In Vivo*

5.4.1 Gene Mutations and Dominant Lethal Mutations

In a study that compared the mutagenic activities of 3 commercially available saccharin samples with a highly purified saccharin sample, Batzinger et al. (1977) administered 2.5 g saccharin/kg to mice (strain not specified) and collected 24-hour urine samples. The urine samples were then assayed for mutagenicity in *S. typhimurium* strains TA98 and TA100 in the presence and absence of S9 and the enzyme β-glucuronidase. In strain TA98, all commercial samples were positive for the induction of reverse mutations, but the purified sample was negative. In strain TA100, all samples were positive. Mutagenic activities of the urine was enhanced in TA98 by β-glucuronidase. In TA100, mutagens were inactivated by S9; in TA98, mutagens were activated by S9. The authors proposed that 2 mutagenic substances were present. A similar study using TA 98, TA 100 and TA 1537 performed on urine obtained from rats treated with 5% dietary sodium saccharin, Hasegawa et al. (1984) failed to show mutagenic activity after 0, 1, 5, or 14 days of treatment.

Batzinger et al. (1977) also conducted a host-mediated assay for the induction of reverse mutations by the 4 saccharin samples (3 commercially available, 1 highly purified). *S. typhimurium* strain TA98 or TA100 was incubated for 6 hours in the peritoneal cavity of mice administered 2.5 g saccharin/kg. The highly purified sample was negative for mutation induction in both bacterial strains. All of the commercially available samples were positive, except for one sample that was negative when incubated with strain TA98.

Both negative and positive results were obtained for sodium saccharin in the mouse spot test, examining somatic cell mutations induced in pup coat color. In his review of the literature, Ashby (1985) reported that the difference may have been due to the different routes of exposure (i.p. vs. orally, respectively) and the 7.5-fold higher oral dose levels in the positive study.

As compiled by Ashby (1985), IARC (1987a,b) and Whysner and Williams (1996) and discussed by Adler and Ashby (1989), saccharin has given conflicting results in the mouse dominant lethal mutation assay, with three positive and three negative studies for sodium saccharin. The strain of mice and the route of exposure were often the same and the doses often

overlapped for both negative and positive studies. The authors of the review articles concluded that the *in vivo* mutagenic ability of saccharin has not been adequately demonstrated.

5.4.2 DNA Damage

Sodium saccharin, when administered orally at doses between 5 and 10 g/kg, was reported to induce up to a two-fold increase in SCE in Chinese hamster bone marrow cells (Renner, 1979). Dropkin et al. (1985) reported that sodium saccharin at doses up to 25 mg/kg/day did not cause sister chromatid exchanges in the fetal pups of female ICR albino mice dosed on the 10th day of gestation and sacrificed on the 17th day.

5.4.3 Chromosomal Aberrations

In the reviews conducted by Ashby (1985), IARC (1987a,b), and Whysner and Williams (1996), both sodium saccharin and saccharin (form unspecified) were reported as negative for the induction of chromosomal damage in somatic and germ cells of rodents in seven studies and positive in somatic and germ cells in one study each. Dropkin et al. (1985) also reported that sodium saccharin at doses up to 2000 mg/kg did not cause chromosome aberrations in the fetal pups of female ICR albino mice dosed on the 10th day of gestation and sacrificed on the 17th day.

5.4.4 Induction of Micronuclei

Sodium saccharin was reported in two studies as negative for micronucleus induction in mouse bone marrow cells.

Table 5-1. Summary of Saccharin Genotoxicity Studies

System	Biol. Endpoint	S9 Metab. Activation	Chemical Form and Purity	Dose-Response; Doses Used	Endpoint Response	Comments	Reference
5.1 Noneukaryotic Systems							
5.1.1 Gene Mutations							
Salmonella typhimurium strains TA98 and TA100	Induction of reverse mutations	+/-	saccharin (4 commercially available samples and 1 highly purified sample); n.p.	No; up to 40 mg/plate (commercially available samples) or up to 80 mg/plate (highly purified sample); incubation time not specified	positive (commercially available samples) negative (highly purified sample)	There was considerable variation in mutagenic activity among the 4 commercially available samples.	Batzinger et al. (1977)
S. typhimurium strains TA98, TA100, and TA1537	Induction of reverse mutations	+/-	5% dietary sodium saccharin, method of production and purity not specified.	Yes; 0. 0.2 and 0.3 mL urine used on days 0, 1, 5, and 14 of treatment.	negative (commercially available samples)	No dose-response relationship was observed. Results were interpreted as no mutagens being present in the urine following freeze-ulceration and/or sodium saccharin feeding.	Hasegawa et al. (1984)
5.1.2 DNA Damage							
Escherichia coli strain WP2	Lambda prophage induction (microscreen assay)	+/-	saccharin, n.p.	No; 100 µg/well for 20 h	negative/ negative	No enhancement of plaque forming units per plate	Rossman et al. (1991)
5.1.3 DNA Synthesis							
S. typhimurium	Stimulation of DNA synthesis	-	saccharin, n.p.	Yes; 1, 10, 20, 30, and 40 µg/ assay for 10 min	negative	Measured [3H]thymidine DNA synthesis	Beljanski et al. (1982)
5.2 Lower Eukaryotic Systems							
5.2.3 Higher Plants							
Allium cepa (onion) and *Vicia faba* (broad bean)	Micronucleus assay	-	saccharin, n.p.	Yes; 40, 80, and 120 mM for 6 h followed by 44 h recovery	positive	Significant increase in micronuclei 80 and 120 mM	Ma et al. (1995)

Table 5-1. Summary of Saccharin Genotoxicity Studies (Continued)

System	Biol. Endpoint	S9 Metab. Activation	Chemical Form and Purity	Dose-Response; Doses Used	Endpoint Response	Comments	Reference
5.3 Mammalian Systems *In Vitro*							
Human RSa embryo cells; SW480 human colon adenocarcinoma cells	Mutations at k-ras codon 12	-	sodium saccharin, n.p.	Yes; 10 to 30 mg/mL for 24 h	positive	DNA was extracted, amplified by PCR, dot-blotted, and hybridized to labeled probes, positive at 15 mg/mL	Suzuki and Suzuki (1993)
5.3.1 Gene Mutations							
Human RSa embryo cells	Mutations to ouabain resistance	-	sodium saccharin, n.p.	Yes; 10 to 22.5 mg/mL for 24 h	positive	Dose dependent increase in mutant frequency with top dose being 45-fold higher than controls	Suzuki and Suzuki (1988)
5.3.3 Inhibition of DNA Repair							
WI-38 human fetal lung fibroblasts	Inhibition of DNA repair synthesis	-	sodium saccharin, n.p.	Yes; 10, 57, 319, 1785, and 10,000 µg/mL for 4 h	negative	Measured incorporation of [3H]thymidine after UV irradiation	Skare and Wong (1985)
5.3.4 DNA Synthesis							
Human B-32 fibroblasts	DNA synthesis inhibition	+	saccharin sodium, n.p.	No; 0.1 M for 0, 30, or 90 min	positive	Measured [3H]thymidine incorporation following treatment	Yanagisawa et al. (1987)
HeLa S3 cells	DNA synthesis inhibition	-	saccharin, n.p.	No; DI50 (concn. which inhibited DNA synthesis by 50%) - 140 mM for 90 min	positive	Measured incorporation of BrdU using anti-BrdU antibody	Heil and Reifferscheid (1992)
5.3.6 Cell Transformation							
BALB/3T3 cells	Morphological cell transformation	-	sodium saccharin, n.p.	No; dose not provided, 72 h treatment followed by 2 wk with or without TPA promotion	negative	No increase in transformed foci with or without TPA	Sakai and Sato (1989)

Table 5-1. Summary of Saccharin Genotoxicity Studies (Continued)

System	Biol. Endpoint	S9 Metab. Activation	Chemical Form and Purity	Dose-Response; Doses Used	Endpoint Response	Comments	Reference
5.4 Mammalian Systems *In Vivo*							
5.4.1 Gene Mutations and Dominant Lethal Mutations							
mice (strain not specified)	Induction of reverse mutations	+/-	saccharin (3 commercially available samples and 1 highly purified sample); n.p.	No; 2.5 g/kg by gavage	TA98: positive (all commercial samples)/ negative (purified sample) TA100: positive (all samples)	The mutagenic activities of 24-hour urine samples were assayed in *S. typhimurium* strains TA98 and TA100. The strains were incubated both in the presence and absence of β-glucuronidase. Mutagenic activities of the urines were enhanced in TA98 by β-glucuronidase. In TA100, mutagens were inactivated by S9; in TA98, mutagens were activated by S9. The authors proposed that 2 mutagenic substances were present.	Batzinger et al. (1977)
mice (strain not specified)	Induction of reverse mutations	-	saccharin (3 commercially available samples and 1 highly purified sample); n.p.	No; 2.5 g/kg by gavage	TA98: positive (all commercial samples)/ negative (purified sample) TA100: positive (2/3 commercial samples)/ negative (1/3 commercial samples; purified sample)	*S. typhimurium* strain TA98 or TA100 was incubated for 6 hours in the peritoneal cavity of mice administered saccharin.	Batzinger et al. (1977)
5.4.2 DNA Damage							
ICR albino mice (pregnant)	SCE	-	saccharin sodium, >99%	Yes; i.p., 5, 10, and 25 mg/kg/day	negative	Dams dosed on 10th day of gestation and sacrificed on day 17	Dropkin et al. (1985)
5.4.3 Chromosomal Aberrations							
ICR albino mice (pregnant)	Chromosomal aberrations	-	saccharin sodium, >99%	Yes; i.p., 1000 and 2000 mg/kg	negative	Dams dosed on 10th day of gestation and sacrificed on day 17	Dropkin et al. (1985)

Figure 5-1. Genetic Activity Profile (GAP) for Saccharin

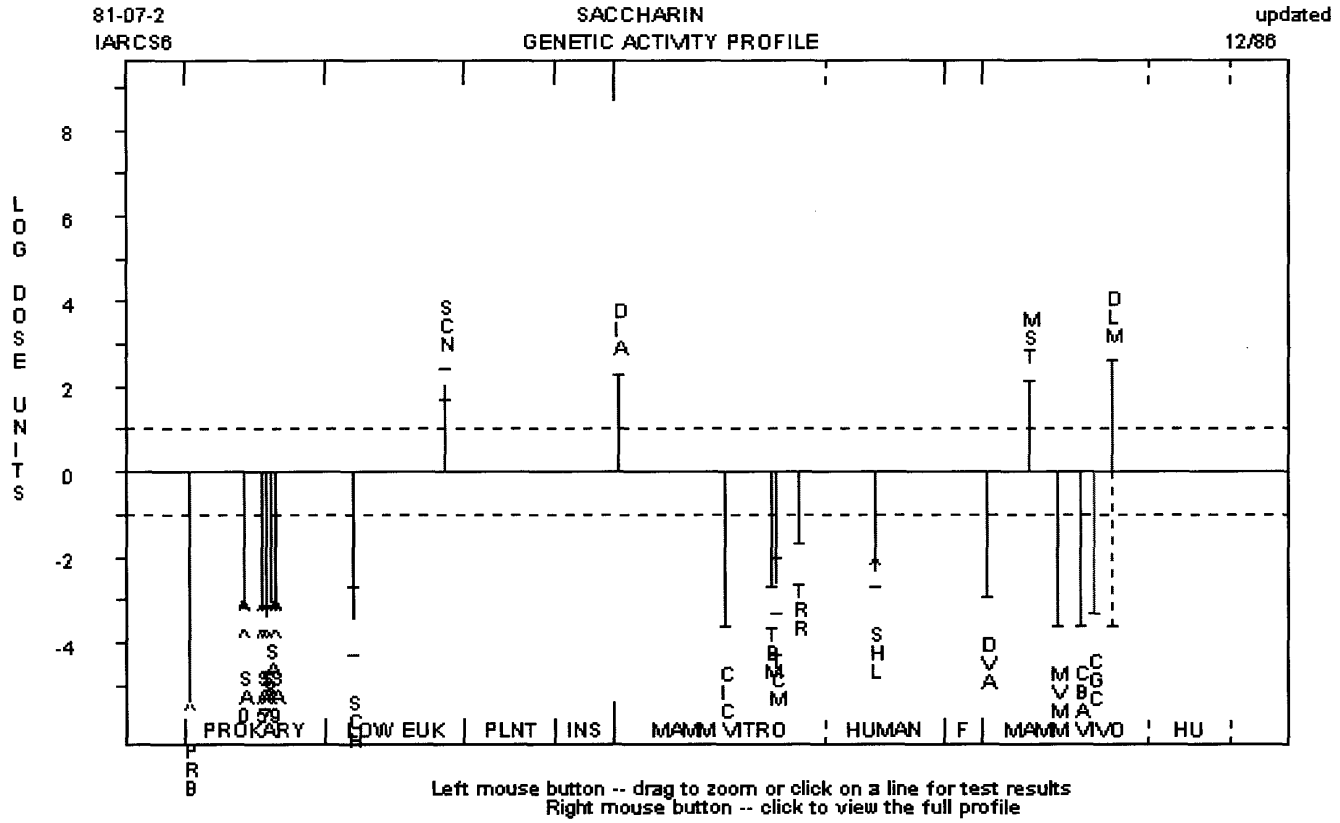


Figure 5-2. Genetic Activity Profile (GAP) for Sodium Saccharin

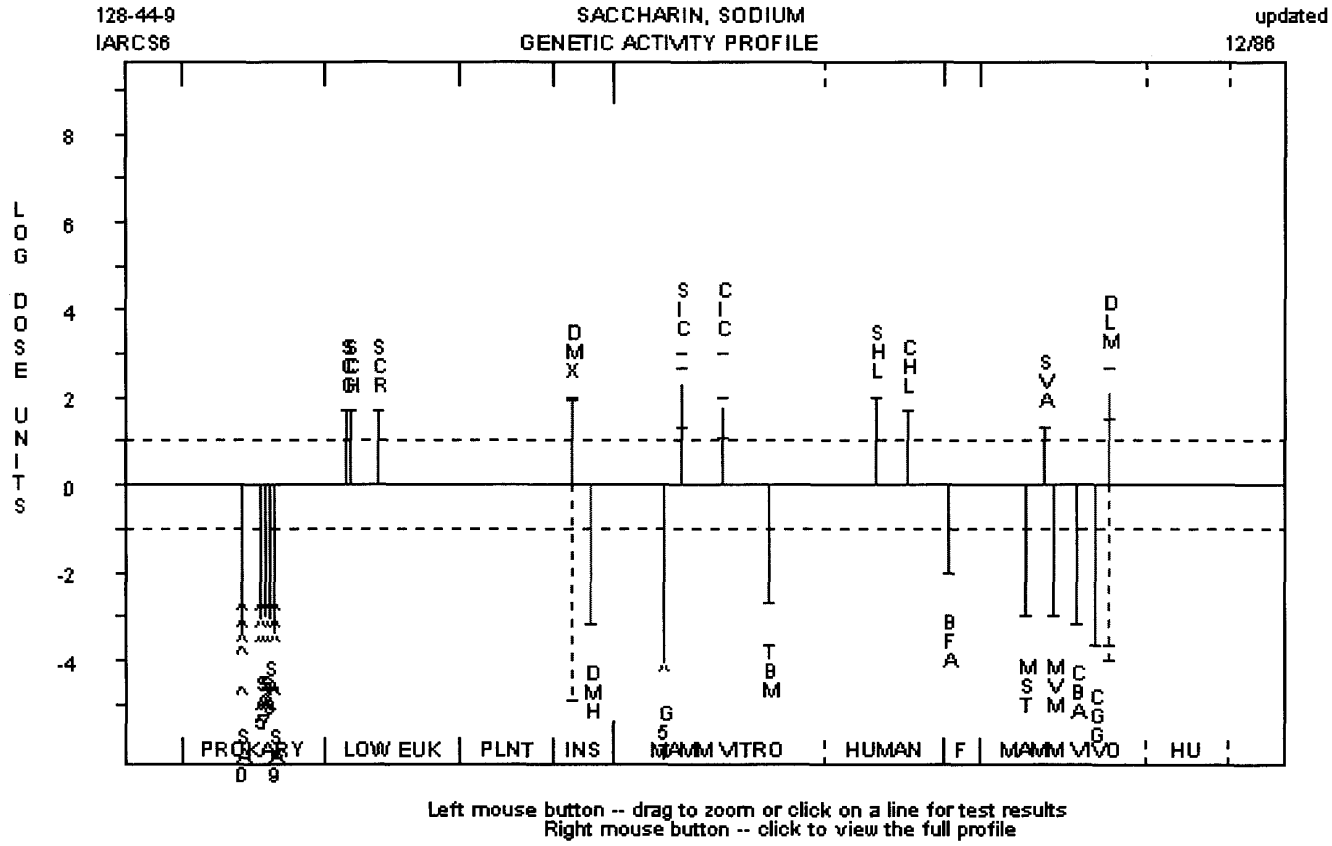
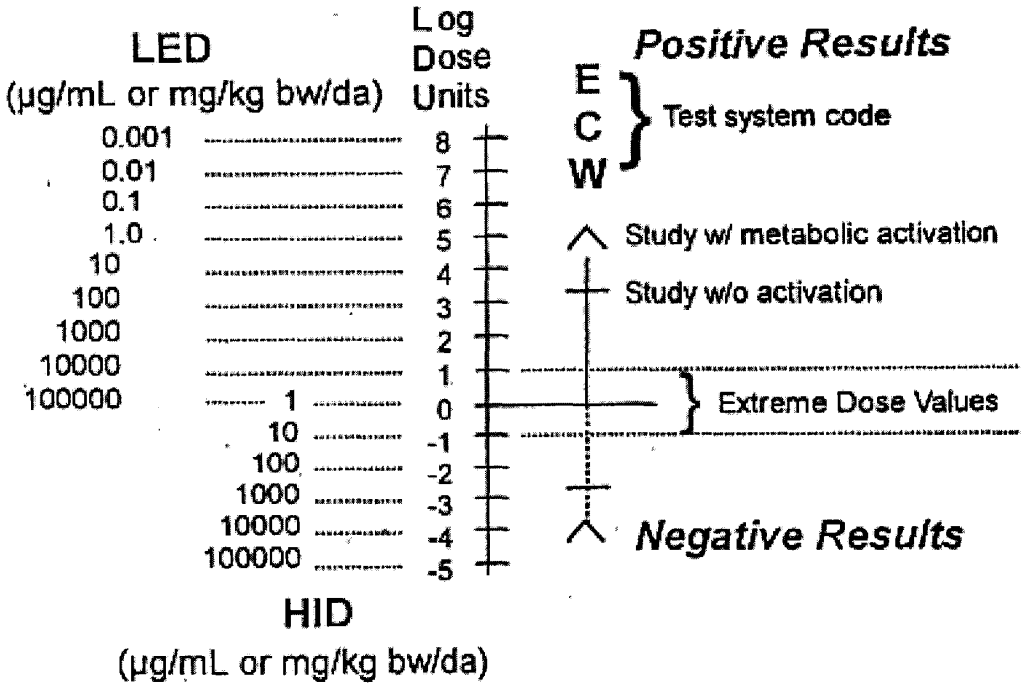


Figure 5-3. Schematic View of a Genetic Activity Profile



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N. E., H. F. Stack, M. R. Gross, and M. D. Waters. 1984. An Analysis of the Spectra of Genetic Activity Produced by Known or Suspected Human Carcinogens. Mutat. Res. 134:89-111.

Waters, M. D., H. F. Stack, A. L. Brady, P. H. M. Lohman, L. Haroun, and H. Vainio. 1988. Use of Computerized Data Listings and Activity Profiles of Genetic and Related Effects in the Review of 195 Compounds. Mutat. Res. 205:295-312.

Waters, M. D., H. F. Stack, N. E. Garrett, and M. A. Jackson. 1991. The Genetic Activity Profile Database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT DATA

Summary: Saccharin is a polar synthetic compound that is not a substrate for normal intermediary metabolism and is not used as an energy source. Earlier metabolic investigations using radiolabeled techniques indicated that saccharin underwent limited metabolism by ring opening to 2-sulfamoylbenzoic and 2-sulfabenzoic acids. However, these findings were not confirmed in later more extensive studies conducted on humans and rats using similar radiolabeled techniques. In humans, saccharin is almost completely (90%) absorbed from the intestinal tract and excreted unchanged in the urine largely (90%) by renal tubular secretion within 24 hours after oral administration. Human data fitted a two compartment model (plasma and renal clearance, half-life [$t_{1/2}$] about 70 minutes) for intravenous (i.v.) administration of a bolus dose of sodium saccharin dihydrate ($NaSac \cdot 2H_2O$).

After rats were i.v. dosed with [5-3H]saccharin, the plasma concentration-time curve clearly showed a biphasic decline during the first 2 hours, and about 90% of the dose was recovered in urine which was found to be consistent with the elimination $t_{1/2}$ (30 minutes). At low doses (100 mg/kg or less) the plasma clearance (about 10 mL min^{-1} kg^{-1}) decreased at high doses (1000 mg/kg) to 5.5 mL min^{-1} kg^{-1}, with the recovery in urine in 2 hours decreasing to 65% of the dose. The elimination $t_{1/2}$ (30 minutes) was found to be similar for all doses less than 1000 mg/kg.

With occasional exceptions, studies in male and female rats dosed with 5% or greater levels of sodium saccharin in the diet typically show alterations in the ultrastructural morphology of urinary bladder urothelium, enhanced proliferation as evidenced by elevated labeling indexes (LIs), and morphological evidence of urothelial hyperplasia. These effects can be seen as soon as 90 days after commencement of in utero treatment and generally within 10 wk when treatment starts shortly after weaning, especially when treatment is preceded or accompanied by treatment with a urinary bladder initiator. It has been shown that the severity of urothelial changes is influenced by diet. Urinary bladder changes have been demonstrated in male and female rats but not in other species tested.

6.1 Absorption, Distribution, and Excretion

Sweatman et al. (1981) dosed three adult human males ages 25 to 37 years with saccharin either orally (2 g in gelatin capsules after an overnight fast or 1 to 2 hours after breaking fast) or i.v. (sodium saccharin dihydrate, 10 mg/kg) and recorded the excretion of saccharin over 96 hours. The results indicated that saccharin was almost completely (90%) absorbed from the intestinal tract after oral administration and excreted unchanged in the urine largely by renal tubular excretion, mostly within the first 24 hours of dosing. This study also found that saccharin administered either orally or intravenously resulted in 90% recovery of the dose in the urine and up to 8% in the feces.

In studies it was found that saccharin does not accumulate in any tissues, including the bladder (Renwick, 1986). Sweatman and Renwick (1980) studied eighteen adult male and six adult female rats fed ad libitum a diet containing 1 to 10%, and 5%, respectively, sodium saccharin dihydrate for 22 days. High-pressure liquid chromatography was used to detect the concentration of saccharin in tissues (well perfused; poorly perfused) and plasma. Saccharin

underwent significant plasma protein binding (69 to 86%) at all dietary levels. The well perfused tissues (adrenal, liver, lung, and spleen) contained 20 to 50% lower concentrations of saccharin than the corresponding plasma concentrations at each dietary level. The lowest levels of saccharin detected (10 to 20% plasma level) were found in poorly perfused tissues (muscle and fat). The highest concentrations were found in the gut wall. A tissue-to-plasma ratio greater than unity was observed in the kidneys (101.6 μg/mL : 29.6 μg/mL [3.43]) and urinary bladder (120.7 μg/mL : 29.6 μg/mL [4.1]). Although the tissue distribution was similar between male and female rats fed a diet containing 5% saccharin, the tissue concentrations of saccharin were higher in females than those found in males (liver, 2.4-fold; lung, 3-fold; muscle, 2.6-fold; kidney, 1.5-fold; bladder, 6.7-fold).

Sweatman and Renwick (1982) studied whether or not a two-generation feeding protocol was associated with uniquely elevated concentrations of saccharin in the bladder or other tissues of rats. Following a single oral dose of [3H]saccharin (sodium saccharin dihydrate; 50 mg/kg; 1.0 to 3.0 mCi; >99.8% pure) to female Sprague-Dawley rats in late pregnancy, concentrations of 3H in tissues of dams at 6 to 12 hr after administration of the dose were higher than those of the fetuses. At 6 hr, maternal liver, kidney, and bladder wall concentrations were ~5-fold, ~33.3-fold and ~16.7-fold, respectively, higher than those of the fetuses. At 12 hr, maternal liver, kidney, and bladder wall concentrations were ~1.4-fold, ~8.3-fold, and ~5.8-fold, respectively, higher than those of fetuses. The concentrations of 3H in fetal tissues decreased more slowly at 48 hr, exceeding the corresponding values obtained for maternal tissues: liver, 3.2-fold; kidney, 0.8-fold, and bladder wall, 5.4-fold. The authors suggested that these findings point to the possible accumulation of saccharin during chronic intake (Sweatman and Renwick, 1982).

In another experiment conducted by Sweatman and Renwick (1982), dams were fed a 5% saccharin diet ad libitum from 4 wk prior to mating until killed during late gestation. The observed liver and kidney concentrations were lower in the fetuses than the corresponding maternal values: liver, 80 μg/g maternal vs. 36.5 μg/g fetal; kidney, 382 μg/g maternal vs. 198 μg/g fetal. However, the average concentration of saccharin in fetal bladder tissue was approximately 3.8-fold higher than the corresponding maternal value. The saccharin levels in the bladder, but no other tissue, of females (189 ± 149 μg/g) were significantly lower than in males (292 ± 261 μg/g) ($p < 0.05$ by unpaired Student's t-test). Between days 17 and 20, the concentration of saccharin in the amniotic fluid increased (males [n = 5], 15 μg/g; females [n = 7], 20 μg/g to males [n = 12] 361 μg/g; females [n = 18] 276 μg/g), which is a similar finding to Ball et al. (1977) who stated that the increase was possibly due to elimination of saccharin in fetal urine.

Liver concentrations of saccharin in F_1 animals exposed to a 5% saccharin diet reached a maximum of approximately 50 μg/g soon after weaning (between days 28 and 45). Due to the variability in the levels of saccharin in the bladder, no distinct maximum concentration was observed in F_1 animals. In previous studies conducted by Matthews et al. (1973), Lethco and Wallace (1975), Ball et al. (1977), and Sweatman and Renwick (1980), variability in the concentration of saccharin in the bladder wall was reported, either after a single dose or after chronic administration. Statistical analysis of the total bladder wall data showed that female levels were significantly (50%; $p < 0.05$) lower than males when each individual result was expressed as a percentage of the mean for the animals killed at that time point (to eliminate temporal variation). Between days 18 and 23, which corresponds to the time of separation from

the mother and consumption of a 5% saccharin diet, the average concentration of saccharin in urine of F_1 animals showed a marked increase (males, [n = 5] 4.6 μg/mL vs. [n = 2] 17.9 μg/mL; females, [n = 2] 8.6 μg/mL vs. [n = 5] 11.1 μg/mL) (Sweatman and Renwick, 1982).

Sweatman and Renwick (1982) studied the distribution and turnover of [3H]saccharin in pregnant rats maintained on a 5% saccharin diet prior to mating and transferred to a 5% saccharin diet radiolabeled with [3H] (6.1 μCi/g) on the 10th day of gestation. On days 10 to 20 of gestation, the concentrations of [3H]saccharin in maternal and fetal livers were similar to the unlabeled concentrations found by HPLC on day 20 (see above), indicating that steady-state concentrations had been reached. In the fetal tissues, the levels of [3H]saccharin showed a relatively uniform distribution. However, markedly lower concentrations were found in the brain. Similar findings were reported by Ball et al. (1977). The concentrations of [3H]saccharin were below the limit of detection in the fetal bladder. Sweatman and Renwick (1982) suggested that this was due to the size of the fetal bladder and the relatively low specific activity of the [3H]saccharin diet given. There was a marked reduction in the 3H concentrations in most maternal and fetal tissues upon transferring back to an unlabeled 5% saccharin diet for 24 hr or 48 hr prior to killing. Tritium concentrations in fetal liver, kidney, and muscle decreased to an average of 29, 45, and 22%, respectively, of the steady-state level after 24 hr on the unlabeled saccharin diet, while the corresponding maternal tissues decreased to 19, 51, and 23%, respectively. Tritium concentrations were not detectable (< 200 μg/g) in the fetal bladder wall throughout the duration of [3H]saccharin diet (10-20 days).

Ball et al. (1977) studied three groups of rats, one on a normal diet without pretreatment with [^{14}C]sodium saccharin for up to 12 months, and the others pre-treated with 1% or 5% saccharin diet for up to 12 months. Individual rats in each group were subsequently administered an oral dose of 16 to 22 mg/kg (5 to 9 μCi) sodium saccharin. In both groups about 95% of the dose was eliminated within 24 hours, with 72 to 92% detected in the urine and 0 to 22% detected in the feces. Within 3 days of dosing, excretion of ^{14}C was essentially complete. The final recovery in 6 days averaged 100%, with the urine containing 77 to 97% and feces containing 6 to 22% of the labeled dose. Pre-treatment of rats with a diet containing 1% and 5% saccharin for up to 12 months did not alter the pattern of absorption and excretion. The only alteration of this pattern was increased concentrations of [^{14}C]saccharin in the feces after continued intake, especially at the 1% dietary level. The authors also investigated the excretion of [^{14}C]saccharin in urine after i.p. injection and very little was associated with the gastrointestinal tract. The authors concluded that the increased concentration of [^{14}C]saccharin in feces after oral administration arose from incomplete absorption in the gut.

Lethco and Wallace (1975) administered [3-^{14}C]saccharin (5, 50, and 500 mg/kg) to male and female rats. The distribution of radioactivity in organs and tissues at various time intervals was monitored. One hour after administration of a 50 mg/kg dose, traces of ^{14}C were found in almost all of the organs. Saccharin reached a peak blood concentration within 8 hours. The kidney, urinary bladder, and liver tissues contained the highest ^{14}C concentration. All of the tissues except brain and spleen contained traces of ^{14}C 72 hours after dosing. The rats excreted 66 to 84 % of the labeled dose of [3-^{14}C]saccharin in the urine and 10 to 40% in the feces. This study also compared the metabolic profiles of a dog, rabbit, guinea pig, and hamster. When

compared, the metabolic profiles indicated that there was very little difference in the pattern due to dose level or animal species.

The absorption, distribution, and excretion of radiolabeled saccharin was studied by Matthews et al. (1973). Male rats (seven groups of three or more) were studied after receiving a single oral dose of [^{14}C]saccharin (1 mg/kg in 0.5 cm3 distilled water). The dose was administered orally to animals that had been fed ad libitum or fasted overnight. Saccharin entered the bloodstream rapidly, most likely due to absorption through the stomach, with peak concentrations in the blood between 7.5 and 15 minutes after administration. Saccharin was absorbed by the fasted animals more rapidly than those that were fed. The saccharin concentration of fasted animals was approximately twice that found in animals fed ad libitum. The time to peak saccharin concentration and the general shape of the curve were similar in the kidney and blood. The authors found that glomerular filtration of saccharin from the blood and its excretion in the urine resulted in temporary accumulation of 5 times more saccharin in the kidneys than in other organs or tissues. Saccharin was detected in the urine taken from the bladders of every rat as soon as 3 minutes post saccharin administration.

The accumulation and clearance of multiple doses of saccharin was also investigated by Matthews et al. (1973). Saccharin (1 mg/kg/day) was administered to two groups of four rats each for 7 days. Saccharin concentrations in the major organs were measured 24 and 72 hours after administering the final dose. At 24 hours, the saccharin concentration was slightly higher in the gastrointestinal tract and considerably higher in the bladder than in any other tissues. The authors suggested that although elevated concentrations of saccharin were not present in these tissues, the tissues may have absorbed saccharin from their contents rather than by distribution of the blood. Most of the saccharin had been cleared from all of the tissues by 72 hours after the last dose, with none of the tissues having a significantly higher concentration than the others at that time. The authors also stated that the ratio of saccharin excreted in the urine and feces was approximately 9:1 when analyzed during the feeding period and after the last dose of saccharin had been administered.

The authors continued this study by treating rats 5 times with a dose of 1 mg/kg at 90-minute intervals for a total dose of 5 mg/kg within a 6-hour period. This dosing regimen was used to simulate the daily dose of saccharin humans would be expected to consume be using saccharin in food or beverages several times throughout the day. The rats receiving multiple doses of saccharin were reported to have a higher saccharin concentration in tissues than in the corresponding tissues of rats which had received single doses of saccharin (1 mg/kg). Rats that were sacrificed 90 minutes after the fifth (last) 1 mg/kg dose were found to have a saccharin concentration in the kidneys equal to or greater than 5 times the concentration of kidneys from rats which received only a single dose of 1 mg/kg. Thereafter, the saccharin concentration in the kidneys of all of these rats approached 9 to 10 times that of the animals which received only a single dose. Twenty-four hours later, the difference had decreased to approximately 2-fold. A 10-fold difference was observed after 24 hours between concentrations in the bladders of rats receiving multiple doses and those of rats receiving single doses. Still, at 24 hours, the concentration in the bladders of rats which received 5 doses was less than 10% of that observed 90 minutes after the last of the 5 doses (Matthews et al., 1973). These data showed that

significant concentrations of saccharin can occur in certain tissues such as the kidney and bladder that appear to be almost completely cleared by the following day.

6.2 Metabolism

Saccharin is a polar synthetic compound that is not a substrate for normal intermediary metabolism and is not used as an energy source (Renwick, 1986). Metabolic investigations using radiolabeling techniques have indicated that saccharin undergoes limited metabolism by ring opening to 2-sulfamoylbenzoic and 2-sulfobenzoic acids (Pitkin et al., 1971; Kennedy et al., 1972; Arnold, 1983; Renwick, 1986). Kennedy et al. (1972) fed [^{14}C]saccharin to two rats (1 male and 1 female). Components of solvent extracts from their acidified urine were separated by thin layer chromatography (TLC) and compared to the authentic 2-sulfamoylbenzoic and sulfobenzoic acids. This experiment showed that in the urine samples collected between 0 to 24 hours after dosing, 0.4% to 0.6% of the dose was excreted as 2-sulfamoylbenzoic acid and less than 0.1% to 0.6% of the dose as 2-sulfobenzoic acid.

Pitkin et al. (1971) studied the metabolism of [^{14}C]saccharin in eight female Rhesus monkeys using the same method as Kennedy et al. (1972), which was unpublished at the time. The authors reported that [^{14}C]saccharin was excreted essentially unchanged in the urine of monkeys. The authors also found that urine samples collected and analyzed between 24 to 48 hours and 48 to 72 hours after dosing contained 1.2% of the dose as 2-sulfamoylbenzoic acid and 0.1% as 2-sulfobenzoic acid.

The Food and Drug Administration also detected 2-sulfamoylbenzoic in a more extensive study that focused on the metabolic profiles of a dog, guinea pig, hamster, rabbit, and six rats exposed to [3-^{14}C]saccharin via gavage (Lethco and Wallace, 1975). In this study six rats (three males and three females) were given oral doses of 5, 50, and 500 mg/kg [3-^{14}C]saccharin (10 to 15 μCi/kg). Twenty-four hours after the dose was administered, the rate of $^{14}CO_2$ expiration, and [^{14}C]carbonate and 2-sulfamoylbenzoic acid excreted via the urine were identified using paper chromatography, TLC, UV spectrophotometry, and reverse isotope dilution techniques. These data showed that both male and female rats expired $^{14}CO_2$ between 0.5 and 8 hours after dosing, while only female rats expired 0.01% of the dose at 24 hours. Male rats expired a total of 0.29, 0.03, and 0.10% of 5, 50, and 500 mg/kg doses, respectively, and female rats expired a total of 0.23, 0.55, and 0.27% of the 5, 50, and 500 mg/kg, doses, respectively, 24 hours post-dose. When 24 rats (12 males and 12 females; 4 rats/dose) were dosed with 5, 50, and 500 mg/kg [3-^{14}C]saccharin, about 0.4% of the dose was excreted as 2-sulfamoylbenzoic acid in the urine with approximately equal amounts identified as ^{14}C carbonate as detected by DEAE cellulose chromatography (Lethco and Wallace, 1975; Renwick, 1986). Generally, more than 99% of the urinary radioactivity was unmetabolized saccharin and all of the species' urine samples contained small amounts of 2-sulfamoylbenzoic acid. Comparative metabolic profiles of a dog, rabbit, guinea pig, and hamster indicated that there was little difference in the pattern due to animal species or dose level (Lethco and Wallace, 1975). The authors suggested that the breakdown of saccharin was due to a chemical reaction as opposed to enzymatic reactions.

Ball et al. (1977) used chromatographic, reverse isotope dilution techniques, and UV spectrophotometric techniques for the detection of radiolabeled metabolites of saccharin (2-sulfamoylbenzoic acid and $^{14}CO_3^{2-}$ in the urine, $^{14}CO_2$ in expired air). The limits of detection

were as low as 0.03% for $^{14}CO_2$ and $^{14}CO_3^{2-}$. Rats were fed a diet containing 1% or 5% of saccharin for up to 12 months prior to receiving a [^{14}C]saccharin dose (20 mg/kg) administered orally. The authors were unable to detect any metabolism in either the urine or in the expired air of the rats dosed with radiolabeled saccharin. Ball et al. (1977) were also unable to detect any metabolites of saccharin in the urine of three adult humans (one female, two males; 55 to 94 kg body weight) who ingested 1 g saccharin/day for 22 days as a treatment prior to receiving a final dose of [3-^{14}C]-saccharin (20 µCi; 13 mg) on the 22nd day. The authors were also unable to detect metabolite production from the three adult humans when they were not subjected to the saccharin pretreatment before a dose of [3-^{14}C]saccharin (20 µCi/13 mg).

Sweatman and Renwick (1979) exposed male rats to saccharin both in utero and during lactation. The authors were unable to detect any metabolites of saccharin in the excreta of rats under these conditions. The authors also reported that after 3-methylcholanthrene treatment (inducer of metabolism), saccharin metabolites were undetectable using reverse isotope dilution with limits of detection as low as 0.01% for 2-sulfamoylbenzoic acid. These results found that significant metabolism is not induced by long term administration of saccharin during the neonatal and weaning-stages of two generations.

Clearly, a discrepancy between some of the earlier reports and later investigations exist. Earlier studies may have used saccharin with slight impurities resulting in metabolism of the impure substance. Pitkin et al. (1971) used benzene ring-labeled [^{14}C]saccharin from Mallinckrodt Chemical Corp. Byard and Golberg (1973) reported that the benzene ring-labeled [^{14}C]saccharin supplied by Mallinckrodt Chemical Works (St. Louis, MO, USA) contained an impurity which produced a 2 to 3% metabolic reaction if given to animals. In brief, the authors found that the metabolite produced *in vivo* from the impurity chromatographed as 2-sulfamoylbenzoic acid but did not recrystallize with added 2-sulfamoylbenzoic acid. Both Matthews et al. (1973) and Byard and Golberg (1973) found that solvent extraction and t.l.c. in neutral solutions would give rise to artifactual metabolites. In neither the Kennedy et al. (1972) study which used [3-^{14}C]saccharin from Monsanto Co. (St. Louis, MO, USA), nor the Pitkin et al. (1971) study, which used [^{14}C]saccharin from Mallinckrodt Chemical Corp, was the purity of the saccharin specified. It seems likely that the results obtained from experiments conducted by Kennedy et al. (1972) and Pitkin et al. (1971) might be due to some unidentified impurity similar to that found by Byard and Golberg (1973). Experiments aimed at the induction of metabolism of [^{14}C]saccharin by pretreatment with phenobarbital (Byard and Golberg, 1973) also failed to induce metabolic reactions producing 2-sulfamoylbenzoic acid, 2-sulfobenzoic acid, CO_2, or the carbonate.

Lethco and Wallace (1975) explained the presence of [^{14}C]saccharin metabolites as a slight breakdown of saccharin due to simple decomposition rather than enzymatic mechanisms. Although the authors' data were substantiated by the large number of animals studied and the consistency of the extent of metabolism over a wide range of doses in various species, the saccharin molecule is resistant to chemical decarboxylation and thus slight breakdown to CO_2 and CO_3^{2-} seems unlikely (Renwick, 1986).

6.3 Pharmacokinetics

The human data generated by Sweatman et al. (1981) fitted a two-compartment model (plasma and renal clearance) for i.v. administration of a bolus dose of saccharin (sodium saccharin dihydrate; 10 mg/kg) in the presence or absence of probenecid (competes for and inhibits renal tubular secretion of organic ions). Probenecid was administered (500 mg) 2 and 12 hours before and 2 hours after the i.v. dose of saccharin. Saccharin was rapidly eliminated via the urine after i.v. administration ($t_{1/2}$ about 70 min). A significant decrease in the elimination rate constant (40%) and in the plasma clearance (36%) rate occurred when the i.v. dose was given during probenecid treatment. Thus, tubular secretion is responsible for the elimination of a minimum of 40% of circulating saccharin in humans, which is consistent with the high renal clearance noted in this study. The fact that plasma clearance values were slightly less than the corresponding renal clearance suggests the absence of significant metabolism. This supports earlier studies using [^{14}C] saccharin in humans (Byard et al., 1974; Ball et al., 1977) that failed to detect significant metabolism after oral administration.

Sweatman and Renwick (1980) dosed ten male rats with low i.v. bolus doses (1, 20, 50 mg/kg). The plasma concentration-time curve clearly showed a biphasic decline during the first 2 hours. The plasma levels fit the equation

$$C_p = 3.12De + 1.35De^{-0.0213t},$$

where C_p is the plasma concentration in $\mu g\ mL^{-1}$ at time (t) and D is the dose in mg/kg. About 90% of the dose was recovered in the urine within 2 hours. This finding is consistent with the elimination $t_{1/2}$ (30 minutes).

6.4 Structure-Activity Relationships

No data were found.

6.5 Cell Proliferation

Experimental details for the studies described in this section are presented in **Table 6-1**.

6.5.1 Hamsters

Neither hyperplasia of the urinary bladder nor significantly increased DNA synthesis was observed in 6-wk-old male Syrian hamsters administered a 5% sodium saccharin diet for 20 wk (Fukushima et al., 1983).

6.5.2 Mice

Neither hyperplasia of the urinary bladder nor significantly increased DNA synthesis was observed in 6-wk-old male B6C3F1 mice administered a 5% sodium saccharin diet for 20 wk (Fukushima et al., 1983).

6.5.3 Rats

Lessel (1971) reported that saccharin was positive for hyperplasia in rats exposed to a 5% saccharin diet for 2 yr. Of 5 bladders from animals exposed to 5% saccharin, 1 male and 1 female had urothelial hyperplasia. IARC (1980) noted the small number of bladders examined histologically.

A 5% sodium saccharin diet fed to 6-wk-old F344 male rats for up to 18 wk induced vacuolar degeneration of the bladder urothelium after 3 wk and simple hyperplasia at 5 wk. The degree of hyperplasia increased with a display of mitotic figures, hyperplastic foci and pleomorphic microvilli starting at 9 wk. Increased thymidine uptake (5- to 8-fold the rate seen in controls) was present in the bladders of exposed rats at all time periods measured through 18 wk (Fukushima and Cohen, 1980).

Hooson et al. (1980) reported mild focal urothelial hyperplasia in 1/50 female Wistar rats exposed to 2 g sodium saccharin/kg/day for 2 yr. IARC (1980) noted that the rats were not started on the test diet at weaning, but after several wk on a normal diet.

Six-week-old male and female F344 rats fed up to 5% sodium saccharin in a stock diet alone for 32 wk did not develop simple, papillary, or nodular hyperplasia (Nakanishi et al., 1980a). However, rats initiated with BBN for 4 wk and then fed 5% sodium saccharin stock diet for 32 wk developed papillary and nodular hyperplasia (Nakanishi et al., 1980a).

In a 2-generation study, male and female F_1 Charles River CD rats exposed to up to 7.5% sodium saccharin in the diet for up to 2 yr had an increased incidence of urinary bladder hyperplasia at the 7.5% dose, but it was not morphologically precancerous. Exposure to 0.01, 0.1, 1.0, or 5.0% sodium saccharin had no effect on the incidence of hyperplasia (Taylor et al., 1980).

Lawson and Hertzog (1981) reported that sodium saccharin did not induce DNA synthesis in male Sprague Dawley rat bladder epithelium, as measured by an LI or by specific activity of DNA. Animals were fed 7.5% sodium saccharin diet for 50 wk with interim sacrifices throughout. [Methyl-^{3}H]thymidine was injected intraperitoneally 1 hour before death.

Murasaki and Cohen (1981) studied the dose response relationship between sodium saccharin exposure and cell proliferation in the urinary bladders of five-week-old male F344 rats fed sodium saccharin in the diet for 10 wk. The results of this experiment showed a dose-related increase in tritiated thymidine LI, the presence of pleomorphic microvilli, and hyperplasia. The no-observable-effect-level (NOEL) for statistically significant changes in LI was 0.1%.

The incidences of simple hyperplasia (25/32 vs. 1/28 controls) and papillary or nodular hyperplasia (20/32 vs. 0/28 controls) were significantly increased in male ACI rats administered 5% sodium saccharin in the diet for 52 wk beginning at 6 wk of age. At least half of the ACI rats were infected with the bladder parasite *Trichosomoides crassicauda* (Fukushima et al., 1983). Females were not evaluated.

Male F344 rats fed a 5% sodium saccharin diet for up to 20 wk beginning at 6 wk of age developed hyperplasia of the urinary bladder and significantly increased DNA synthesis at 20 wk (Fukushima et al., 1983).

Sodium saccharin induced hyperplasia of the urinary bladder in male ACI rats but not in F344, Sprague Dawley, or Wistar rats administered 5% sodium saccharin in the diet for 52 wk beginning at 6 wk of age. However, the concentration of urinary $MgNH_4PO_4$ crystals was greater in all strains of treated rats than in their respective controls (Fukushima et al., 1983). The ACI rats also developed urinary bladder papillomas and carcinomas. Females were not evaluated.

The effects of sodium saccharin on freeze ulceration-induced cell proliferation in male F344 rats were studied by Murasaki and Cohen (1983b). The authors found that the degree of microvilli formation and hyperplasia was similar for the 2-wk period following freeze ulceration

whether or not 5% sodium saccharin was administered immediately following the procedure. In another experiment, Murasaki and Cohen (1983b) found that sodium saccharin administered 2 or 8 wk following freeze ulceration produced a similar increase in hyperplasia, LI, and microvilli.

Hasegawa and Cohen (1986) studied the impact of the cation associated with different dosage forms of saccharin. In male F344 rats fed a 5% sodium saccharin, acid saccharin, potassium saccharin, or calcium saccharin diet for 10 wk, the LI was increased approximately 2-fold for calcium saccharin, 3-fold for potassium saccharin, and 9-fold for sodium saccharin. No increased LI was found for acid saccharin, and only the increased LI associated with sodium and potassium salt exposures reached statistical significance. A statistically significant increase in rats with hyperplasia was found in the sodium saccharin-treated group. Evidence of simple hyperplasia following potassium saccharin and calcium saccharin exposure, and increases in microvilli with potassium saccharin exposure were found. However, these changes were not statistically significant.

Tatematsu et al. (1986) found that a 5% sodium saccharin diet for 21 wk did not increase DNA synthesis in the bladder epithelium of male Fischer rats.

A 2-generation study conducted by Masui et al. (1988 abstr.), evaluated the urinary bladder proliferative effects upon fetal and neonatal Sprague-Dawley rats of both sexes, when their dams were fed a 5% sodium saccharin diet prior to mating and up to weaning. In control and sodium saccharin-treated fetuses at days 17 and 21 of gestation, the LIs were similar in both groups. Similar LIs were also found for both exposed and control rats at day 7 after birth. However, the LI was greater in sodium saccharin-treated rats (higher in females than in males), at day 21 after birth, compared to controls.

Garland et al. (1989b) found that the proliferative effects of sodium saccharin were dependent upon diet. In Experiment 1, five-week-old male F344 rats were given 5 or 7.5% sodium saccharin in Prolab 3200, NIH-07 or AIN-76 diet for 4 or 10 wk. In Experiment 2, male F344 rats and 4-wk-old male Sprague-Dawley rats were dosed with 5 and 7.5% sodium saccharin in Prolab 3200 or Purina 5002 diet for 10 wk. The results of Experiment 1 showed that sodium saccharin had a greater effect on bladder urothelium in the rats fed the Prolab diet compared with those on the NIH diet. In addition, there was little effect in the rats on the AIN diet. Effects included urothelial hyperplasia at 4 and 10 wk and an increased thymidine LI for the Prolab and NIH diet at 10 wk. In Experiment 2, the response was greater in F344 rats than in Sprague-Dawley rats and greater for the Prolab rather than the Purina diet for hyperplasia, increased LI, and evidence of urothelial damage.

Male F344 rats were exposed to 3, 5, or 7.5% sodium saccharin diet (Prolab feed) for 4, 7, or 10 wk in a dose-response experiment conducted by Cohen et al. (1990). Cell exfoliation and necrosis were evident at 10 wk in the group fed 3% sodium saccharin. An apparent progression from mild to more severe necrotic changes during the 4- to 10-wk period was found in the 5 and 7.5% sodium saccharin group. In the 5% sodium saccharin-exposed group, a doubling of the LI with extensive cell damage was noted. In the 7.5% sodium saccharin-exposed group, the LI was increased several fold, with evidence of hyperplasia.

The effects of diet on cell proliferation induced by sodium saccharin were also studied by Debiec-Rychter and Wang (1990). Male F344 rats were exposed to 5% sodium saccharin in either Wayne or AIN-76A diet for 2, 4, 6, 10, or 16 wk. Both diets increased the LI

approximately 5-fold when measured at 2, 4, 6, 10, or 16 wk. The authors also found that 2% sodium bicarbonate increased the LI for the AIN-76A diet 6- to 9-fold. In addition, a sodium saccharin and sodium bicarbonate combination proved to have an additive effect on cell proliferation, except at the 2-wk interval. A similar study was not conducted for the Wayne diet.

Garland et al. (1991) reported that sodium saccharin at 7.5% dietary concentration was positive for hyperplasia in male SD rats exposed *in utero* from conception up to 90 days of age. Urothelial hyperplasia was not present at 30 days of age.

Two separate studies conducted by Garland et al. (1994) and Uwagawa et al. (1994) demonstrated that NCI-Black-Reiter (NBR) rats, which do not produce 2-globulin (the male rat-specific, low molecular weight urinary protein), do not exhibit sodium saccharin-induced urinary bladder cell proliferation. Male NBR, F344, and castrated F344 rats were fed 7.5% sodium saccharin in Prolab 3200 diet for 10 wk. The most severe changes were found in both normal and castrated sodium saccharin-exposed F344 rats. Hyperplastic changes were found in the bladders of 7/10 intact F344 rats compared with 1/10 NBR rats. Hyperplasia was not found in the bladders of control rats. Although the 2μ-globulin urinary content in castrated F344 rats has been reported to be only 10% of that in intact normal F344 rats (Roy and Neuhaus, 1967; cited by Garland et al., 1994), examination of the saccharin-treated castrated F344 rats urinary bladders revealed that 4/10 showed signs of hyperplasia (Garland et al.,1994).

Uwagawa et al. (1994) exposed 6-wk-old F344 and NBR rats to 5% sodium saccharin, 5% sodium ascorbate, or 3% uracil for 8 wk. In both strains, the most severe urothelial changes were induced by uracil as shown by scanning electron microscopy (SEM). Sodium ascorbate-induced simple hyperplasia was found in the bladders of F344 rats but not in NBR rats. Sodium saccharin did not induce hyperplasia in the bladders of NBR; uracil-induced hyperplasia, however, was found in both strains. Increases in the BrdU LIs were found in F344 rats administered uracil (> 50-fold), ascorbate (36-fold), or sodium saccharin (20-fold).

Fischer 344 rats exposed to a 7.5% sodium saccharin diet for 10 wk developed hyperplasia. Amorphous precipitate was present in exposed rats along with an increased incidence of urothelial simple hyperplasia (Cohen et al., 1995a).

Sodium saccharin was positive for cell proliferation in male and female F344 rats exposed to a 5% sodium saccharin diet for 21 or 91 days; the effects were reversible over time (Cohen et al., 1995b).

6.5.4 Guinea Pigs

Neither hyperplasia of the urinary bladder nor significantly increased DNA synthesis was observed in 6-wk-old male Hartley guinea pigs administered a 5% sodium saccharin diet for 20 wk (Fukushima et al., 1983).

6.5.5 Nonhuman Primates

Sodium saccharin was negative for cell proliferation in Macaca mulatta monkeys fed 20, 100, or 500 mg/kg/day in diet for 79 months. Histopathological examination of urinary bladders, kidneys, and testis of surviving and deceased animals showed no abnormal pathology (McChesney et al., 1977 abstr.; cited by IARC, 1980).

6.6 Cell Proliferation with Co-Administration of Known Carcinogens

6.6.1 *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)

Urinary bladder hyperplasia was enhanced in 6-wk-old male and female F344 rats exposed to 2000, 10,000, or 50,000, but not 400 ppm, sodium saccharin in the diet following BBN pretreatment. Exposure to sodium saccharin without BBN pretreatment did not induce any changes in urinary bladders of rats of either sex (Nakanishi et al., 1980a).

The effects of sequential administration (initiation/promotion protocol) of 0.01% BBN in drinking water and 5.0% sodium saccharin in feed and concurrent administration of 0.001% BBN in drinking water and 5.0% sodium saccharin in feed, were studied in 8-wk-old male Wistar rats by Nakanishi et al. (1980b). In the first experiment (sequential administration), rats received BBN for 4 wk and then sodium saccharin for an additional 32 wk. In the second experiment (concurrent administration), rats were fed both BBN and sodium saccharin for 40 wk. There was an enhancement of urinary bladder hyperplasia and bladder tumors when rats were exposed to BBN and sodium saccharin either sequentially or concurrently, while sodium saccharin alone caused urinary bladder urothelial hyperplasia.

Nakanishi et al. (1982) reported that there was a significant increase ($p < 0.05$) in the incidences of simple, papillary, or nodular urinary bladder hyperplasia in male F344 rats (age not specified) initiated with 0.01% BBN in drinking water for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered BBN alone (simple hyperplasia: 27/29 vs. 19/28; papillary or nodular hyperplasia: 24/29 vs. 11/28).

6.6.2 2-Acetylaminofluorene (AAF)

Sodium saccharin was positive for hyperplasia in female Horton SD rats fed a 5% sodium saccharin diet for 40 wk with co-administration of AAF. Hyperplasia of the urinary bladder mucosal lining occurred in all control and treated females fed AAF. The hyperplasia was most pronounced in the AAF/sodium saccharin-exposed animals, with one of these rats displaying squamous metaplasia and precancerous changes in the mucosal epithelium. IARC noted that the small number of animals used, and the fact that food consumption was not measured, prevented the evaluation of AAF and sodium saccharin exposure (Ershoff and Baja, 1974; cited by IARC, 1980).

Nakanishi et al. (1982) reported that there was a significant increase ($p < 0.05$) in the incidences of simple, papillary, or nodular urinary bladder hyperplasia in male F344 rats (age not specified) initiated with 0.02% AAF in the diet for 4 wk and then fed 5% sodium saccharin in the diet for an additional 32 wk, as compared to rats administered BBN alone (simple hyperplasia: 6/29 vs. 0/28; papillary or nodular hyperplasia: 4/29 vs. 0/28).

6.6.3 *N*-Methyl-*N*-nitrosourea (MNU)

There was an increase in the number of proliferative bladder lesions in female Wistar rats (age not specified) administered a single 0.15 mL intravesicular dose of MNU, followed 2 wk later by daily administration of either 2 g/kg *o*-toluenesulfonamide-free sodium saccharin or 2 g/kg sodium saccharin containing 40 mg/kg *o*-toluenesulfonamide for 2 yr, as compared to a control group given MNU alone (incidence not given) (Hooson et al., 1980).

Table 6-1. Cell Proliferation

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6.5.1 Hamsters							
6-wk-old Syrian golden hamsters	50M	35M	sodium saccharin, >99.5% pure [7 ppm *o*-toluenesulfonamide]; method of production not specified	5% diet	up to 20 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder or significantly increase DNA synthesis.	Fukushima et al. (1983)
6.5.2 Mice							
6-wk-old B6C3F1 mice	50M	35M	sodium saccharin, >99.5% pure [7 ppm *o*-toluenesulfonamide]; method of production not specified	5% diet	up to 20 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder or significantly increase DNA synthesis.	Fukushima et al. (1983)
6.5.3 Rats							
Boots-Wistar rats (age not specified)	40M, 40F	20M, 20F	saccharin[a], made by Remsen-Fahlberg method, purity not specified	0.005, 0.05, or 5% diet	2 yr	Positive with highest dose Of 5 bladders from animals exposed to the highest dose, 1 male and 1 female had urothelial hyperplasia. IARC (1980) noted the small number of bladders examined histologically.	Lessel (1971)
6-wk-old Charles River F344 rats	24M	6M	sodium saccharin, methods of production and purity not specified	5% diet	<18 wk	Positive Three treated rats were killed at 1, 3, 5, 7, 9, 12, 15, and 18 wk. Three controls killed at 0 and 18 wks. Vacuolar degeneration of the epithelial cells at 3 wk and simple hyperplasia at 5 wk were observed. At 9 wk, the degree of hyperplasia increased with occurrences of mitotic figures, hyperplastic foci and pleomorphic microvilli. Thymidine LIs were increased in bladders of exposed rats at all time periods measured.	Fukushima and Cohen (1980)
Wistar rats (age not specified)	50F	63F	sodium saccharin, made by Maumee process, purity not specified	2 g/kg body weight/day	2 yr	Negative Mild focal urothelial hyperplasia was seen in one rat fed sodium saccharin. IARC (1980) noted that animals were started on the test diet not at weaning, but after several wk on a normal diet.	Hooson et al. (1980)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old inbred Charles River F344 rats	302M, 311F	29M, 30F	sodium saccharin, methods of production and purity not specified	0.04, 0.2, 1, or 5% diet with or without 4 wk of BBN pretreatment	32 wk	Negative Sodium saccharin alond did not induce simple, papillary or nodular hyperplasia except after pretreatment with BBN in the 5% groups of males and females.	Nakanishi et al. (1980a)
in utero Charles River CD rats	240M, 240F	8M, 48F	sodium saccharin, made by Remsen-Fahlberg method, 350 ppm *o*-toluenesulfonamide	0.01, 0.1, 1, 5, or 7.5% diet	2 yr	Positive with highest dose This was a 2-generation study. Males and females exposed to 7.5% sodium saccharin had an increased incidence of urinary bladder hyperplasia, but it was not morphologically precancerous. Exposure to 0.01, 0.1, 1, or 5% sodium saccharin had no effect on the incidence of hyperplasia.	Taylor et al. (1980)
3-wk-old Sprague-Dawley CD weanling rats	labeling index (LI) measurement group: 8M sacrificed at 3 defined durations of treatment specific activity of DNA measurement group: varying numbers (19-24M) sacrificed at 9 defined durations of treatment	LI measurement group: 8M sacrificed at 3 defined durations of treatment specific activity of DNA measurement group: varying numbers (19-23M) sacrificed at 9 defined durations of treatment	sodium saccharin incorporated in the diet and then pelleted, purity not specified	7.5% sodium saccharin diet plus [Methyl-^{3}H]thymidine injected intraperitoneally 1 h before death	LI measurement group: 1, 15, and 50 wk specific activity of DNA measurement group: 1, 2, 3, 6, 10, 15, 20, 30, and 50 wk	Negative Sodium saccharin did not increase bladder epitheleal DNA synthesis (measured by the LI and by specific activity of DNA).	Lawson and Hertzog (1981)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old Fischer F344 rats	10M (for each dose level)	10M	sodium saccharin, made by Maumee process, purity not specified	0.1, 0.5, 1, 2.5, 5% diet	10 wk	Positive above 0.1% Sodium saccharin did not induce formation of papillary or nodular hyperplasia, papilloma, or cancer. The LI increased significantly in a dose response manner at dose above 0.1%. Administration of 1, 2.5, or 5% sodium saccharin increased the number of foci containing ropy microridges or uniform microvilli in intestines.	Murasaki and Cohen (1981)
rats (strain and age not specified)	M (number not specified)	M (number not specified)	sodium saccharin, methods of production and purity not specified.	5% diet	10 wk	Positive Dose-related increase in tritiated thymidine LI and the presence of uniform and pleomorphic microvilli and hyperplasia were observed. The no-observable-effect-level (NOEL) for statistically significant changes in LI was 0.1%.	Murasaki and Cohen (1981)
6-wk-old ACI rats	48M	45M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	52 wk	Positive The incidences of urinary bladder simple hyperplasia (25/32 vs. 1/28 controls) and papillary or nodular hyperplasia (20/32 vs. 0/28 controls) were significantly increased. At least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda*.	Fukushima et al. (1983)
6-wk-old F344 rats	50M	35M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	up to 20 wk	Positive Sodium saccharin induced hyperplasia of the urinary bladder and significantly increased DNA synthesis at 20 wk.	Fukushima et al. (1983)
6-wk-old F344 rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	52 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder, but the concentration of $MgNH_4PO_4$ crystals in the urine of treated rats was greater than that in controls.	Fukushima et al. (1983)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6-wk-old SD rats and Wistar rats	40M	40M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	52 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder, but the concentration of $MgNH_4PO_4$ crystals in the urine of treated rats was greater than that in controls.	Fukushima et al. (1983)
5-wk-old inbred Fischer 344 rats	5-13M sacrificed at 9 defined durations up to 8 wk	5-13M sacrificed at defined durations up to 8 wk: 4 sacrifice dates for group receiving freeze ulceration + control diet and 7 sacrifice dates for groups receiving either sodium saccharin or control diet alone	sodium saccharin mixed in the diet and pelleted, purity not specified	5% diet either immediately after or 2 wk after freeze ulceration	8 wk	Positive Nodular and papillary hyperplasia and luminal surface abnormalities were detected when rats were fed sodium saccharin either immediately after freeze ulceration or 2 wk after freeze ulceration. Incidences high for entire 8 wk of the experiment.	Murasaki and Cohen (1983b)
5-wk-old inbred Fischer 344 rats	M (number not specified)	M (number not specified)	sodium saccharin mixed in the diet and pelleted, purity not specified	5% diet administered 8 wk after freeze ulceration 5% diet administered 2 wk after freeze ulceration	16 wk	Positive Development of nodular and papillary lesions, surface abnormalities, and increased LI were similar to results reported in the two groups above.	Murasaki and Cohen (1983b)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old F344 rats	M (number not specified)	M (number not specified)	sodium saccharin acid saccharin potassium saccharin calcium saccharin Methods of production and purity not specified	5% diet 5% diet 5% diet 5% diet	10 wk	Positive Sodium saccharin induced significant urinary bladder epithelial proliferation. Potassium saccharin also did, but not as much. Calcium saccharin and acid saccharin did not induce a significant increase in proliferation.	Hasegawa and Cohen (1986)
Fischer rats (age not specified)	not specified	not specified	sodium saccharin, methods of production and purity not specified	5% diet	21 wk	Negative Exposure did not increase DNA synthesis in the bladder epithelium.	Tatematsu et al. (1986)
fetal and neonatal Sprague-Dawley rats	not specified	not specified	sodium saccharin, methods of production and purity not specified	5% diet fed to dams before mating until weaning	fed to dams before mating until weaning	Positive At day 21 after birth, the LI in bladder was greater for exposed rats than control rats. The LI was higher in exposed females than in exposed males.	Masui et al. (1988 abstr.)
5-wk-old F344 rats and 4-wk-old Sprague-Dawley rats	105M	60M	sodium saccharin, 99.9% pure; method of production not specified	5 or 7.5% diet	4 or 10 wk	Positive when diet made urine alkaline One of 3 diets was fed: Prolab 3200, NIH-07, or AIN-76A. There was a higher incidence of simple or nodular hyperplasia of urothelium in rats fed Prolab than those fed NIH diet. There was little response with AIN diet. Urinary pH in rats fed AIN diet was 6.0 ± 0.0. Rats fed NIH diet had a urinary pH of 6.3 ± 0.2 and rats fed Prolab had a urinary pH of 6.4 ± 0.2. The response to sodium saccharin was greater in F344 rats than SD rats.	Garland et al. (1989b)
28-day-old F344 rats	30M (for each dose level)	30M	sodium saccharin, methods of production and purity not specified	3, 5, or 7.5% diet	4, 7, or 10 wk	Positive with highest dose Light microscopic changes in bladder and an increase in LI in bladder were seen at all time points but only in rats fed 7.5% dose level. Scanning electron microscopic changes were seen beginning at 4 wk, with increasing severity at higher doses.	Cohen et al. (1990)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
Weanling F344 rats (age not specified)	18-20M per group	20M (AIN-76A), M (number not specified; Wayne diet)	sodium saccharin, methods of production and purity not specified	5% diet AIN-76A or Wayne diet	2,4,6,10, or 16 wk	Positive Sodium saccharin in both diets caused a significant increase in the thymidine LI. Sodium bicarbonate alone increased the LI and in combination with sodium saccharin had an additive effect on bladder urothelial LI. A sodium bicarbonate study was not done with the Wayne diets.	Debied-Rychter and Wang (1990)
in utero and 30-day-old Sprague-Dawley rats	7M, 7F for each dose level (*in utero*); 7M, 7F for each dose level (30-day-old)	7M, 7F (*in utero*); 7M, 7F (30-day-old)	sodium saccharin, 99.2% pure; method of production not specified	1, 3, or 7.5% diet	30, 60, or 90 days	Positive with highest dose *In utero* rats were exposed to sodium saccharin from conception to 30 days. Thirty-day-old rats were exposed for 60 days. Mild simple hyperplasia of the urinary bladder occurred in 90-day-old male rats (4 cases) fed 7.5% sodium saccharin, one 30-day-old female rat fed 7.5% sodium saccharin, and eight 90-day-old female rats fed 7.5% sodium saccharin. There were 2 cases of moderate or severe hyperplasia in 90-day-old female rats fed 7.5% sodium saccharin and 1 case in a 30-day-old female control rat. One 30-day-old female control rat exhibited moderate or severe hyperplasia. Significance values were not included.	Garland et al. (1991)
4- to 5-wk-old intact F344, castrated F344, and NBR rats	10M (intact), 10M (castrated), 10M (NBR)	10M (intact), 10M (castrated), 10M (NBR)	sodium saccharin, 98.1% pure with no impurities > 1 ppm; method of production not specified	7.5% diet	10 wk	Positive only in rats that synthesized α2μ-globulin NBR rats don't synthesize α2μ-globulin. Castrated rats have lower levels than intact rats. Sodium saccharin produced less bladder proliferation in NBR rats than in intact F344 rats. Intermediate proliferation was seen in castrated rats.	Garland et al. (1994)
6-wk-old NBR and F344 rats	6M (NBR), 6M (F344)	10M (NBR), 5M (F344)	sodium saccharin, methods of production and purity not specified	5% diet	8 wk	Positive only in rats that synthesized α2μ-globulin NBR rats do not synthesize α2μ-globulin. Only F344 rats had an increase in cell proliferation in urinary bladder after exposure to sodium saccharin.	Uwagawa et al. (1994)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
5-wk-old F344 rats	10M	10M	sodium saccharin, pure, method of production not specified	7.5% diet	10 wk	Positive Amorphous precipitate was present in exposed rats along with an increased incidence of urothelial simple hyperplasia.	Cohen et al. (1995a)
8-wk-old and 6-wk-old F344 and Sprague-Dawley rats	M, F (numbers not specified)	not specified	sodium saccharin acid saccharin Methods of production and purity not specified	5% diet 5% diet	21 or 91 days	Positive (sodium saccharin) Sodium saccharin and acid saccharin were evaluated. Neither increased bladder proliferation when fed at birth through 7 days of age. Sodium saccharin increased proliferation at later times but acid saccharin did not. The effects of sodium saccharin were reversible over time.	Cohen et al. (1995b)
6.5.4 Guinea Pigs							
6-wk old Hartley guinea pigs	30M	20M	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	5% diet	up to 20 wk	Negative Sodium saccharin did not induce hyperplasia of the urinary bladder or significantly increase DNA synthesis.	Fukushima et al. (1983)
6.5.5 Nonhuman Primates							
Macaca mulatta monkeys (age not specified)	7M, 7F	3M, 3F	sodium saccharin, made by Remsen-Fahlberg method, containing 2.4 or 3.2 mg/kg *o*-toluene-sulfonamide	20, 100, or 500 mg/kg bw/day in diet	79 mo	Negative Histopathological examination of urinary bladders, kidneys, and testis of surviving and deceased animals showed no abnormal pathology.	McChesney et al. (1977 abstr.; cited by IARC, 1980)
6.6 Cell Proliferation with Co-Administration of Known Carcinogens							
6.6.1 *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)							
6-wk-old F344 rats	242M, 249F	60M, 62F	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]; method of production not specified	400, 2000, 10,000, or 50,000 ppm diet with or without BBN pretreatment	32 wk	Positive with higher doses and BBN pretreatment Urinary bladder hyperplasia was enhanced in both sexes by exposure to 2000-50,000 ppm sodium saccharin following BBN pretreatment. Exposure to sodium saccharin without BBN pretreatment did not produce any changes in urinary bladders of rats of either sex.	Nakanishi et al. (1980a)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
8-wk-old Wistar rats	40M (BBN/sodium saccharin)	36M (BBN alone), 32M (sodium saccharin alone), 18M (no chemicals)	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]	sodium saccharin: 5% diet; 0.01% in drinking water	Rats pretreated with BBN for 4 wk and then given sodium saccharin for 32 wk	Positive with BBN treatment There was an enhancement of urinary bladder papillary or nodular hyperplasia (21/31 vs. 6/23 BBN controls).	Nakanishi et al. (1980b)
8-wk-old Wistar rats	40M (BBN/sodium saccharin)	24M (BBN alone), 24M (sodium saccharin alone), 18M (no chemicals)	sodium saccharin, >99.5% pure [7 ppm *o*-toluene-sulfonamide]	sodium saccharin: 5% diet BBN; 0.001% in drinking water	Rats were co-administered BBN and sodium saccharin for 40 wk	Positive with BBN treatment There was an enhancement of urinary bladder hyperplasia (simple hyperplasia, 24/24 vs. 2/22 BBN controls; papillary or nodular hyperplasia, 20/24 vs. 2/22).	Nakanishi et al. (1980b)
F344 rats (age not specified)	31M	30M (BBN alone)	Sodium saccharin [7 ppm *o*-toluenesulfonamide]; methods of production and purity not specified	0.01% BBN in drinking water for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Positive with BBN pretreatment There was a significant increase in the incidences of simple and papillary or nodular hyperplasia in the urinary bladder (simple hyperplasia: 27/29 vs. 19/28; papillary or nodular hyperplasia: 24/29 vs. 11/28).	Nakanishi et al. (1982)
6.6.2 2-Acetylaminofluorene (AAF)							
Horton Sprague-Dawley rats (age not specified)	62F	62F	sodium saccharin, methods of production and purity not specified	5% diet	40 wk	Positive with co-administration of AAF Hyperplasia of the urinary bladder mucosal lining occurred in all animals but was more severe in AAF/sodium saccharin-exposed animals, with one of these animals displaying squamous metaplasia and precancerous changes in the mucosal epithelium. No animals had malignant lesions of the urinary bladder. IARC noted the small number of animals used and the fact that food consumption was not measured, preventing the evaluation of AAF and sodium saccharin exposure.	Ershoff and Baja (1974; cited by IARC, 1980)
F344 rats (age not specified)	31M	30M (BBN alone)	sodium saccharin [7 ppm *o*-toluenesulfonamide]; methods of production and purity not specified	0.02% AAF in diet for 4 wk followed by 5% sodium saccharin in diet for 32 wk	see dose	Positive with AAF pretreatment There was a significant increase in the incidences of simple and papillary nodular hyperplasia of the urinary bladder (simple hyperplasia: 6/29 vs. 0/28; papillary or nodular hyperplasia: 4/29 vs. 0/28).	Nakanishi et al. (1982)

Table 6-1. Cell Proliferation (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration	Results/Comments	Reference
6.6.3 *N*-Methyl-*N*-nitrosurea (MNU)							
Wistar rats (age not specified)	63F (MNU + sodium saccharin containing 40 mg/kg *o*-toluene-sulfonamide) 63F (MNU + sodium saccharin free of *o*-toluene-sulfonamide)	63F (MNU alone)	MNU sodium saccharin prepared by the Remsen-Fahlberg method, containing 40 mg/kg *o*-toluene-sulfonamide sodium saccharin prepared by the Maumee process (no *o*-toluenesulfonamide)	0.15 mL instilled into bladder 2 g/kg/day in drinking water 2 g/kg/day in drinking water	single dose 2 yr (started 2 wk after MNU) 2 yr (started 2 wk after MNU)	Positive with MNU pretreatment There was an increase in the number of proliferative bladder lesions in rats treated with MNU and sodium saccharin (incidence not given).	Hooson et al. (1980)

Abbreviations: F = females; LI = labeling index; M = males

7.0 MECHANISMS

Summary: Bladder tumors found predominantly in male rats exposed to high dietary concentrations of sodium saccharin (equal to or greater than 1% of the diet) prior to birth, at birth, or starting at up to 35 days of age are thought to occur and proceed in association with elevated urinary sodium ion concentration and pH above 6.5. Implications that the sodium ion, itself, may be at least partially responsible for the carcinogenic effects observed in the male rat bladder stem from studies involving many other sodium salts (e.g., of succinic acid and ascorbic acid) eliciting similar effects in the male rat. In addition, when rat bladder epithelial cells were incubated with sodium saccharin, calcium saccharin, potassium saccharin, sodium ascorbate, sodium chloride, sodium citrate, potassium chloride, or calcium chloride *in vitro* for 24 hours, all of the sodium salts proved to be cytotoxic, while the other salts did not display similar effects. Studies using diets varying in pH have shown that sodium saccharin does not significantly promote proliferation in the male rat urinary bladder when fed in the acidic AIN-76A diet, but sodium saccharin did increase urothelial proliferation when fed in the Prolab 3200 (alkaline) diet.

A number of studies have shown that pH above 6.5 and increased urinary sodium ion concentration in the male rat urinary bladder enhance the formation of urinary silicate crystals. These crystals have been shown to form by the binding of urinary proteins to saccharin, and may act as microabrasives in the rat urinary bladder, causing regenerative hyperplasia (increase in cell number) and increased cell proliferation, which, when sustained over a lifetime, provide the basis for urinary bladder tumorigenesis. The anatomy of the rat bladder is thought to play a role in rendering the rat susceptible to bladder tumorigenesis. It is known that the horizontal position of the rat during urination leaves the rat prone to the retention of calculi in the bladder, and the formation and retention of precipitate in the rat bladder has been linked to the induction of tumors predominantly in the male rat.

Other factors associated with induction of urinary bladder tumors in the rat include high urine volume, low urine osmolality, and intrinsically high urinary protein, especially in male rats. It is noteworthy that saccharin binds to urinary proteins, including $\alpha 2\mu$-globulin which is common in male rats, and that the most extensive mechanistic studies have been conducted only in male rats. Whether the female rat positive urinary bladder response seen in initiation/promotion studies is associated with increased urinary protein and urinary crystal formation has not been adequately studied. Furthermore, extensive mechanistic studies in mice exposed to high doses of sodium saccharin, with or without previous exposure to a urinary bladder initiator, have not been done to definitively rule out the possibility that mice could also develop urinary bladder neoplasia under specific experimental conditions.

The constellation of physiological characteristics of urine in rats fed high doses of sodium saccharin, particularly commencing at times when intrinsic bladder urothelial proliferation is high, would not be expected in humans exposed to normal usage levels of sodium saccharin.

7.1 Mechanism of Urinary Bladder Tumorigenesis Found Predominantly in Male Rats

Long-term studies of sodium saccharin have shown that bladder tumors are the most common malignancies and that they occur predominantly in the male rat. Tumors found in the bladder are detected only when sodium saccharin is fed at high dietary levels (equal to or greater than 1% in rats) beginning at birth or when fetal rats are exposed *in utero* by feeding the dams 5% sodium saccharin diet (Schoenig et al., 1985; for review, see Velazquez et al., 1996). Schoenig et al. (1985) also found that *in utero* exposure was not necessary and that the incidence of bladder tumors in rats given 5% sodium saccharin from birth was essentially identical to that in rats fed 5% sodium saccharin prior to conception and throughout life (for review, see Renwick, 1993; Williams and Whysner, 1996).

Cohen et al. (1991b) offered the following hypothesis to describe the events leading to urinary bladder tumorigenesis in male rats: When sodium saccharin is fed to male rats at high dietary levels (about 2.5%), the concentration of urinary sodium is increased and the pH level is elevated (above 6.5). Under these conditions, binding of saccharin and male-rat-specific α2μ-globulin results in the formation of silicon-containing crystallized precipitate in the bladder (for review, see Ellwein and Cohen, 1990; Burin et al., 1995a; Cohen et al., 1995d; Velazquez et al., 1996). After binding, the precipitate enters the bladder urothelial cells and is cytotoxic. Acting as microabrasives, the silicate and precipitate particles irritate the mucosa and cause focal necrosis. The loss of urothelial cells results in regenerative hyperplasia and increased cell proliferation, which, when sustained over the rats' lifetime, provides the basis for urinary bladder tumorigenesis. Cohen et al. (1991a) further hypothesized that diet-, dose-, species-, and sex-specific effects of saccharin may be related to the formation of the particles (for review, see Burin et al., 1995a; Velazquez, 1996).

7.1.1 The Role of pH in the Promotion of Bladder Carcinogenesis in Male Rats

Studies indicate that a urinary pH higher than 6.5 promotes the tumorigenicity of sodium saccharin in male rats (for reviews, see Murai et al., 1997; Cohen et al., 1995d). For instance, Okamura et al. (1991) compared the effects of sodium saccharin on 5-wk-old male F344 rats initiated with 0.2% FANFT for 4 weeks followed by administration for 100 weeks of either 0 or 5% sodium saccharin in either Prolab 3200 or AIN-76A diet. In rats, administration of AIN-76A diet results in a strongly acidic urine, with a pH lower than 6.0 (for review, see Cohen, 1995c; Velazquez et al., 1996) while Prolab 3200 produces a neutral or slightly alkaline urinary pH (Fisher et al., 1989). [Humans tend to have acidic urine, with a pH between 5.0 and 6.0, although diet can alter this (Cohen, 1995c)]. The data from the study by Okamura et al. (1991) demonstrated that sodium saccharin did not significantly promote urinary bladder tumors in the male rat if fed an AIN-76A diet. However, there was a significant increase the incidence of bladder tumors if male rats were fed the Prolab 3200 diet.

A study by Garland et al. (1989b) also evaluated the responses of 5-wk-old male F344 rats to sodium saccharin administered in different diets. However, while Okamura et al. (1991) used tumor formation as an endpoint, Garland et al. (1989b) looked only at cellular proliferation in the urinary bladder, presumably because of the short duration of the study (10 weeks). Rats were either administered 0 or 7.5% sodium saccharin in Prolab 3200, AIN-76A, or NIH-07 diet and killed after 4 weeks, or they were administered 0, 5, or 7.5% sodium saccharin in these same

diets and killed after 10 weeks. In rats killed after 4 weeks of treatment, there was a significantly higher incidence of hyperplasia with administration of sodium saccharin and this incidence was higher in rats fed the Prolab diet than in rats fed the NIH diet. There was little response when sodium saccharin was administered in the AIN-76A diet. In the group of rats killed after treatment for 10 weeks, there was a similar trend (these rats also demonstrated a dose-dependent increase in hyperplasia). Since the urinary pH of rats fed sodium saccharin in the NIH-07 diet is known to be slightly lower than the urinary pH in rats fed Prolab 3200, and the urinary pH of rats fed AIN-76A is known to be even lower than that of rats fed NIH-07 (ibid.), these results are consistent with the hypothesis that urinary pH participates in the mediation of the proliferative response in urinary bladders of male rats exposed to sodium saccharin.

The findings of Okamura et al. (1991) and Garland et al. (1989b) imply that alkaline urinary pH alone was responsible for mediating urothelial proliferation, but other factors might also explain this phenomenon. For instance, while different diets have been shown to produce different urinary pH levels, they also can produce different levels of ions such as calcium, potassium and sodium, and silicates (Cohen, 1995c). Other studies, however, have supported a role for urinary pH in saccharin-induced carcinogenesis, showing that a pH above 6.5 greatly enhances the formation of the bladder epithelium-irritating urinary silicate crystals in male rats fed sodium saccharin (for review, see Cohen et al., 1991a). For a review of the role of pH in oncogenesis, see Harguindey et al. (1995).

7.1.2 The Role of Sodium Concentration in the Promotion of Bladder Carcinogenesis in Male Rats

There is evidence indicating that induction of bladder carcinogenesis in male rats exposed to saccharin is increased under conditions of high urinary sodium ion concentration. For instance, Hasegawa and Cohen (1986) fed weanling male F344 rats the sodium, potassium, or calcium salt of saccharin, or acid saccharin as 5% of the diet for ten weeks. They found that sodium saccharin induced a significantly higher level of urinary bladder epithelial proliferation than potassium saccharin. Calcium saccharin and acid saccharin, on the other hand, did not significantly change the bladder epithelium. Anderson et al. (1988) found similar results in weanling male CD rats. Like Hasegawa and Cohen (1986), they fed sodium saccharin, potassium saccharin, calcium saccharin, or acid saccharin to rats for 10 weeks and noticed that only sodium saccharin and potassium saccharin produced hyperplasia in the bladder. In a later study by Cohen et al. (1991b), after a 6-wk initiation period with 0.2% FANFT, sodium saccharin, administered as 3% or 5% of the diet for 72 weeks, was shown to be tumorigenic in male F344 rat bladders while calcium saccharin was only slightly so and acid saccharin was not at all.

In a review written by Cohen et al. (1997), it was noted that in rats, oral administration of sodium saccharin causes an increase in cell proliferation in the urothelium that is more pronounced than that induced by potassium saccharin, whereas calcium saccharin produces only slight changes and acid saccharin has no effect on the urinary bladder. It was also noted that these differences in potency occur even though urinary saccharin concentrations do not vary greatly among rats administered the different forms of saccharin. Refer to **Table 7-1** for a summary of the effects of various forms of saccharin on the rat urinary bladder. Refer to **Table 7-2** for results of urine analyses in rats given various forms of saccharin.

While sodium saccharin has been shown to induce carcinogenesis in the male rat bladder, so have many other sodium salts including those of vitamin C (Fukushima et al., 1986), glutamate and bicarbonate (for review see Cohen, 1995b), and succinic acid (Otoshi et al., 1993) (most of these studies did not evaluate the responses of female rats). This implies that the sodium ion, itself, may be at least partially responsible for these effects. Studies supporting this idea include those by Shioya et al. (1994) and Shibata et al. (1989), both of which only evaluated the responses of male rats. A list of sodium salts that produce changes in the rat bladder is provided in **Table 7-3**.

The results of an *in vitro* study performed by Garland et al. (1989a) suggest that the carcinogenic effect on the bladder of a high urinary sodium ion concentration could be mediated by the cytotoxicity of these ions. Transformed rat bladder epithelial cells (sex of donor animals not specified) were incubated in sodium saccharin, calcium saccharin, potassium saccharin, sodium ascorbate, sodium chloride, sodium citrate, potassium chloride, or calcium chloride for 24 hours and then attachment and viability of the cells were assessed. All of the sodium salts (and potassium saccharin) decreased cell attachment and viability, while potassium chloride and calcium chloride did neither. Calcium saccharin decreased only cell viability.

Another possible mechanism for sodium-induced carcinogenesis is direct induction of cellular proliferation and/or DNA synthesis by sodium ions (for review, see Cohen, 1995c). Several *in vitro* studies support this hypothesis. For example, Burns and Rozengurt (1984) used confluent quiescent Swiss mouse 3T3 cells to demonstrate that initiation of DNA synthesis in these cells by various stimulants was inhibited by limiting extracellular sodium ion concentration. Normally, 3T3 cells will initiate DNA synthesis when growth factors are included in their incubation media. However, when Burns and Rozengurt (1984) included one growth factor (i.e., epidermal growth factor, vasopressin, or insulin) in the media (serum-free), and removed extracellular sodium ions, there was no initiation of DNA synthesis.

Another study by Cameron et al. (1980) evaluated intracellular sodium ion concentrations in slowly and rapidly dividing cells, and in tumor cells. They found that sodium ion concentrations were highest in tumor cells and lowest in slowly dividing cells. They concluded that high sodium ion concentrations were, associated with mitogenesis while very high levels were associated with oncogenesis. However, the studies do not necessarily provide any support for the hypothesis that extraneous high sodium ion concentrations were responsible for induction of cellular proliferation or oncogenesis.

The most likely mechanism for a carcinogenic response to sodium saccharin mediated by sodium ions is the interation of sodium ions with proteins in the urine (Cohen, 1995c). It has been shown that urinary proteins in rats bind to saccharin to produce a crystallized precipitate (Cohen, 1995b; Cohen et al., 1995a), which may act as an abrasive in the rat bladder, causing regenerative hyperplasia (Cohen et al., 1990; Hicks, 1984). The formation of this precipitate is greatly enhanced by high sodium ion concentrations (Cohen et al., 1991a), thus raising the possibility that high sodium ion concentration is a necessary condition for precipitate formation.

Renwick (1993) stated that the urinary concentration of the anion of sodium saccharin does not play a role in the overall mechanism for tumorigenesis in the rat bladder. In addition, Renwick (1993) suggests that dietary sodium saccharin provides a vehicle for the delivery of "massive" but non-toxic amounts of sodium ions to the urinary bladder. However, the sodium

ion concentrations in the feed containing carcinogenic doses of sodium saccharin are not much higher than in the rat feed alone. For example, Purina Rodent Chow consists of 0.3% sodium ions or 3000 ppm. When comparing this concentration to the highest sodium saccharin concentration known to promote tumorigenesis (7.5% or 75,000 ppm), we calculated that the sodium ion concentration in the feed at this dose was approximately 3-fold that found in a typical rat chow (75,000 ppm x 12.5% sodium ions in sodium saccharin = 9400 ppm). Although a 7.5% sodium saccharin diet increased the concentration of sodium ions approximately 3-fold, this concentration scarcely represents a large increase from the usual daily dietary intake of sodium ions.

7.1.3 The Combined Effect of pH Level and Sodium Concentration

While both pH and sodium ions have been shown to affect cell proliferation in the bladder, most likely these two parameters do not act in isolation but are part of a set of parameters that regulate tumorigenesis. This hypothesis is supported by a study conducted by Fukushima et al. (1988) in which male F344 rats were initiated with 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) and then fed a diet containing either 3% sodium bicarbonate, 1% sodium chloride, or a control diet. Sodium bicarbonate was found to increase urinary pH and sodium ion concentration and promote urinary bladder carcinogenesis. Administration of sodium chloride produced an increase in urinary sodium ions but not pH, and did not promote urinary bladder carcinogenesis.

Ito and Fukushima (1989) also found that both elevated pH and elevated sodium ion concentration were necessary conditions for induction of bladder tumorigenesis. They initiated male rats with 0.05% BBN and then administered either ascorbic acid, sodium ascorbate, sodium bicarbonate, or ammonium chloride alone or in several different combinations. Promotion of urinary bladder carcinogenesis occurred only under conditions of both elevated urinary pH and elevated urinary sodium ion concentration, induced by the administration of sodium bicarbonate and sodium ascorbate.

7.1.4 The Association Between Increased Urinary Output and Sodium Saccharin-Induced Bladder Tumors

Schoenig et al. (1985) found that rats that ingested 7.5% sodium saccharin in a two-generation bioassay and developed bladder tumors had a higher urine volume throughout their lives than did those that did not develop bladder tumors. Schoenig et al. (1985) also found that the difference in urine volume between the non-tumor bearing group and the untreated controls was almost as great as the difference between the sodium saccharin-treated tumor bearing and non-tumor bearing rats.

Anderson et al. (1987b) studied the effect of inherent urine output (high urine volume or low urine volume) on the response of male rats fed 7.5% dietary sodium saccharin for 10 weeks. Rats exposed to 7.5% dietary sodium saccharin for 10 weeks showed an increased incidence of bladder epithelial hyperplasia (12/20 rats exposed to sodium saccharin vs. 2/20 controls). The incidence of hyperplasia was similar (6/10) in the sodium saccharin high and low urine output groups. One of the two control rats that had hyperplastic lesions in the bladder showed evidence

of inflammation and had a higher than average urine output, while the other had the highest urine output in the control group (73 g/kg).

Anderson et al. (1987a) found that high urine output rats in the control group consumed more feed than those in the low urine output control group. Therefore, the authors compared the mean daily sodium saccharin consumption between the exposed high and low urine output groups (g/kg bw) for the 10-week period. The authors found that the high urine output group consumed 8.0 ± 0.2 g/kg bw and the low urine output group consumed 7.8 ± 0.2 g/kg bw feed containing 7.5% sodium saccharin on an average daily basis. However, urinary concentrations of saccharin were similar in the high and low urine mass groups (41 ± 3 and 46 ± 2 mg/mL, respectively). Thus, Anderson et al. (1987a) concluded that it is unlikely that a difference in urinary saccharin concentration or total saccharin exposure can account for the role of high urinary volume in saccharin-associated bladder tumorigenicity.

7.2 Dose Response in Cell Proliferation and Tumorigenesis

Numerous studies have been conducted that suggest high doses of sodium saccharin produce urinary bladder tumors in male rats. For example, Cohen et al. (1991b) and Fukushima et al. (1986) have demonstrated that the effects of high dietary concentrations of sodium saccharin on male rat bladder epithelium are associated with increased urinary bladder tumor promotion. Cohen et al. (1989 abstr.) found that feeding male rats high doses of sodium saccharin (7.5%) beginning 5 weeks after birth increased cell proliferation in the bladder urothelium. Cohen and Ellwein (1991) suggested that approximately one-third of the total mitoses of the urothelium occurs within the first 3 weeks of a rat's life. Therefore, when sodium saccharin dosing begins at birth, rather than after weaning, rats are somehow more susceptible to sodium saccharin-induced tumors in later life due to the increased cell proliferation occurring at this time. The increases of cell proliferation observed after short periods of high sodium saccharin administration are dose-responsive. Details of carcinogenesis experiments mentioned herein are in **Table 4-1**.

Schoenig et al. (1985) conducted a 2-generation rat bioassay on sodium saccharin. This study involved 2500 second-generation male Charles River CD rats (F_1, between 21 and 38 days of age; 6 treatment groups, 125 to 700 rats per group) receiving 1, 3, 4, 5, 6.25, and 7.5% sodium saccharin in their diet for up to 30 months. The parents (F_0) of the F_1 generation had been maintained on diets containing between 1 and 7.5% sodium saccharin. Except during mating, gestation, and lactation, all animals were housed individually in a single environmentally controlled room. The data resulting from this experiment, designed to determine the dose-response for urinary bladder tumors, indicated that a 1% dietary level of sodium saccharin represented a no-effect level. Higher dietary concentrations showed a very steep dose-response, indicating that tumor incidence increased rapidly with an increase in the dose. For example, significant increases in the incidence of primary neoplasia (benign and malignant tumors) in the urinary bladder of F_1 male rats sacrificed during month 15 of this study were not found in the 1.0 or 3.0% sodium saccharin group. However, pairwise comparisons between the control group (0.0% total primary neoplasia) and all groups treated with 4, 5, 6.25, and 7.5% sodium saccharin showed significant increases in the incidence of benign (2.1, 3.3, 10.0, and 15.3%, respectively) and malignant tumors (4.2, 9.2, 6.7, and 16.1%, respectively) alone as well as of total primary

bladder neoplasia (6.3% to 31.4%). Total primary bladder neoplasia was also significantly higher in the 3.0% sodium saccharin group (1.7%). Therefore, the 1.0% sodium saccharin dietary level was considered to be a no-effect level for bladder neoplasia. However, 5 bladder tumors were found in the 1% sodium saccharin group and none were found in the concurrent controls. This finding prompted the authors to review the historical control data for the incidence of primary urinary bladder tumors in male Charles River CD rats at IRDC (Squire, 1985). The review included 10 studies that examined the urinary bladders from 982 male controls either *in utero* or over a lifetime. Of these animals, 863 survived for 67 weeks, which corresponds to the appearance of the first urinary bladder tumor observed in the bioassay conducted by Schoenig et al. (1985). No primary urinary bladder tumors appeared prior to week 67 in the controls of the ten studies reviewed. The percentage incidence of tumors was calculated from historical controls by using the number of rats that survived until the first bladder tumor was observed as the denominator. These data showed that total primary bladder neoplasia ranged from 0.0 to 3.3% with a mean of 0.8%. The corresponding incidence of total primary bladder neoplasia at the 1% dietary sodium saccharin observed by Schoenig et al. (1985) was also 0.8%. These findings suggest that the NOEL (1% sodium saccharin dietary level) proposed by Schoenig et al. (1985) is not significantly different from the results obtained from the controls (0.8%) studied by Squire (1985), and that the background tumor incidence for this strain of rat at IRDC was identical to that observed in the 1% sodium saccharin group (0.8%) studied by Schoenig et al. (1985).

Murusaki et al. (1981), who studied the light microscopic and electron microscopic changes in the bladder of rats fed sodium saccharin (dietary concentrations between 0.1 and 5%), also reported a steep dose-response curve over a narrow range of dose levels above 1%. Furthermore, Nakanishi et al. (1980a) and West et al. (1986), using light microscopy, autoradiography, and scanning electron microscopy, detected cellular responses in male rat bladders only with sodium saccharin dietary concentrations of 2.5% to 5% beginning at 6 to 8 weeks of age. Chappel (1992) reviewed and assessed the biological risk of sodium saccharin. The author stated that the steep dose-response curves representing both physiological changes in the urine and morphological changes in the urothelium provide strong evidence of a common threshold at a sodium saccharin dietary concentration between 1 and 3%. To Chappel (1992), these results provided strong evidence that these phenomena are interrelated.

Ellwein and Cohen (1988), using model-based simulations, demonstrated that the proliferative effects (hyperplasia; increase in LI) following high doses of sodium saccharin are sufficient to explain tumorigenic effects in the rat urinary bladder without having to postulate a genotoxic influence. Their database was generated from a large series of experiments dealing with the increase in LI and hyperplasia after the administration of high doses of sodium saccharin. The authors postulated a tumorigenic effect secondary to sodium saccharin administration only if it is administered during the neonatal period at a dose which will generate a cell proliferative response in the urothelium; and after weaning when ulcerations of the bladder occur. The authors suggested that a dietary level of at least 1% sodium saccharin is necessary for a cellular response to occur in the rat bladder, even though most experiments aimed at cellular responses detected by light microscopy, autoradiography, or scanning electron microscopy (West et al., 1986; Murusaki et al., 1981; Nakanishi et al., 1980a) have found these effects only at doses of 2.5% or higher.

Like Chappel (1992), Ellwein and Cohen (1990) suggested that saccharin exhibits a biological threshold.

7.3 Relevance of Animal Cancers To Humans

Numerous studies have investigated the carcinogenicity of sodium saccharin in rats (Cohen et al., 1995b; Cohen et al., 1990; Anderson, 1988; for review, see Oser, 1985; Williams and Whysner, 1996), mice (for review, see Oser, 1985), non-human primates (Thorgeirsson et al., 1994) and humans (Risch et al., 1988; for reviews, see Elcock and Morgan, 1993; Chappel, 1992; Ellwein and Cohen, 1990; Morgan and Wong, 1985). These studies have revealed that it is mainly the male rat which is susceptible to the formation of bladder tumors following chronic exposure to high doses of sodium saccharin (Cohen, 1995b; Chappel, 1992), i.e., greater than or equal to 1% of the diet (Ellwein and Cohen, 1990). A summary of positive mammalian carcinogenicity studies is presented in **Table 7-4**. An interspecies comparison of the effects of sodium saccharin on the urinary bladder is presented in **Table 7-5**, and a comparison of the effects of sodium saccharin in various rat strains is presented in **Table 7-6**.

Results from animal studies suggest that there is an intrinsic difference between male rats and other animals in how they react to sodium saccharin exposures and, in particular, they imply that there may be a peculiarity of the male rat bladder which makes the male rat uniquely susceptible to cancer of this organ following sodium saccharin exposures. Most likely, this peculiarity is not of a genetic origin but is, rather, physiologically based (Weisburger, 1990), since sodium saccharin has been shown to be non-genotoxic *in vivo* (Ellwein and Cohen, 1990; Ashby, 1985; Lutz and Schlatter, 1977).

If the male rat bladder is indeed a unique organ with respect to its response to sodium saccharin, it would have to be concluded that male rats do not accurately represent humans when considering such a response and that it would therefore not be appropriate to extrapolate data from male rat exposure studies to humans. This section will investigate the validity of these statements by comparing the anatomy and physiology of the male rat bladder with the human bladder.

7.3.1 Comparative Bladder Anatomy and Urine Chemistry

The anatomy of the rat bladder is significantly different than that of the human bladder. For instance, the rat bladder is an abdominal organ, while the human bladder progresses from an abdominal organ in infancy and childhood to a pelvic organ in adulthood when the pelvis is fully developed and upright posture of the body is achieved (DeSesso, 1995).

The upright/vertical posture of mature humans versus the horizontal posture of rats is highly relevant to the nature of bladder response to sodium saccharin when the process of urination is considered. Specifically, it is known that the vertical position of humans allows for a more efficient elimination of calculi from the bladder while the horizontal position of the rat during urination leaves the rat more prone to retention of such material (Burin et al., 1995b; Cohen, 1995b).

Although other animals (e.g., mice) that maintain a horizontal position may also be susceptible to calculus retention, this phenomenon is uniquely relevant to rats when exposure to sodium saccharin is considered. This is due to the fact that sodium saccharin has been shown to

induce precipitate formation solely in male rat urine (see **Table 7-5**) (Cohen, 1995b; Cohen et al., 1995a; Cohen et al., 1991a), and the formation and retention of this precipitate has been linked to the formation of tumors of the male rat bladder (Cohen 1995b). Tumor formation may be the result of chronic irritation, and the damage it causes to bladder urothelium (Burin et al., 1995b; Clayson et al., 1995; Ellwein and Cohen, 1988). The precipitate is composed of mainly calcium phosphate, but also contains silicate, protein, saccharin, sulfur-containing substances, potassium, and chloride (Cohen, 1995b), and is jagged in nature (Cohen et al., 1989 abstr.).

When the urothelium is damaged by abrasion, regenerative hyperplasia is likely to occur (Cohen et al., 1990; Hicks, 1983). This results in an increase in the number of urothelial cell divisions (Cohen and Lawson, 1995) which may lead to tumor formation (Cohen and Ellwein, 1991).

After sodium saccharin exposure, the formation of precipitate in the male rat urine is thought to be the result of an interaction in the urine between saccharin and the male rat-specific protein, α2μ-globulin (Murai et al., 1997; Garland et al., 1994; Swenberg et al., 1992). Alpha 2μ-globulin is a low-molecular-weight protein, weighing less than 40 kDa (Hard, 1995). It is synthesized in the liver and is quantitatively the major protein found in male rat urine (Roy and Neuhaus, 1966). It is not present in significant quantities in female rat urine and is not synthesized by humans (Hard, 1995).

It has been shown that rats lacking α2μ-globulin are not as subject to bladder cell proliferation following sodium saccharin exposure as are rats producing this protein. Uwagawa et al. (1994) used the male NBR rat, which does not synthesize α2μ-globulin, and the male F344 rat, which does, to demonstrate this. After chronic administration (starting at 6 weeks of age) of a diet containing 5% sodium saccharin, the F344 rat showed signs of cellular proliferation in the bladder urothelium, but the NBR rat did not.

A study by Garland et al. (1994) supports the findings of Uwagawa et al. (1994). Four- to 5-week-old intact F344, castrated F344, and NBR rats were administered 7.5% sodium saccharin in the diet for 10 weeks. Less cellular proliferation occurred in the bladders of the castrated rats, which had reduced levels of α2μ-globulin, than in the bladders of intact F344 rats. Even less proliferation was seen in the bladders of NBR rats, which had lower levels of α2μ-globulin than the castrated rats.

Since α2μ-globulin is normally specific to the male rat and since this protein is thought to be at least partially responsible for the carcinogenicity of sodium saccharin in the bladder, α2μ-globulin in the urinary bladder is probably the physiologic peculiarity that renders the male rat bladder susceptible to a carcinogenic response to sodium saccharin (for review, see Swenberg et al., 1992). However, it is important to note that while Uwagawa et al. (1994) and Garland et al. (1994) demonstrated an association between the presence of α2μ-globulin in the male rat bladder and the occurrence of cellular proliferation of the bladder, no studies were found which evaluated the role of α2μ-globulin in the formation of tumors in these animals.

It is also noteworthy that saccharin binds to other proteins besides α2μ-globulin and that most extensive mechanistic studies have been conducted only in male rats. Whether the female

rat positive bladder response seen in I/P studies is associated with an increase in protein has not been studied.

Another problem with the accuracy of this hypothesis arises from integrating studies investigating the critical age of sodium saccharin administration to male rats for induction of bladder tumors with those investigating the age-dependent expression of α2μ-globulin. It is thought that sodium saccharin produces urinary bladder tumors in male rats only if it is administered before the rats reach 35 days of age (Cohen et al., 1995b) unless exposure occurs after administration of an initiating agent (for review, see Cohen et al., 1995a). In several studies in which rats were exposed to sodium saccharin beginning after this time period, there was no increase in the incidence of bladder tumors in male rats (e.g., Homma et al., 1991; Murasaki and Cohen, 1981; Hooson et al., 1980; Schmähl, 1973; cited by IARC, 1980; for reviews, see Cohen and Ellwein, 1991a; National Academy of Sciences-National Research Council, 1974; cited by Arnold et al., 1980). It has also been shown that hepatic synthesis of α2μ-globulin in the male rat does not begin until 35 to 40 days of age (Roy et al., 1983) and is thus undetectable in male rats below this age (Neuhaus and Flory, 1978). Therefore, the time of susceptibility to induction by sodium saccharin of cellular proliferation in the bladders of male rats does not correlate with the presence of α2μ-globulin in these rats. While this does not necessarily preclude a role for α2μ-globulin in sodium saccharin carcinogenesis, it does raise some doubts.

While the possibility of a role for α2μ-globulin in sodium saccharin carcinogenesis is attractive because it can account for differences in species (e.g., rat and human) and sex (e.g., male and female rats) responses to sodium saccharin, other mechanisms of sodium saccharin carcinogenesis could exist that would also successfully explain these differences. For instance, proteins other than α2μ-globulin may be responsible for the unique vulnerability of male rats to sodium saccharin-induced bladder tumorigenesis. Since male rats have up to 10 times more total protein in their urine than female rats (Lehman-McKeeman and Caudill, 1991) and about 90 times more total urinary protein than humans (Olson et al., 1990), the idea that a protein other than α2μ-globulin can account for species and sex differences in sodium saccharin response is not implausible (for an interspecies comparison of urine chemistries see **Table 7-7**). Few studies have investigated this hypothesis, although the role of albumin was examined by Homma et al. (1991). This group compared the response of analbuminemic rats to sodium saccharin exposure to the response of Sprague-Dawley rats. Neither strain developed abnormal bladder growths and the study was inconclusive. Since albumin levels in humans are known to be higher than levels in male rats (Hard, 1995), future studies should probably focus on investigating low-molecular-weight proteins other than α2μ-globulin that are more abundant in male rats than in female rats or humans (Olson et al., 1990).

7.3.2 Dose-Response Extrapolation

Two major issues to consider when deciding if dose-extrapolation from rats to humans is appropriate are the nature of the carcinogenic mechanism (i.e., does it operate in both rats and humans?) and the presence or absence of a threshold in dose-response. In fact, the majority of data summarized in previous sections of this document indicate that the carcinogenic mechanism of sodium saccharin may be unique to male rats, and that there is a threshold dose.

There is significant data indicating that the mechanism of sodium saccharin-induced bladder carcinogenesis in male rats is related to the formation and retention of urinary precipitate formed under conditions of high urinary pH and high sodium concentration and that this precipitate does not form in other species.

Studies using diets varying in pH have shown that sodium saccharin does not significantly promote proliferation in the male rat urinary bladder when fed in the acidic AIN-76A diet, but sodium saccharin does increase urothelial proliferation when fed in the alkaline Prolab 3200 diet. It has also been shown that in rats, oral administration of sodium saccharin causes an increase in cell proliferation in the urothelium that is more pronounced than that induced by potassium saccharin, whereas calcium saccharin produces only slight changes, and acid saccharin has no effect on the urinary bladder, even though urinary saccharin concentrations do not vary greatly between the different groups of rats. In addition, while sodium saccharin has been shown to induce carcinogenesis in the male rat bladder, so have other sodium salts.

There is also evidence from a number of studies that a threshold dose exists in male rats for sodium saccharin-induced bladder carcinogenesis, suggesting that use of a linear dose-response model is not appropriate to estimate risk in humans.

7.4 Additional Mechanistic Information

7.4.1 Inhibition of Apoptosis (Programmed Cell Death)

Wright et al. (1994) reported that pretreatment with saccharin inhibited apoptosis (specifically the DNA fragmentation induced by UV light or tumor necrosis factor) in human histiocytic (U937) lymphoma cells.

7.4.2 Intercellular Communication

A review by IARC (1987a,b) reported that saccharin (form unspecified) inhibited intercellular communication in mammalian cells *in vitro* in two studies but not a third. These studies administered doses that were 1/2 those used in the tumor-positive rat studies. In a later review by Klaunig and Ruch (1990), the authors reported that saccharin inhibited intercellular communication in Chinese hamster lung V79 cells but not in primary mouse hepatocytes.

Table 7-1. Effect of Various Forms of Saccharin on the Rat Urinary Bladder

Treatment[a]	Simple Hyperplasia	Microvilli on SEM		Labeling Index (%)
		Uniform	Pleomorphic	
sodium saccharin	5/12[b]	2/6	2/6	0.55 ± 0.20 (5)[c]
potassium saccharin	2/12	2/6	0/6	0.18 ± 0.09 (6)[d]
calcium saccharin	2/12	1/6	0/6	0.12 ± 0.11 (6)
acid saccharin	0/12	0/6	0/6	0.07 ± 0.04 (6)
control	0/12	0/6	0/6	0.06 ± 0.04 (6)

[a] 5% in diet for 10 wk
[b] significantly different from acid saccharin and control group, $p < 0.02$
[c] significantly different from all other groups, $p < 0.01$
[d] significantly different from control group, $p < 0.05$

Source: Cohen (1994a)

Table 7-2. Urine Analysis in Rats Given Various Forms of Saccharin

Treatment[a]	Urine Volume (mL/day)	Saccharin (mmol/mL)	pH	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{2+} (mEq/L)	Osmolality (mOsm/L)
sodium saccharin	10.4	0.17	7.2	291	151	24.8	1520
potassium saccharin	13.5	0.14	6.8	153	298	23.9	1463
calcium saccharin	6.3	0.14	5.7	158	236	41.2	2145
acid saccharin	8.8	0.19	5.5	139	164	51.6	2029
control	6.7	0	7.1	158	201	34.5	1678

[a] 5% in diet for 4 wk

Source: Cohen (1994b)

Table 7-3. Sodium Salts That Produce Urothelial Hyperplasia and Increase the Incidence of Bladder Tumors in Rats Fed High Doses (> 1%)

Sodium ascorbate
Sodium aspartate
Sodium bicarbonate
Sodium chloride
Sodium citrate
Sodium erythorbate
Sodium glutamate
Sodium phosphate
Sodium phytate
Sodium saccharin
Sodium succinate

Source: Cohen et al. (1997)

Table 7-4. Summary of Positive Mammalian Carcinogenicity Studies

Age, Strain, Species	Chemical Form	Effective Dose and Duration	Primary Tumor Location	Comments on Mechanism of Action	Reference
Mice					
'stock' mice (age not specified)	saccharin[a]	2 mg saccharin/8 mg cholesterol pellets implanted in urinary bladder lumina for 52 wk (1-generation study)	urinary bladder	The presence of the cholesterol pellet in the bladder had a promoting action; saccharin was an incomplete carcinogen.	Allen et al. (1957)
6-wk-old albino mice	saccharin[a]	1.5 g/kg in 1 mL distilled water, force fed for 1 yr (1-generation study)	thyroid	Mechanism unknown, but results are questionable because control incidence was not reported, statistical analysis was not performed, sample size was small, purity of saccharin was not reported, and results have not been replicated.	Prasad and Rai (1986)
18- to 19-wk-old BALB/c mice	sodium saccharin	5.0% diet for 117 wk (1-generation study)	Harderian gland	No dose-response demonstrated. Marginally significant for trend. Probably not applicable to humans, since they only have rudimentary Harderian gland.	Frederick et al. (1989)
Rats					
Charles River CD rats (age not specified)	sodium saccharin	7.5% in diet for 28 mo (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Taylor and Friedman (1974 abstr.)
Weanling SD rats (age not specified)	sodium saccharin	5% in diet for 100 wk (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Tisdel et al. (1974)
32-day-old SD rats	sodium saccharin	5% in diet for 90 days (adults) or ~700 days (pups) (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Arnold et al. (1980)
in utero Charles River CD rats	sodium saccharin	7.5% in diet for ≤ 2 yr (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Taylor et al. (1980)
6-wk-old ACI rats[b]	sodium saccharin	5% in diet for 12 mo (1-generation study)	urinary bladder	Mechanism specific to males fed high dose. *Trichosomoides crassicauda* infection enhanced sodium saccharin-induced cell proliferation in urinary bladder.	Fukushima et al. (1983)
6-wk-old F_0 and 28- to 38-day-old F_1 Charles River CD rats	sodium saccharin	3.0, 4.0, 5.0, 6.25, or 7.5% in diet for 30 mo (2-generation study)	urinary bladder	Mechanism specific to males fed high dose.	Schoenig et al. (1985)

[a] No distinction was made between saccharin and its sodium salt
[b] At least half of these rats were infected with the bladder parasite *Trichosomoides crassicauda*

Table 7-5. Interspecies Comparison of the Effects of Sodium Saccharin on the Urinary Bladder

Species	Bladder Hyperplasia[a]		Bladder Carcinogenesis[a]		Bladder Promotion[b]	Urinary Precipitate[b]
	1 Generation	2 Generation	1 Generation	2 Generation		
Hamster	+ (0) - (1)	NE	+ (0) - (1)	NE	NE	NE
Mouse	+ (0) - (1)	NE	+ (2)[c] - (6)	NE	-	-
Rat	+ (17) - (7)	+ (1) - (0)	+ (1)[d] - (14)	+ (5) - (0)	+	+
Guinea Pig	+ (0) - (1)	NE	NE	NE	NE	NE
Monkey	+ (0) - (1)	NE	+ (0) - (4)	NE	NE	-

NE = not evaluated

[a] Number of positive (+) and negative (-) studies in parentheses; data summarized from Tables 4-1 and 6-1
[b] Adapted from Cohen (1994c)
[c] These two studies were equivocal.
[d] This study was equivocal.

Table 7-6. Interstrain Comparison of the Effects of Sodium Saccharin on the Rat Urinary Bladder[a]

Rat Strain	Bladder Hyperplasia		Bladder Carcinomas	
	1 Generation	2 Generation	1 Generation	2 Generation
ACI	+ (1)[b] - (0)	NE	+ (1)[b] - (0)	NE
Charles River CD	NE	+ (1) - (0)	+ (0) - (2)	+ (3) - (0)
F344	+ (10) - (4)	NE	+ (0) - (1)	NE
NBR[c]	+ (0) - (1)	NE	NE	NE
Osborne-Mendel	NE	NE	+ (0) - (1)	NE
Sprague-Dawley	+ (4) - (1)	NE	+ (0) - (4)	+ (2) - (0)
Wistar	+ (1) - (2)	NE	+ (0) - (6)	NE

NE = not evaluated

[a] Number of positive (+) and negative (-) studies in parentheses; data summarized from Tables 4-1 and 6-1
[b] This study was equivocal; at least half of the rats were infected with the bladder parasite *Trichosomoides crassicauda*.
[c] NBR rats do not synthesize α2μ-globulin.

Table 7-7. Interspecies Comparison of Fresh Void Urine Chemistry

Species Treatment	pH	Protein (mg/mL)	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mg/dL)	Creatinine (mg/dL)	Phosphorus (mg/dL)	Urea (mg/dL)	Chloride (mEq/L)	Magnesium (mg/dL)
Human										
Male	6.4 ± 0.23	0.02 ± 0.00	160 ± 18.8	63 ± 10.7	16.6 ± 3	119 ± 15.8	42 ± 7.7	780 ± 87	160 ± 17.2	9.2 ± 1.9
Female	5.8 ± 0.16	0.03 ± 0.01	140 ± 16.9	62 ± 10.1	11.9 ± 2	103 ± 17.3	42 ± 7.7	721 ± 114	162 ± 20.7	6.6 ± 1.3
Monkey-Cyano										
Control Male	7.2 ± 0.67	0.02 ± 0.02	15 ± 5.0	13 ± 11.0	4 ± 1.0	15 ± 6	1.5 ± 0.5	119 ± 98	15 ± 0	1.5 ± 0.5
NaSac Male	7.0 ± 0.56	0.09 ± 0.02	10 ± 0	47.5 ± 43.5	58 ± 52	71.5 ± 58.5	1 ± 0	468 ± 406	16 ± 1.0	14 ± 12
Control Female	6.7 ± 0.19	0.14 ± 0.13	46.5 ± 36.5	58.5 ± 29.5	55 ± 54	131 ± 102	1 ± --	792 ± 588	39 ± 24	11.5 ± 10.5
NaSac Female	6.8 ± 0.32	0.17 ± 0.07	16.5 ± 6.5	19 ± 7	13 ± 4	30 ± 7	2 ± 0	376 ± 114	14 ± 1	7.5 ± 3.5
Monkey-Rhesus										
Control Male	7.0 ± 0.11	0.13 ± 0.05	13.5 ± 3.5	21 ± 13	12 ± 2	25.5 ± 11.5	1 ± 0	219 ± 115	15 ± 0	5 ± 0
NaSac Male	7.0 ± 0.24	0.10 ± 0.03	15 ± 5	31 ± 25	10 ± 6	40.5 ± 24.5	1 ± 0	326 ± 249	25 ± 10	5.5 ± 3.5
Control Female	6.8 ± 0.75	0.07 ± 0.01	10 ± 0	11 ± 5	5.5 ± 0.5	18.0 ± 1.0	1 ± 0	138 ± 8.5	15 ± 0	2.5 ± 0.5
NaSac Female	6.5 ± 1.4	0.08 ± 0.08	10 ± 0	9.5 ± 7.5	3 ± 1	16 ± 6	1.5 ± 0.5	94 ± 61	15 ± 0	1.5 ± 0.5
Mouse										
Control Male	7.2 ± 0.07	1.10 ± 0.03	214 ± 22	216 ± 11.2	3 ± 0.38	20.2 ± 0.65	130 ± 6.5	2845 ± 131	218 ± 19	27.8 ± 3.5
Rat										
Control Male	6.8 ± 0.13	1.7 ± 0.08	199 ± 9.7	406 ± 19.4	6.8 ± 0.4	117 ± 4.0	223 ± 6.4	4069 ± 163.0	242 ± 11.6	29 ± 2.1
NaSac Male	6.5 ± 0.05	0.30 ± 0.05	251 ± 17.3	130 ± 7.5	12.5 ± 0.6	37 ± 2.6	136 ± 9.4	1328 ± 88.0	80.9 ± 6.0	43.6 ± 3.9
Control Female	7.2 ± 0.09	0.20 ± 0.06	222 ± 20.3	437 ± 38.7	14.8 ± 0.9	113 ± 7.0	250 ± 14.8	4281 ± 270.0	264 ± 22.5	36 ± 4.6
NaSac Female	6.6 ± 0.07	0.10 ± 0.02	274 ± 15.1	141 ± 3.7	14.9 ± 1.3	40 ± 1.7	142 ± 7.2	1591 ± 62.0	88 ± 3.4	56.3 ± 3.2

NaSac = sodium saccharin

Source: Cohen (1994d)

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APPENDIX A

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Volume 22 (Some Non-Nutritive Sweetening Agents) Saccharin, pp. 111-185, 1980

APPENDIX B

Excerpts from the IARC Monograph on the
Evaluation of the Carcinogenic Risk of Chemicals to Humans
Supplement 4 (Chemicals, Industrial Processes and Industries
Associated with Cancer in Humans, IARC Monographs Volumes 1 to 29)
Saccharin
pp. 224-226, 1982

APPENDIX C

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Supplement 7 (Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42) Saccharin pp. 334-339, 1987

APPENDIX D

Description of Online Searches for Saccharin and Saccharin Salts

DESCRIPTION OF ONLINE SEARCHES FOR SACCHARIN AND SACCHARIN SALTS

Initial online searches for saccharin [CASRN 128-44-9] and its sodium [81-07-2, anhydrous; 6155-57-3, dihydrate] and ammonium [6381-61-9] salts were performed in February and March 1996 in databases on the systems of STN International, DIALOG, NLM's TOXNET, and the Chemical Information System. Toxicology information was sought in the databases CCRIS (Chemical Carcinogenesis Research Information System), CHEMHAZIS (from the NTP Chemical Repository), EMIC, EMICBACK, GENETOX, RTECS (one record for each), and TOXLINE (name and CASRNs combined with terms for metabolism and the MESH heading for all neoplasms). Since that time, we have monitored 1200 life sciences journals for saccharin information using Current Contents on Diskette® (and cumulative issues on CD-ROM). We monitored not only for saccharin but also for information on rat bladder carcinogenesis induced by other chemicals and for articles by S. Cohen. We had requested and received many reprints on these topics by the time work resumed on this compound in 1997.

Market information, including production, shipments, sales and consumption, labor use, and workers by type was sought in IAC PROMT and the FOODLINE files Food Science and Technology and International Food Market Data in March 1996.

Regulatory information was sought in March 1996 from CHEMTOX and the FOODLINE file CURRENT FOOD LEGISLATION and more recently from the in-house FESA CD-ROM containing the latest *Code of Federal Regulations* and the *Federal Register* pertaining to the title 21 (FDA) and title 40 (EPA) regulations.

APPENDIX E

Listing of GAP Test Codes in Phylogenetic Order
For Saccharin and Sodium Saccharin

LISTING OF GAP TEST CODES IN PHYLOGENETIC ORDER FOR SACCHARIN AND SODIUM SACCHARIN

Prokaryotic Systems:

PRB = Prophage, induction, SOS repair, DNA strand breaks or cross-links
SA5 = Salmonella typhimurium TA1535, reverse mutation
SA7 = Salmonella typhimurium TA1537, reverse mutation
SA8 = Salmonella typhimurium TA1538, reverse mutation
SA9 = Salmonella typhimurium TA98, reverse mutation
SA0 = Salmonella typhimurium TA100, reverse mutation

Lower Eukaryotic Systems:

SCG = Saccharomyces cerevisiae, gene conversion
SCH = S. cerevisiae, homozygosis by recombination or gene conversion
SCR = Saccharomyces cerevisiae, reverse mutation
SCN = Saccharomyces cerevisiae, aneuploidy
DMX = Drosophila melanogaster, sex-linked recessive lethal mutation
DMH = Drosophila melanogaster, heritable translocation test

Mammalian Systems in vitro:

DIA = DNA strand breaks, cross-links or rel. damage, animal cells in vitro
G5T = Gene mutation, mouse L5178Y cells in vitro, TK locus
SIC = Sister chromatid exchange, Chinese hamster cells in vitro
CIC = Chromosomal aberrations, Chinese hamster cells in vitro
TBM = Cell transformation, BALB/C3T3 mouse cells
TCM = Cell transformation, C3H10T1/2 mouse cells
TRR = Cell transformation, RLV/Fischer rat embryo cells
SHL = Sister chromatid exchange, human lymphocytes in vitro
CHL = Chromosomal aberrations, human lymphocytes in vitro

Mammalian Systems in vivo:

BFA = Body fluids from animals, microbial mutagenicity
DVA = DNA strand breaks, cross-links or rel. damage, animals in vivo
MST = Mouse spot test
SVA = Sister chromatid exchange, animal cells in vivo
MVM = Micronucleus test, mice in vivo
CBA = Chromosomal aberrations, animal bone-marrow cells in vivo
CGC = Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG = Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
DLM = Dominant lethal test, mice

*** Alternative test codes (not shown in profiles)**

BVD = Binding (covalent) to DNA, animal cells in vivo
ICR = Inhibition of intercellular communication, rodent in vitro
ICR = Inhibition of intercellular communication, rodent in vitro
ICR = Inhibition of intercellular communication, rodent in vitro
SPM = Sperm morphology, mouse

APPENDIX F

Listing from the Eighth Report on Carcinogens

SACCHARIN
CAS No. 128-44-9
First Listed in the *Second Annual Report on Carcinogens*

CARCINOGENICITY

There is sufficient evidence for the carcinogenicity of saccharin in experimental animals (IARC V.22, 1980; IARC S.4, 1982; IARC S.7, 1987). Saccharin is produced commercially as calcium and sodium salts (6485-34-3 and 128-44-9, respectively) as well as the free acid, and the name saccharin has been applied to all these chemicals. When saccharin was administered in the diet or drinking water, increased incidences of lymphomas/leukemias and transitional cell carcinomas of the urinary bladder were seen in rats. In multigeneration studies using rats, administration of saccharin in the diet induced transitional cell carcinomas and papillomas of the urinary bladder in the first generation male offspring. In one study when administered in the diet, saccharin induced papillary adenocarcinomas of the thyroid in mice. Several studies in which saccharin was administered orally to mice, rats, hamsters, and monkeys were considered inadequate for evaluation by IARC Working Groups. Surgical insertion of pellets containing saccharin resulted in urinary bladder cancer in mice and urinary bladder carcinomas in female mice. Other studies involving topical administration of saccharin to mice and intraperitoneal injection of female mice were considered to be inadequate for complete evaluation by IARC Working Groups. Transplacental exposure of rats to sodium saccharin and to saccharin did not produce any treatment-related neoplasms. Pretreatment with a single instillation in the urinary bladder of a low dose of *N*-methyl-*N*-nitrosourea or feeding of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide and subsequent oral administration of sodium saccharin for long periods increased the incidence of urinary bladder neoplasms in rats over that induced by the nitrosourea or the amide alone. Simultaneous administration of *N*-nitroso-*N*-(4-hydroxybutyl)butylamine and sodium saccharin significantly enhanced the induction of urinary bladder papillomas over that seen after treatment with the nitrosamine alone.

An IARC Working Group reported that there is no adequate evidence for the carcinogenicity of saccharin in humans (IARC S.7, 1987). Since the positive report of Howe et al. (1980), the results of seven case-control studies and one population study of urinary bladder cancer have been inconsistent. The largest was a population-based study in 10 areas of the United States. Significant trends of increasing risk with increasing average daily consumption were found in female nonsmokers and male heavy smokers. Subsequent, independent reanalysis of the same data by a different statistical technique (multiple logistic regression) confirmed the original findings overall but cast doubt on the significance of the findings in the two subgroups because of inconsistent dose-response trends, especially among the male heavy smokers. Three other case-control studies have also shown increased risks among subgroups, but other studies have given negative results. In another study of patients hospitalized for cancer and control patients, a greater proportion of artificial sweetener users was found only among women with cancer of the stomach. Little information was available on urinary tract cancer. No overall association was found between artificial sweetener use and cancer.

PROPERTIES

Saccharin is a white crystalline powder with an intensely sweet taste. It is soluble in water, acetone, ethanol, and glycerol and slightly soluble in chloroform and diethyl ether. Saccharin is also available as the calcium and sodium salts. Calcium saccharin is a free-flowing white powder that is odorless or has a faint aromatic odor. Sodium saccharin occurs as white, nondusting granules with no odor. Both salts are soluble in water. When heated to decomposition, saccharin and its calcium and sodium salts emit toxic fumes of nitrogen oxides (NO_x) and sulfur oxides (SO_x). Saccharin is

available as a grade containing up to 98-101% active ingredients. Calcium saccharin is available as a grade 95% pure. Sodium saccharin is available as a grade 98-101% pure.

USE

Saccharin is used primarily as a nonnutritive sweetening agent, with usage increasing substantially after cyclamates were banned in food in 1970. In 1976, the estimated U.S. consumption for all forms of saccharin was 45% in soft drinks; 18% in tabletop sweeteners; 14% in fruit juices, sweets, chewing gum, and jellies; 10% in cosmetics and oral hygiene products; 7% in drugs, such as coatings on pills; 2% in smokeless tobacco products; 2% in electroplating; and 2% for other uses (IARC V.22, 1980).

PRODUCTION

The USITC identified one U.S. producer for saccharin and its sodium salt from 1980 to 1988, but no production data were provided (USITC, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989). The USITC also reported that one U.S. company produced saccharin, calcium salt, from 1982 to 1984, but no production data were provided. U.S. imports of saccharin have steadily declined from 5.9 million lb in 1983 to 3.7 million lb in 1984, about 1.8 million lb in 1985, and to 1.6 million lb in 1987 (USITCa, 1984; USDOC Imports, 1985 1986, 1988). The 1979 TSCA Inventory identified three U.S. companies producing 1.1 million lb of saccharin in 1977, and 6.3 million lb were imported. Two U.S. companies produced 1.6 million lb of saccharin, sodium salt, and 281,000 lb were imported in 1977. Imports of saccharin, calcium salt, amounted to 5,500 lb, and one U.S. company produced 550,000 lb of saccharin, ammonium salt, in 1977 (TSCA, 1979).

EXPOSURE

The primary routes of potential human exposure to saccharin are ingestion and dermal contact. Potential exposure occurs through the consumption of dietetic foods and drinks and some personal hygiene products, such as certain toothpastes and mouthwashes. The FDA has authorized the use of saccharin and its salts in beverages in concentrations not to exceed 12 mg/oz, as a sugar substitute not to exceed 20 mg for each expressed teaspoonful of sugar sweetening equivalency, and in processed food not to exceed 30 mg per serving. In 1983, the Calorie Control Council estimated that in the United States, 44 million adults consumed saccharin-sweetened products. Saccharin consumption is greatest among diabetics and others whose medical conditions require the restriction of calories or carbohydrates. Exposure to saccharin has possibly decreased in recent years due to the introduction of Nutra-Sweet® and Equal® (aspartame). The risk of potential occupational exposure exists for workers involved in the production of saccharin or its salts, in the manufacture and formulation of saccharin-containing products, and during the packaging of the consumer products. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that about 28,000 workers were potentially exposed to saccharin in the workplace (NIOSH, 1976). The National Occupational Exposure Survey (1981-1983) estimated that 12,994 total workers, including 11,182 women, potentially were exposed to saccharin and 18,952 total workers, including 11,801 women, potentially were exposed to its sodium salt (NIOSH, 1984). The Toxic Chemical Release Inventory (EPA) listed four industrial facilities that produced, processed or otherwise used saccharin in 1988 (TRI, 1990). In compliance with Community Right-to-Know Program, the facilities reported releases of saccharin to the environment which were estimated to total 750 lb.

REGULATIONS

The EPA regulates saccharin and its salts under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), and Superfund Amendments and Reauthorization Act (SARA). Saccharin is subject to reporting and recordkeeping rules under CERCLA, RCRA, and SARA. The EPA proposed raising the statutory reportable quantity (RQ) of 1 lb, established under CERCLA, to 100 lb for saccharin and its salts. The final rule adjusts the RQ from 1 lb to 100 lb. Saccharin is regulated as a hazardous constituent of waste under RCRA, and threshold amounts for facilities which may release saccharin have been established under SARA. The FDA regulates saccharin under the Food, Drug, and Cosmetic Act (FD&CA) as a food ingredient not to exceed specific concentrations. In compliance with the Delaney Clause, the FDA proposed to ban saccharin as a food additive in 1977 because of the available evidence of its carcinogenicity in animals. However, final regulations are pending because of congressional action in 1977 requiring further study and labeling of saccharin. OSHA regulates saccharin under the Hazard Communication Standard and as a chemical hazard in laboratories.

Guidance for Industry

Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing Cross-Contamination

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

April 2013
Current Good Manufacturing Practices (CGMPs)

Guidance for Industry

Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing Cross-Contamination

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U.S. Department of Health and Human Services
Food and Drug Administration
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Current Good Manufacturing Practices (CGMP)

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Guidance for Industry[1]

Non-Penicillin Beta-Lactam Drugs: A CGMP Framework for Preventing Cross-Contamination

I. INTRODUCTION

This guidance describes the importance of implementing manufacturing controls to prevent cross-contamination of finished pharmaceuticals and active pharmaceutical ingredients (APIs) with non-penicillin beta-lactam drugs. This guidance also provides information regarding the relative health risk of, and the potential for, cross-reactivity in the classes of sensitizing beta-lactams (including both penicillins and non-penicillin beta-lactams). Finally, this guidance clarifies that manufacturers generally should utilize separate facilities for the manufacture of non-penicillin beta-lactams because those compounds pose health risks associated with cross-reactivity.

Drug cross-contamination is the contamination of one drug with one or more different drugs. Penicillin can be a sensitizing agent that triggers a hypersensitive exaggerated allergic immune response in some people. Accordingly, implementing methods for preventing cross-contamination of other drugs with penicillin is a key element of manufacturing penicillin and current good manufacturing practice (CGMP) regulations require the use of such methods. See, e.g., 21 CFR §§ 211.42(d), 211.46(d), and 211.176. Non-penicillin beta-lactam drugs also may be sensitizing agents and cross-contamination with non-penicillin beta-lactam drugs can initiate the same types of drug-induced hypersensitivity reactions that penicillins can trigger, including life-threatening allergic reactions. Therefore, manufacturers of non-penicillin beta-lactam drugs should employ similar control strategies to prevent cross-contamination, thereby reducing the potential for drug-induced, life-threatening allergic reactions.

The information in this guidance is intended for manufacturers of finished pharmaceuticals and APIs, including repackagers. Other establishments that handle drugs, such as pharmacy compounders, may find this information useful.

[1] This guidance was developed by the Office of Compliance, Office of Manufacturing and Product Quality, in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance documents describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Regulatory Framework

Section 501(a)(2)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 351(a)(2)(B)) requires that, with few exceptions, all drugs be manufactured in compliance with current good manufacturing practices (CGMPs). Drugs that are not in compliance with CGMPs are considered to be adulterated. Furthermore, finished pharmaceuticals are required to comply with the CGMP regulations at 21 CFR parts 210 and 211.

Several CGMP regulations directly address facility and equipment controls and cleaning. For example, § 211.42(c) requires building and facility controls in general to prevent cross-contamination of drug products. Specifically, the regulation states, "[t]here shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mix-ups" during manufacturing, processing, packaging, storage, and holding.

With respect to penicillin, § 211.42(d) requires that "[o]perations relating to the manufacture, processing, and packing of penicillin shall be performed in facilities separate from those used for other drug products for human use." However, FDA has clarified that separate buildings may not be necessary, provided that the section of the manufacturing facility dedicated to manufacturing penicillin is isolated (i.e., completely and comprehensively separated) from the areas of the facility in which non-penicillin products are manufactured.[2] Under § 211.46(d), manufacturers must completely separate air handling systems for penicillin from those used for other drugs for human use. Additionally, § 211.176 requires manufacturers to test non-penicillin drug products for penicillin where the possibility of exposure to cross-contamination exists, and prohibits manufacturers from marketing such products if detectable levels of penicillin are found.[3]

Although FDA has not issued CGMP regulations specific to APIs, the Agency has provided guidance to API manufacturers in the guidance for industry, ICH[4] Q7, *Good Manufacturing*

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[2] Preamble to the final rule, "Current Good Manufacturing Practice, Processing, Packing, or Holding." 43 FR 45014 at 45038 (September 29, 1978).

[3] See "A Review of Procedures for the Detection of Residual Penicillins in Drugs" (Appendix I, *Procedures for Detecting and Measuring Penicillin Contamination in Drugs*, FDA By-Lines No. 8 (November 1977)), available at http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM095812.pdf. NB: This link works as of 5/18/2012.

[4] International Conference on Harmonization.

Practice Guidance for Active Pharmaceutical Ingredients (ICH Q7 guidance).[5] Because some APIs are sensitizing compounds that may cause anaphylactic shock, preventing cross-contamination in APIs is as important as preventing cross-contamination in finished products. The ICH Q7 guidance recommends using dedicated production areas, which can include facilities, air handling equipment and processing equipment, in the production of highly sensitizing materials, such as penicillins and cephalosporins.[6]

B. Beta-Lactam Antibiotics

Beta-lactam antibiotics, including penicillins and the non-penicillin classes, share a basic chemical structure that includes a three-carbon, one-nitrogen cyclic amine structure known as the beta-lactam ring. The side chain associated with the beta-lactam ring is a variable group attached to the core structure by a peptide bond; the side chain variability contributes to antibacterial activity. As of the date of this publication, FDA has approved over 34 beta-lactam compounds as active ingredients in drugs for human use.[7] Beta-lactam antibiotics include the following five classes[8]:

- penicillins (e.g., ampicillin, oxacillin)
- cephalosporins (e.g., cephalexin, cefaclor)
- penems (e.g., imipenem, meropenem)
- carbacephems (e.g., loracarbef)
- monobactams (e.g., aztreonam)

Allergic reactions associated with penicillins and non-penicillin beta-lactams range from rashes to life-threatening anaphylaxis. Immunoglobulin E (IgE) antibodies mediate the immediate hypersensitivity reactions that are responsible for the symptoms of hay fever, asthma, hives, and anaphylactic shock. IgE-mediated hypersensitivity reactions are of primary concern because they may be associated with significant morbidity and mortality. There is evidence that patients with a history of hypersensitivity to penicillin may also experience IgE-mediated reactions to other beta-lactams, such as cephalosporins and penems.[9]

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[5] We update guidance documents periodically. To make sure you have the most recent version of a guidance, check the Guidance Page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

[6] See section IV.D Containment (4.4) of the ICH Q7 guidance.

[7] Approved beta-lactam antibiotics are listed in FDA's *Approved Drug Products with Therapeutic Equivalence Evaluations*, generally known as the *Orange Book* (available on the Internet at http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm). The Orange Book is searchable by active ingredient and updated as newer drug products are added.

[8] Yao, JDC, and RC Moellering, Jr., Antibacterial agents, in *Manual of Clinical Microbiology*, 9th edition, edited by PR Murray et al., Washington D.C., ASM Press, 2007.

[9] Saxon, A, DC Adelman, A Patel, R Hajdu, and GB Calandra, 1988, Imipenem cross-reactivity with penicillin in humans, J Allergy Clin Immunol, 82:213-217; Saxon, A, GN Beall, AS Rohr, and DC Adelman, 1987, Immediate hypersensitivity reactions to beta-lactam antibiotics, Ann Intern Med, 107(2):204-215; Prescott, Jr., WA, DD

All non-penicillin beta-lactams also have the potential to sensitize individuals, and subsequent exposure to penicillin may result in severe allergic reactions in some patients. Although the frequency of hypersensitivity reactions due to cross-reactivity between beta-lactam classes can be lower than the risk within a class,[10] the hazard posed is present[11] and potentially life-threatening. The potential health hazard of non-penicillin beta-lactams therefore is similar to that of penicillins. Further similarities between non-penicillin beta-lactams and penicillins are as follows:

- It is difficult to define the minimal dose below which allergic responses are unlikely to occur in humans. [12]
- There is a lack of suitable animal or receptor testing models that are predictive of human sensitivity. [13]
- The threshold dose at which allergenic response could occur is extremely low and difficult to detect with current analytical methods.[14]

While beta-lactam antibiotics are similar to one another in many ways, they may differ in pharmacokinetics, antibacterial activity, and potential to cause serious allergic reactions. Because allergy testing methods have not been well-validated,[15] it is clinically difficult to determine the occurrence and rate of cross-reactivity between beta-lactam antibiotics in humans. Therefore, undiagnosed or underreported cases of cross-reactivity likely exist. Some beta-lactam antibiotics have negligible potential for cross-reactivity with beta-lactams of other classes, whereas other beta-lactam compounds may exhibit sensitizing activity as derivatives before the incorporation of side chains that confer antibacterial activity.

Regardless of the rate of cross-reactivity between beta-lactam drugs or the mechanism of action by which such cross-reactivity may occur, the potential health risk to patients indicates that drug

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DePestel, JJ Ellis, and RE Regal, 2004, Incidence of carbapenem-associated allergic-type reactions among patients with versus patients without a reported penicillin allergy, Clin Infect Dis, 38:1102-1107.

[10] Salkind, AR, PG Cuddy, and JW Foxworth, 2001, Is this patient allergic to penicillin? An evidence-based analysis of the likelihood of penicillin allergy, JAMA, 285:2498-2505.

[11] Khan, D. and R Solensky , 2010, Drug Allergy, J Allergy Clin Immunol. 125(2): S131.

[12] Dayan, AD, 1993, Allergy to antimicrobial residues in food: assessment of the risk to man, Vet Microbiol, 35:213-226; Blanca, M, J Garcia, JM Vega, A Miranda, MJ Carmona et al., 1996, Anaphylaxis to penicillins after non-therapeutic exposure: an immunological investigation, Clin Exp Allergy, 26:335-340.

[13] Olson, H, G Betton, D Robinson, K Thomas, A Monro et al., 2000, Concordance of the toxicity of pharmaceuticals in humans and in animals, Regul Toxicol Pharmacol, 32:56-67.

[14] Perez Pimiento, A, M Gomez Martinez, A Minguez Mena, A Trampal Gonzalez, S de Paz Arranz, and M Rodriguez Mosquera, 1998, Aztreonam and ceftazidime: evidence of in vivo cross-allergenicity, Allergy, 53:624-625; Shepard, GM, 1991, Allergy to B-lactam antibiotics, Immunol Allergy Clin North Am, 11(3):611-633.

[15] Bernstein, IL, JT Li, DI Bernstein, et al., 2008, Allergy diagnostic testing: an updated practice parameter, Ann Allergy Asthma Immunol, 100:S1-S148.

manufacturers should take steps to control for the risk of cross-contamination for all beta-lactam products.[16]

C. Beta-Lactamase Inhibitors

Beta-lactam compounds such as clavulanic acid, tazobactam, and sulbactam have weak antibacterial activity but are irreversible inhibitors of many beta-lactamases. These compounds, which are potential sensitizing agents, are typically used in combination with specific beta-lactam agents to preserve antibacterial activity (e.g., amoxicillin-clavulanate, piperacillin-tazobactam). Because these compounds are almost always used in combination with specific beta-lactam agents, any clinical observations of hypersensitivity reactions likely would be attributed to the beta-lactam antibiotic component rather than the inhibitor. Although there have been no case reports confirming anaphylactic reactions to a beta-lactamase inhibitor that is also a beta-lactam, these compounds are potentially sensitizing agents, and manufacturers should implement controls to reduce the risk of cross-contamination with beta-lactamase inhibitors as with all other beta-lactam products.

D. Beta-Lactam Intermediates and Derivatives

Some beta-lactam intermediate compounds and derivatives also possess similar sensitization and cross–reactivity properties. Beta-lactam intermediate compounds usually are API precursor materials that undergo molecular change or purification before use in the manufacture of beta-lactam antibiotic APIs. As a result of these changes, the intermediate compounds may develop antigenic characteristics that can produce allergic reactions. For example, 6-aminopenicillanic acid (6-APA) serves as the intermediate for the formation of all synthetic penicillins that are formed by attaching various side chains. The structure of 6-APA includes unbroken beta-lactam and thiazolidine rings. The beta-lactam ring is relatively unstable, and it commonly breaks open. In the case of 6-APA, this breakage leads to the formation of a penicilloyl moiety, which is the major antigenic determinant of penicillin. This moiety is thought to be a common cause of penicillin urticarial reaction.[17] Degradation of 6-APA can also result in the formation of minor antigenic determinants, including penicilloic acids, penaldic acid, and penicillamine. Anaphylactic reactions to penicillins usually are due to the presence of IgE antibodies to minor determinants in the body. Although 6-APA is not a true antibiotic, it still carries with it a potential to induce allergenicity.

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[16] Following publication of the draft version of this guidance (76 FR 14024), several commenters suggested that monobactams, specifically aztreonam, have a lower risk profile than other beta-lactam products and therefore should be exempted from the separation and control recommendations set forth in this guidance. We have reviewed relevant scientific and medical literature and determined that the relative risk of cross-reactivity associated with aztreonam, when compared to other beta-lactams, is a matter of scientific uncertainty. Accordingly, at this time, FDA does not recommend manufacturing controls that treat aztreonam differently from other beta-lactam products. As with any non-binding recommendations offered in guidance to industry, manufacturers can use an alternative approach if the alternative approach satisfies the requirements of the applicable statutes and regulations. Manufacturers who wish to discuss an alternative separation and control strategy for a non-penicillin beta-lactam such as aztreonam with FDA are invited to do so through the application submission and review process.

[17] Middleton's Allergy: Principles and Practice,7th ed. (electronic) (2009). Chapter 68: Drug Allergy.

Derivatives are unintended by-products that occur during the manufacturing process (i.e., an impurity or degradant). Like intermediates, beta-lactam derivatives could have sensitizing properties and may develop antigenic properties that can produce allergic reactions. Beta-lactam chemical manufacturing processes including, but not limited to, fermentation and synthesis, may create beta-lactam intermediates or derivatives with unknown health consequences. Although the health risk of sensitization and cross-reaction is difficult to predetermine for beta-lactam intermediates and derivatives and is not always well-defined, manufacturing controls intended to reduce the risk of cross-contamination should be considered for operations that produce beta-lactam intermediates or derivatives.

III. RECOMMENDATIONS

Because of the potential health risks associated with cross-reactivity (cross-sensitivity) of beta-lactams, manufacturers should assess and establish stringent controls (including appropriate facility design provisions assuring separation) to prevent cross-contamination. Just as FDA considers the separation of production facilities for penicillins to be current good manufacturing practice, FDA expects manufacturers to treat sensitizing non-penicillin beta-lactam-based products similarly. Specifically, FDA recommends that manufacturers establish appropriate separation and control systems designed to prevent two types of contamination: (1) the contamination of a non-penicillin beta-lactam by any other non-penicillin beta-lactam, and (2) the contamination of any other type of product by a non-penicillin beta-lactam. Accordingly, FDA recommends that the area in which any class of sensitizing beta-lactam is manufactured be separated from areas in which any other products are manufactured, and have an independent air handling system.

As with penicillin, the section of a facility dedicated to manufacturing a sensitizing non-penicillin beta-lactam should be isolated (i.e., completely and comprehensively separated) from areas in the facility in which other products are manufactured. This control applies to each of the five classes of sensitizing beta-lactams; the area in which any class of sensitizing beta-lactam is manufactured should be separated from areas in which any other products are manufactured, including any other class of sensitizing beta-lactam. Manufacturing that is restricted to a specific class of beta-lactam compound (e.g., the cephalosporin family of products) generally would not mandate separate facilities and air handling systems, and could permit production campaigning and cleaning as sufficient control.

Finally, as discussed above, beta-lactam intermediates and derivatives may induce allergic reactions and therefore pose risks of cross-contamination. Accordingly, firms that manufacture beta-lactam intermediates or receive them for further processing, as well as firms whose manufacturing processes result in beta-lactam derivatives, should evaluate their manufacturing operations for the possibility of cross-contamination and implement appropriate controls to reduce or mitigate the potential for cross-contamination. As with penicillin and non-penicillin beta-lactam drugs, such controls could include, but are not limited to, isolation and separation of intermediate and derivative materials, facilities, equipment, and personnel.

Clinical Pharmacology of Human Insulin

LUTZ HEINEMANN, PHD
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Nowadays, human insulin is used daily by millions of diabetic patients. The biological effect of human insulin is comparable to that of porcine insulin. However, after subcutaneous injection, pharmacological and clinical studies showed pharmacokinetic and pharmacodynamic differences between human and animal insulins. Human insulin tends to have faster absorption and shorter duration of action compared with animal insulin. These differences are more pronounced and can be of clinical relevance with intermediate- and long-acting insulin preparations. Optimal metabolic control can be achieved with either human or highly purified animal insulin preparations, provided appropriate insulin replacement strategies are used.

The development of manufacturing techniques for human insulin has made it possible to treat IDDM patients with a hormone that has an amino acid sequence identical to endogenous insulin. After characterization of the biological activity of human insulin in vitro and in animal studies, a series of efficacy and safety trials with human insulin in humans was performed (1,2). In the first years, several studies compared the potency of human insulin and animal insulin preparations with regard to their pharmacological properties. Later, such studies were performed to compare human insulin preparations manufactured using different methods (3,4).

It is surprising how much of the literature on human insulin, including proceedings of commercially sponsored symposia as well as papers and reports published in books and supplements to well-known journals, was printed 10 years ago, all non-peer-reviewed, compared with the number of original papers published on human insulin that have passed a peer-review system. This is disturbing, because pharmacological differences between human insulin and animal insulin might have practical implications for the daily therapy of millions of patients.

In this paper, we will review the properties of human insulin preparations available today for clinical practice. Furthermore, we will describe the pharmacological differences between human insulin and highly purified (monocomponent) insulin preparations of animal origin. We attempt to give a balanced overview of the results of all studies, comparing various pharmacological aspects of human insulin and animal insulin. As a result, it was necessary to quote papers that were not peer-reviewed.

A major emphasis of this review is the presentation of the time-action profiles of the most widely used human insulin preparations. A mere discussion of differences between human insulin and animal insulins would be somewhat out of date, because, in many countries, human insulin is already used by most patients.

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IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

STRUCTURE, PRODUCTION, PURITY, AND POTENCY OF HUMAN INSULIN

Structure

The structure of animal insulin has minor but potentially important differences from human insulin: Porcine insulin differs by one amino acid (alanine instead of threonine at the carboxy-terminal of the B-chain, i.e., position B30), and beef insulin differs by two additional alterations of the sequence of the A-chain (threonine and isoleucine on positions A8 and A10 are alanine and valine). Thus, there is nearly a complete homology between human insulin and porcine insulin in the amino acid sequence.

None of the differences between human insulin and animal insulins is thought to be at sites crucial to the binding or action of insulin. Therefore, it could be expected that the receptor binding and cellular interactions of human insulin would not differ significantly from those of pork or beef insulin (2). The amino acid on position B30 is near one of the parts of the insulin molecule thought to be involved in the self-association of two insulin molecules into dimers. Thus, the self-association tendency could be different between human insulin and porcine insulin (5).

The physicochemical properties of human, pork, and beef insulins differ somewhat because of their different amino acid sequence. Threonine adds

one extra hydroxyl group to the human insulin molecule. This increases its hydrophilic properties and decreases the lipophilic properties, as compared with that of porcine insulin. Thus, the solubility of human insulin in aqueous solutions is higher than that of porcine insulin.

Production

One way to mass produce human insulin was to exchange alanine in position B30 of porcine insulin with threonine, using an enzymatic-chemical method (semi-synthetic technique) (6). During the last decades, biosynthetic production of human insulin was made possible through advances in genetic engineering, especially in recombinant DNA technology (7,8). Methods used to produce human insulin have changed considerably during the last decade. At the end of the 1980s, the semi-synthetic production of human insulin was essentially stopped and replaced by biosynthetic production. In the beginning of the biosynthetic production of human insulin, the A and B chains were produced separately and had to be combined. At present, biosynthetic human insulin is produced with a perfect three-dimensional structure; that is, all foldings and disulfide bridges of the insulin precursor produced by the bacteria or yeast cells are identical to endogenous insulin. The correct spherical structure is important for the insulin-insulin receptor interaction, and hence for the biological action of insulin. Porcine insulin has a slightly different three-dimensional structure when compared with human insulin (9).

Purity

To ascertain a low immunogenicity of human insulin preparations, impurities had to be avoided. The semi-synthetic human insulin production could take advantage of the well-established production and purification methods for porcine insulin, which was used as the original substrate. Possible contaminations with proinsulinlike or glucagonlike substances, pancreatic polypeptide, somatostatin, and vasoactive intestinal peptides were avoided by using monocomponent porcine insulin. Contamination by enzymes or waste products, as a result of the enzymatic-chemical exchange of one amino acid during the secondary production step, also could be avoided (10). In contrast, the insulin production methods that use recombinant DNA technology have a higher propensity for contamination of the insulin product with various bacterial or yeast cell polypeptides. The first biosynthetic human insulin production using bacteria had more obstacles in achieving purity, attributable to the fact that the A-and B-chains had to be extracted separately, and the two chains had to be combined with an intact insulin molecule. Thus, proteins and other substances of bacterial origin, as well as waste products of the insulin recombination, had to be eliminated. Later, purification methods were developed to obtain insulin preparations free of any potentially harmful contamination by *Escherichia coli*-derived peptides (11–13). Antibodies to such peptides could not be detected in 10 patients treated with human insulin for 6 mo (12). Some of the problems of the recombinant DNA technique were circumvented when it became possible to produce homologous proinsulin by *E. coli* (13). Thus, only the C-peptide-like sequence had to be cleaved to achieve human insulin. Human insulin produced biosynthetically from yeast cells with a different insulin precursor (not identical to human proinsulin) was even easier to clear from impurities because the precursor is secreted into the medium, and after cleavage of C-peptide, the intact molecule can be obtained (14,15). Because of the sophisticated purification techniques, it can be assumed that advanced human insulin preparations are pure and free of any significant contamination (16). In regular insulin preparations, insulin molecules self-associate to dimers and large oligomers. In addition, a small amount of covalently aggregated dimers and other insulin-transformation products is formed in commercial insulin. These transformation products prevail in the blood of insulin-treated diabetic patients because they have a slower metabolic clearance relative to insulin monomers (17–19). Human insulin was reported as more susceptible to the production of such products than beef insulin (19). These transformation products are claimed to be highly immunogenic. In addition, degradation of the injected insulin occurs in the subcutaneous depot, resulting in degradation products that also might have immunogenic activity (20).

It has to be emphasized that even with a hormone identical to the human insulin, there are still major differences compared with the naturally occurring hormone. The route of insulin administration is different, and the insulin preparations contain additives like antiseptics, stabilizers, and, with NPH-insulins (Isophane), xenomorphous proteins like protamine.

Potency

In the first study that reports the effects of short-acting human insulin produced by recombinant DNA technology in healthy men, the plasma glucose decrement after subcutaneous injection of human insulin was similar to that of highly purified porcine insulin (21,22). The potency of semi-synthetic human insulin or biosynthetic human insulin also was reported to be similar to that of animal insulin after intravenous insulin infusion at various doses or after subcutaneous injection in diabetic patients (2).

In the rabbit hypoglycemia bioassay, used to estimate insulin strength, porcine and human insulin also had a similar potency (11,23). However, in this model, human insulin showed a more rapid onset and a shorter duration of action, along with a lower potency, compared with bovine insulin (23). Most investigators came to the conclusion that there is no difference in the biological potency of human insulin and animal

insulins (1,2). However, this seems to apply only for the intravenous route and not for subcutaneously injected insulin. Differences in the absorption properties of human insulin and animal insulins, and the results of clinical studies (see below), led to the suggestion that the daily dose of insulin should be reduced by 10 to 25% when switching from animal insulin to human insulin (24). Such a dosage reduction may be needed especially in those patients previously treated with bovine insulin or with mixed animal insulins.

The *British Pharmacopoeia; Codex medicamentarius* and the *Pharmacopeia of the United States* permit deviations from the declared concentration of commercial insulins of ± 5 and ± 10%, respectively. Thus, it cannot be excluded that some of the differences in the reported potencies could be attributable to variations in insulin dose.

HUMAN INSULIN PREPARATIONS

Shortly after its introduction human insulin became available in short-, intermediate-, and long-acting formulations. In principle, these formulations are identical to their porcine or bovine counterparts with respect to the content of auxiliary substances. Because most brands with animal insulins are still available, clinicians and patients are faced with a plethora of different insulin preparations. Even professionals find it difficult to keep track of the insulin preparations available in different countries, because various names may be used for the same insulin with different compositions and concentrations. Some of the insulin preparations marketed are of questionable usefulness, for example, mixtures of short- and intermediate-acting human insulin in 10% steps ranging from 10%:90% to 50%:50%. However, this comment should not be misinterpreted as a suggestion to withdraw animal insulin preparations from the market altogether. Some manufacturers of insulin have tried to withdraw animal insulins from the market (and some have actually done so). This is understandable from a commercial point of view (standardization of production). However, because human insulin has no clear clinical benefit, animal insulins should stay available.

PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF HUMAN INSULIN PREPARATIONS

Methods used to study the pharmacological properties of insulin preparations

In many studies investigating insulin absorption (pharmacokinetic studies) and/or insulin action (pharmacodynamic studies), inappropriate methods, different doses, and sites of administration have been used. This makes the comparison of the results difficult. In some studies, the diabetic patients investigated had been previously treated with animal insulins. As a result, these patients might have had insulin antibodies, which might have influenced the pharmacological properties of exogenous insulin preparations. In fact, the variable dissociation rates of insulin from circulating antibodies are likely to contribute to the high variability in the bioavailability of any insulin preparation.

In principle, the pharmacokinetic properties of insulin preparations could be studied using the direct method (i.e., measurement of serum insulin concentration) or an indirect method (i.e., injection of radiolabeled insulin and registration of the disappearance from the subcutaneous tissue). The problems and pitfalls that limit the use of the indirect method have been discussed in detail elsewhere (25).

Pharmacodynamic properties can be studied by following the blood glucose-lowering effect of a subcutaneous insulin injection over time. This test of insulin activity results in a stimulation of the counterregulatory response caused by hypoglycemia. The effect of the counterregulatory hormones tends to increase blood glucose, thereby leading to an underestimation of the response to the injected insulin. Thus, relevant pharmacodynamic differences can only be detected if doses or activities of the insulins investigated are substantially different. To avoid hypoglycemic episodes, blood glucose can be kept constant by an intravenous glucose infusion targeted to maintain blood glucose at normoglycemic values (euglycemic glucose clamps). Because the glucose requirement is proportional to the biological activity of insulin, it provides a direct measure of potency, at least with regard to glucose metabolism. Endogenous insulin secretion in healthy volunteer subjects can be suppressed by a low-dose intravenous insulin infusion. In our opinion, the euglycemic glucose clamp technique is the best method currently available to study pharmacodynamic properties of various insulin preparations. Moreover, pharmacokinetic properties can be studied simultaneously (2,26,27)

A recent survey of the literature showed that time-action profiles of many insulin preparations are not well-defined because different methods, patient-selection criteria, insulin doses, methods of insulin administration, insulin concentrations, and injection sites are used (28). This survey also highlights the large differences in the reported pharmacological properties of the same insulin preparations caused by the method used. For example, in the 22 studies analyzed, the onset of action after subcutaneous injection of human regular insulin ranged from 0.08–0.5 h, with peak action from 0.75–4 h, and duration of action from 4–12 h.

The direct comparison of pharmacokinetic and pharmacodynamic results obtained with the same group of volunteer subjects showed a considerable difference between the insulin concentration-time profile and the glucose infusion rate-time profile. Thus, an increase in serum insulin concentration does not result in an instantaneous increase in glucose metabolism (Fig. 1).

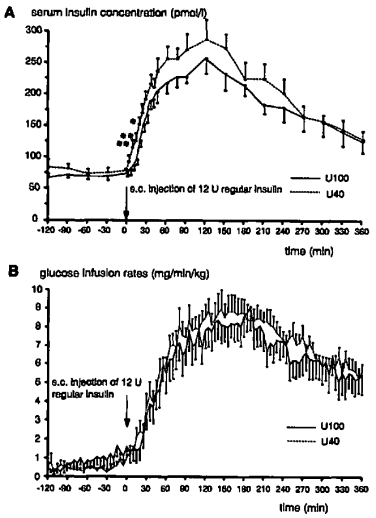

Figure 1—A: *Serum insulin concentrations during an 8-h euglycemic glucose clamp in 8 normal subjects. A subcutaneous injection of 12 U of regular human insulin was given at time 0, with a U40 formulation (mean + SE) on one day and a U100 formulation (mean − SE) on another day. Asterisks mark significantly different serum insulin concentrations.* *, $P < 0.05$; **, $P < 0.02$; *paired Student's t test) (55);* B: *Glucose infusion rates on the U40- (mean + SE) and on the U100- (mean − SE) insulin injection day.*

This phenomenon becomes more clear in view of more recent studies about the importance of the endothelial barrier on insulin transport across the capillary wall (29,30). A long series of events is interposed between the appearance of insulin in blood and changes in glucose metabolism. Thus, the time-dependent characteristics used to describe the pharmacological characteristics of insulin preparations have to be different for its kinetic and dynamic properties.

Short-acting preparations

Pharmacological studies. Pharmacokinetic properties of short-acting human insulin individually assessed by decline of radioactivity of subcutaneously injected ^{125}I-labeled insulin showed a similar insulin absorption process of human and porcine insulin (31,32). However, in another study with the same method, human insulin was more rapidly absorbed than porcine insulin (33). Administration of human or porcine insulin by intravenous bolus in healthy volunteer subjects and IDDM patients showed that both insulins have similar biological activities (34). In studies with intravenous infusion of human or porcine insulin, plasma insulin concentrations and metabolic effects were comparable and strictly dose dependent (35–37). Combining intravenous insulin infusion with the euglycemic clamp technique showed that the pharmacodynamic properties of semi-synthetic human insulin and porcine insulin were indistinguishable in normal individuals as well as in diabetic patients (26,38–40).

The appearance of human insulin in plasma after subcutaneous injection was more rapid than after a similar dose of porcine insulin (32,33,41–43). However, no dose-dependent changes in pharmacokinetic parameters could be demonstrated after a subcutaneous insulin injection measuring blood glucose decline (21,44).

Measurement of the time-action profile of short-acting human insulin after its subcutaneous injection by the glucose clamp technique showed a more rapid onset of action and an earlier peak action than after injection of porcine insulin in healthy volunteer subjects as well as in IDDM patients (42).

In summary, in 11 of 16 studies analyzed, the authors concluded that human insulin was absorbed slightly faster from the subcutaneous injection site, independent of its semi-synthetic or biosynthetic origin (3,22,32,33,41–43,45–48). No difference in insulin absorption kinetics was seen in five studies (31,44,49–51). The mechanism of the faster absorption of human insulin in comparison to pork-regular insulin might be explained by the greater hydrophilicity of the human insulin molecule (9). X-ray studies of the tertiary structures of human and porcine insulin show differences only at the B30 region, where changes in the water attraction are located. Another explanation for the faster absorption of human insulin was the influence that the amino acid in position B30 has on the strength by which the dimers are held together within the hexamer (5). The changed solvent structure in the B28-B30 region and alterations in the intermolecular contacts have a weakening effect on the hexamer stability, resulting in a greater tendency to dissociate with decreasing concentration of insulin (5,9).

Clinical studies. In double-blind crossover studies in type I diabetic patients, treated either conventionally or with subcutaneous insulin infusion, blood glucose control, insulin requirement, and number of hypoglycemic episodes were not substantially different between human insulin and porcine insulin (46,52,53). However, in one double-blind study in 21 diabetic children who were in poor metabolic control, significantly higher HbA_1 values were reported during the treatment period with human insulin, compared with that with porcine insulin (15.7 ± 2.3 vs. 14.2 ± 2.3%; $P < 0.01$) (54).

Time-action profile and influence of insulin concentrations. Studies of short-acting human insulin in different concentrations (U40 vs. U100; Actrapid HM, Novo/Nordisk, Bagsvaerd, Denmark) found the onset of action occurred within 15–30 min, and peak action was observed 150–180 min after subcutaneous injection of 12 U (Fig. 1*B*) (55). No significant differences were observed in the glucose infusion rates needed to keep blood glucose constant after injection of insulin, with either U40 or U100 concentrations. However, serum insulin concentrations showed small but significant differences shortly after injection (Fig. 1*A*): Serum insulin concentrations were significantly higher 10–20 min after injection of the U40 formulation in comparison with the U100 formulation.

However, glucose infusion rates during this time were not significantly different. In this experiment, 6 h after injection of a moderate dose of "short-acting" insulin, still more than 50% of maximal glucose infusion rates were needed to keep blood glucose concentration constant. Therefore, compared with the endogenous insulin response to a meal, onset of action and peak action occurred considerably later. In addition, duration of action was longer, requiring consumption of a snack 2–3 h after insulin injection to prevent hypoglycemia. Moreover, it has to be emphasized that considerable deviations from the described time-action profile can occur depending on the subject's insulin sensitivity (i.e., in diabetic patients, depending on the degree of metabolic control or depending on the insulin doses used).

Clinical implications. Rapid initial delivery of insulin plays a crucial role in the control of meal-related glycemic excursions. Thus, the more rapid onset of action of human insulin might have an advantage over short-acting animal insulins. It was shown in two studies that subcutaneously injected human insulin was superior to porcine insulin in the control of meal-related glycemic excursions in IDDM patients (48,56). In another study with IDDM patients, no differences in postprandial glycemic excursions could be demonstrated (51). The preprandial glucose levels were elevated in this study (>13.5 mM), and, therefore, prandial glycemic increases were small, ranging from 0–4.4 mM. In this context, the slightly faster absorption of human insulin did not result in clinically important differences.

Obviously, the pharmacodynamic characteristics of human short-acting human insulin are far from ideal. In other words, the time-action profile of these preparations differs considerably from the prandial insulin requirements. Development of short-acting insulin analogues with a significantly faster onset of action might help to improve prandial control (5,57,58).

Intermediate-acting preparations (NPH and lente)

Pharmacological studies. Intermediate-acting human insulin preparations injected subcutaneously showed variable results in pharmacological studies when compared with their animal insulin counterparts. No differences in the decline of blood glucose concentrations after injection of biosynthetic human insulin or porcine insulin could be observed in the first pharmacodynamic study with NPH insulins (44). However, NPH insulins with human insulin showed a more rapid onset and shorter duration of action than corresponding animal insulins in a series of later pharmacological studies (4,27,41,59,60). In contrast to these results, the disappearance rates of ^{125}I-labeled human or porcine NPH insulin preparations were not significantly different when given to diabetic patients (32,61).

The differences in the pharmacological properties were attributed to the more hydrophilic properties of human insulin and to differences in the interaction of human insulin and animal insulin with protamine (41). Also, formulation differences, such as the nature and quantity of the protamine in the formulas used were implied.

Direct comparison of semi-synthetic and biosynthetic human NPH insulin after injection in healthy volunteer subjects showed a similar maximal hypoglycemic effect within 3–5 h after administration (4). Thereafter, with semi-synthetic NPH insulin, plasma glucose remained significantly lower than with biosynthetic NPH insulin. These results suggested that the biosynthetic human NPH insulin had a less potent glucose-lowering effect and a relatively shorter duration of action compared with semi-synthetic NPH insulin.

Comparison of human protamine-sodium insulin with human NPH insulin in normal subjects during a euglycemic clamp showed a slightly earlier peak in plasma insulin concentrations with the protamine sodium insulin and a longer duration of action with the NPH insulin (62). In a disappearance study in diabetic patients, human NPH insulin showed a decline of radioactivity similar to the Monotard (Monotard MC, Novo/Nordisk) (61). A semi-synthetic human insulin preparation (Monotard HM, Novo/Nordisk) showed similar disappearance rates compared with a porcine lente preparation in 11 IDDM patients (31). In accordance with this, no significant differences were found in serum insulin concentrations between human and porcine Monotard in short-term studies with healthy volunteer subjects (41,46).

Clinical studies. In the first clinical trial with diabetic patients, significantly higher blood glucose levels were observed with human insulin before the morning and evening injection compared with the levels when treated with animal insulin. This was attributed to a more rapid absorption of the human NPH insulin (63). In a 15-mo double-blind crossover study, Home et al. (64) found a small but significant difference in the metabolic control between human and porcine insulin in 96 insulin-treated diabetic patients. The fasting blood glucose concentration and HbA_1 were significantly higher with human insulin than with porcine insulin (11.1 vs. 9.3 mM and 11.7 vs. 11.1%, respectively). A short-term double-blind crossover study in 8 IDDM patients, comparing human with porcine lente insulin, resulted in no differences in blood glucose control (31).

Thus, the use of human NPH insulin instead of animal NPH insulin could be a disadvantage. This finding was tested by another 6-mo double-blind, crossover study in 22 IDDM patients, which resulted in similar 24-h blood glucose profiles, fasting blood glucose levels, HbA_{1c} levels, number of hypoglycemic events, and insulin-dose requirements when using semi-synthetic human NPH insulin and porcine NPH insulin (65). The authors discuss the possibility that it might be of clinical importance whether semi-synthetic or

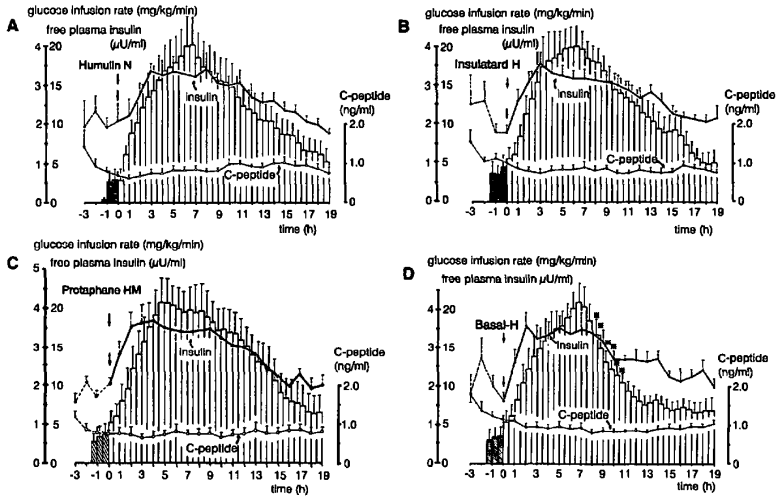

Figure 2—*Glucose infusion rates (▭), plasma free insulin (– – –), and C-peptide (———) concentrations after subcutaneous injection of 12 U of 4 different human NPH insulin formulations (biosynthetic origin: Humulin N [A], Lilly, Indianapolis, IN; semi-synthetic origin: Insulatard H [B] and protaphane HM [C], Novo/Nordisk; Basal H-Insulin [D], Hoechst AG, Frankfurt/Main, Germany; all U40) at time 0 during 19-h euglycemic glucose clamps in 6 normal subjects. (▨), Basal glucose infusion rate, expressed as means + SD. *, Significantly different glucose infusion rates of Basal-H human insulin as compared with the other NPH insulins (P < 0.05; ANOVA and Student's t test [67]).*

biosynthetic human NPH insulin preparations are used.

Time-action profile. Human NPH insulins were absorbed at a faster rate than human zinc insulins (lente insulin) in an euglycemic clamp study over 8 h with healthy volunteer subjects. The result was an increased metabolic effect within the first 4 h after injection (66). Thus, early after injection, the metabolic effects of human NPH and human zinc insulin preparations are different from each other.

The time-action profiles of four widely used human NPH insulin preparations were investigated in healthy subjects using the euglycemic clamp technique (Fig. 2) (67). The overall time-action profiles were interchangeable. The onset of action (defined as half-maximal action) of all NPH insulins tested was within 2.5–3 h, with peak action after 5–7 h, and duration of action (defined as >25% of maximal action) between 13–16 h. This study showed that there are no clinically important differences in the duration of action of human NPH insulins from different insulin manufacturers.

Clinical implications. The more rapid absorption and shorter duration of action of intermediate-acting human insulin preparations have clinical implications. Injecting human NPH insulin before dinner instead of at bedtime might impair metabolic control during the night. Higher fasting blood glucose concentrations in the morning, attributable to a waning of insulin action, have been observed in diabetic patients using human NPH insulins compared with porcine NPH insulins (54,63).

Use of NPH insulin and long-acting insulin preparations. The problem of elevated fasting blood glucose concentrations when human NPH insulin was used as the evening injection led to trials in which the evening injection was moved to bedtime, or long-acting human insulin preparations (Ultratard HM) were used. Fasting blood glucose concentrations were significantly lower when the evening dose of human NPH insulin was given at bedtime instead of at dinner (7.5 ± 1.1 vs. 10.0 ± 1.6 mM; $P < 0.02$) (68). Human ultralente insulin injected at bedtime, with its longer duration of action, resulted in lower fasting blood glucose concentrations compared with human NPH insulin (69,70).

In a crossover, randomized double-blind trial of 82 IDDM patients, the use of human lente (Monotard HM, Novo/Nordisk) or NPH insulin, given twice daily in combination with regular human insulin, resulted in comparable metabolic control (71). With both regimens, the major problem was elevated blood glucose concentrations before breakfast (NPH insulin versus lente insulin: 8.8 ± 0.5 vs. 9.0 ± 0.5 mM, NS). Thus, the use of human lente insulin instead of NPH insulin does not appear to result in better metabolic control during the night.

In the above study (and others quoted), the diabetic patients mixed the regular insulin with the lente insulin immediately before the injection. It is well known that this procedure results in modifications of the time-action profile of regular insulin (see below).

Long-acting human insulin preparations

Ultralente insulin preparations made with bovine or porcine insulin have a different pharmacokinetic profile from those made with human insulin (72,73). It is known that human zinc insulin crystals bind water more avidly than pork insulin crystals. It may be that this causes a faster dissociation of those zinc insulin complexes (2,9). Thus, a better solubility of the crystals of the human insulin ultralente preparations compared with

those of bovine insulin could possibly explain the faster absorption (74).

Pharmacological studies. The ultralente formulation with bovine insulin does not show a peak action. Its long duration of action lasts up to 32 h (72,73,75). In contrast, the human ultralente insulin preparations show a peak of action after 8.5 h (73). In one study, the duration of action of human ultralente was reported to be no shorter than that of bovine ultralente (73).

Hildebrandt et al. (74) reported that human ultralente had a substantially faster absorption than bovine ultralente, when comparing the disappearance rates of ^{125}I-labeled insulin preparations at different doses in IDDM patients. The faster absorption of human ultralente compared with bovine was confirmed in healthy volunteer subjects by measuring blood glucose decline after injection (72).

A comparison between a human insulin zinc suspension (Humulin Zn, Lilly), which was entirely crystalline in its formulation (like ultralente), and the intermediate-acting porcine lente insulin zinc suspension (Monotard MC, Novo/Nordisk; 30% amorphous and 70% crystalline formulation) showed no differences in the duration of hypoglycemic action in a single-dose crossover study in 10 healthy men (76).

Clinical studies. Ultratard HM was studied in a double-blind crossover study in 18 insulin-treated IDDM and NIDDM patients and found to be as effective as bovine ultralente in controlling basal plasma glucose with once-daily morning injections (77). The authors concluded that Ultratard HM is suitable for meeting basal insulin requirements in diabetic patients. In this study, there was no indication that Ultratard HM has a faster absorption from subcutaneous tissue than bovine ultralente.

Time-action profile. The variable results of the pharmacological and clinical studies do not provide a definite answer to the clinically important question of whether the duration of action of human ultralente falls between that of intermediate-acting and long-acting insulin, or whether it is similar to that of long-acting insulin.

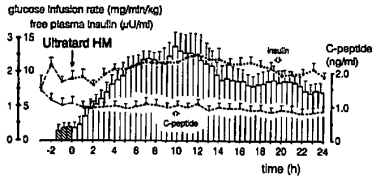

Figure 3—*Glucose infusion rates (□), plasma free insulin (– – –), and C-peptide (———) concentrations after subcutaneous injection of 12 U of a human lente insulin formulation (Ultratard HM) at time 0 during 24-h euglycemic glucose clamps in 7 normal subjects. (▨), Basal glucose infusion rates; means + SD (78).*

A study of the time-action profile of Ultratard HM using the euglycemic clamp technique (injection of 12 U in healthy subjects) revealed that peak action (reached after 10 h) was two-thirds that of a NPH insulin (Fig. 3, in comparison to Fig. 2*c*) (78). With both insulins, after 20 hours free plasma insulin concentrations had returned to basal values and glucose infusion rates indicated that the metabolic effect had nearly returned to basal values. Thus, the duration of action of human ultralente is not considerably longer than that of NPH insulin.

Clinical implications. Thus, once-daily injections of Ultratard HM in the given dose (12 U) will not provide sufficient basal insulinemia during the whole day. Twice-daily injections of human ultralente insulin are necessary to achieve basal insulin requirements.

Clinical trials showed that such an insulin regimen resulted in lower fasting blood glucose concentrations than twice-daily injections of human lente insulin (79,80). If only once-daily injection of human ultralente was used, injection in the morning resulted in a higher fasting blood glucose concentration than injection at bedtime (81,82). In a study of IDDM patients that used a multiple-injection regimen to compare human isophane insulin with human ultralente at bedtime, blood glucose at 0800 was significantly lower with isophane insulin than with the ultralente preparation (10.2 ± 1.2 vs. 14.3 ± 1.3 mM); although the dose of the bedtime ultralente insulin injection (0.35 U · kg^{-1} · day^{-1}) was significantly higher than the dose of isophane insulin (0.25 U · kg · day^{-1}; $P < 0.005$) (83).

Treatment with a once-daily injection of human ultralente over a period of 6 mo resulted in a significant improvement in metabolic control (a drop in HbA_{1c} from 13.2 to 10.6%) in 22 NIDDM patients with secondary sulfonylurea failure (84). However, there were frequent episodes of hypoglycemia. In the same study, 10 patients who were receiving once-daily injections of a bovine lente insulin preparation showed a similar improvement in metabolic control (from 13.1 to 11.2%), but the frequency of hypoglycemic episodes was significantly lower. In this study, the pharmacodynamic properties of human ultralente in comparison with the more flat action profile of bovine lente insulin are clearly unsuitable as a single-daily injection in NIDDM patients when aiming at improved metabolic control.

In contrast to the comment we made regarding the shorter duration of action of human NPH insulins, no clinical disadvantage could be seen with the shorter duration of action of human ultralente compared with its bovine counterpart. For example, we do not recommend use of bovine ultralente to our patients because of the prolonged duration of action, which potentially could cause an overlapping interaction between the metabolic activity of the insulin of the current injection and that of the previous day. This unpredictable accumulation of insulin action can result in prolonged and severe hypoglycemias. Moreover, the patient cannot adapt the dose to changing insulin needs, for example, when exercise is planned. Thus,

the shorter duration of action of human ultralente appears to be an advantage and not a disadvantage in clinical practice.

Miscibility. One problem of Ultratard (and other human lente insulin preparations) is that it cannot be premixed with short-acting insulins in one syringe without a considerable change in the time-action profile (i.e., a retardation of the onset of action of the short-acting insulin). This effect is pronounced even when the mixed human lente insulin preparations are injected immediately after being drawn into the syringe (25,85–88). This delay is caused by a binding of the added regular insulin to zinc, present in excess in the ultralente (and lente) insulins, which results in an amorphous precipitation of zinc insulin. Mixing of human regular and NPH insulin does not result in blunting of the action of the soluble component, regardless of whether it is readily mixed or premixed (3,89).

Another problem with ultralente insulin preparations is the high variability of its insulin bioavailability after injection, a phenomenon well known to the clinician. However, data showing this variability are only available for bovine ultralente (75,90), and, to our knowledge, no formal investigations of this aspect have been published for Ultratard.

EFFECT OF HUMAN INSULIN ON INTERMEDIARY METABOLITES AND LIPID METABOLISM

In vitro studies with insulin receptors from human lymphocytes, as well as measurements of lipid metabolism in rat adipocytes and hepatocytes, showed that the biological actions of biosynthetic human insulin and porcine insulin were identical (91,92). Injection of 0.075 U/kg of either human insulin or porcine insulin by intravenous bolus were reported to result in differences in intermediate metabolites and counterregulatory hormones (93). However, no statistical analysis was given and the reported differences appear to be small. Effects on intermediary metabolite concentrations (blood lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate) were similar after subcutaneous injection of human insulin, porcine, and bovine insulin (49,50), or during euglycemic clamp studies with intravenous infusion of human insulin or porcine insulin (26,38, 39,94).

Although, the initial studies showed differences in hepatic action between human insulin and porcine insulin (21), this was not confirmed in later turnover studies. Suppression of hepatic glucose production and stimulation of peripheral glucose utilization were basically identical with human insulin and porcine regular insulin (94)

CONCLUSIONS

Human insulin preparations of both biosynthetic and semi-synthetic origin have similar, but not identical, pharmacological properties when compared with purified porcine insulin. Pharmacodynamic differences between human insulin and animal insulin preparations in clinical pharmacology are small with short-acting insulin preparations, considerable with NPH insulins, and substantial concerning long-acting insulin preparations. Development and introduction of human insulin has not revolutionized insulin treatment of IDDM patients. Obviously, the change from animal to human insulin per se does not improve metabolic control.

The choice of insulins with appropriate pharmacological characteristics, purity, and origin of the insulin preparations are important prerequisites for optimal therapy. A successful insulin regimen must consider insulin replacement strategies that are appropriate for the patient's lifestyle and individual treatment goals (95–97). And, most important, the patients must receive instruction on the time-action profiles of the insulins they use, and information on how to adapt the doses to achieve good metabolic control while avoiding hypoglycemic episodes.

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Generic Name or Active Ingredient	Status
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Caffeine Anhydrous (125mg/mL); Sodium Benzoate (125mg/mL) Injection	***Currently in Shortage***
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Calcium Gluconate Injection	***Currently in Shortage***
Cefazolin Injection	***Resolved***
Cefepime Injection	***Currently in Shortage***
Cefotaxime Sodium (Claforan) Injection	***Currently in Shortage***
Cefotetan Disodium Injection	***Currently in Shortage***
Chloramphenicol Sodium Succinate Injection	***Currently in Shortage***
Chloroquine Phosphate Tablets	***Resolved***
Desmopressin Acetate Injection	***Currently in Shortage***
Dexamethasone Sodium Phosphate Injection	***Currently in Shortage***
Dextrose 5% Injection Bags	***Currently in Shortage***
Dextrose Injection USP, 70%	***Currently in Shortage***
Disopyramide Phosphate (Norpace) Capsules	***Currently in Shortage***

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Doxorubicin Lyophilized Powder for Injection	***Currently in Shortage***
Epinephrine Injection	***Currently in Shortage***
Eptifibatide (Integrilin) Injection	***Resolved***
Ethiodized Oil (Lipiodol) Injection	***Currently in Shortage***
Fentanyl Citrate (Sublimaze) Injection	***Currently in Shortage***
Fomepizole Injection	***Currently in Shortage***
Gemifloxacin Mesylate (Factive) Tablets	***Currently in Shortage***
Haloperidol Lactate Injection	***Resolved***
Imipenem and Cilastatin for Injection, USP	***Currently in Shortage***
Indigotindisulfonate Sodium (Indigo Carmine) Injection	***Currently in Shortage***
Ketorolac Tromethamine Injection	***Resolved***
L-Cysteine Hydrochloride Injection	***Currently in Shortage***
Leucovorin Calcium Lyophilized Powder for Injection	***Currently in Shortage***
Leuprolide Acetate Injection	***Currently in Shortage***
Levetiracetam (Keppra) Injection	***Resolved***
Lidocaine Hydrochloride (Xylocaine) Injection	***Currently in Shortage***
LifeCare PCA™ Sterile Empty Vial and Injector	***Currently in Shortage***
Liotrix (Thyrolar) Tablets	***Currently in Shortage***
Mecasermin [rDNA origin] (Increlex) Injection	***Currently in Shortage***
Memantine Hydrochloride (Namenda) XR	***Resolved***

Capsules	
Meropenem for Injection, USP	***Resolved***
Methyldopate Hydrochloride Injection	***Currently in Shortage***
Methylphenidate Hydrochloride ER Capsules/Tablets	***Resolved***
Methylprednisolone Sodium Succinate for Injection, USP	***Currently in Shortage***
Metoprolol Injection	***Resolved***
Morphine Sulfate Injection, USP, CII, (Preservative-Free)(For PCA Use Only)	***Currently in Shortage***
Multi-Vitamin Infusion (Adult and Pediatric)	***Currently in Shortage***
Mupirocin Calcium Nasal Ointment	***Currently in Shortage***
Nebivolol (BYSTOLIC) Tablets	***Resolved***
Nimodipine (Nymalize) Oral Solution	***Currently in Shortage***
Penicillin G Benzathine (Bicillin L-A) Injection	***Currently in Shortage***
Peritoneal Dialysis Solutions	***Currently in Shortage***
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Piperacillin and Tazobactam (Zosyn) Injection	***Currently in Shortage***
Potassium Acetate Injection, USP	***Resolved***
Potassium Chloride Injection	***Currently in Shortage***
Reserpine Tablets	***Currently in Shortage***
Sacrosidase (Sucraid) Oral Solution	***Currently in Shortage***
Sodium Acetate Injection, USP	***Currently in Shortage***
Sodium Bicarbonate Injection, USP	***Currently in Shortage***

Sodium Chloride 0.9% Injection Bags	***Currently in Shortage***
Sodium Chloride 23.4% Injection	***Currently in Shortage***
Sufentanil Citrate (Sufenta) Injection	***Currently in Shortage***
Sumatriptan (Imitrex) Nasal Spray	***Currently in Shortage***
Technetium Tc99m Succimer Injection (DMSA)	***Currently in Shortage***
Theophylline Extended Release Tablets and Capsules	***Currently in Shortage***
Tigecycline (Tygacil) Injection	***Currently in Shortage***
Tiopronin (Thiola)	***Resolved***
Tobramycin Injection	***Currently in Shortage***
Tretinoin Capsules	***Currently in Shortage***
Triamcinolone Hexacetonide Injectable Suspension (Aristospan)	***Currently in Shortage***
Trimipramine Maleate (SURMONTIL) Capsules	***Currently in Shortage***
Vancomycin Hydrochloride for Injection, USP	***Currently in Shortage***

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Center for Drug Evaluation and Research (CDER)
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CMC

Revision 1

Guidance for Industry

Changes to an Approved NDA or ANDA

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
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*Contains Nonbinding Recommendations**

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This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public.** You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

** Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect. If you have any questions about the effect of any portion of this guidance, contact the Office of Pharmaceutical Science, Center for Drug Evaluation and Research (HFD-003), Food and Drug Association, 5600 Fishers Lane, Rockville, MD 20857.

I. INTRODUCTION AND BACKGROUND

This guidance provides recommendations to holders of new drug applications (NDAs) and abbreviated new drug applications (ANDAs) who intend to make postapproval changes in accordance with section 506A of the Federal Food, Drug, and Cosmetic Act (the Act) and § 314.70 (21 CFR 314.70). The guidance covers recommended reporting categories for postapproval changes for drugs other than specified biotechnology and specified synthetic biological products. It supersedes the guidance of the same title published November 1999. Recommendations are provided for postapproval changes in (1) components and composition, (2) manufacturing sites, (3) manufacturing process, (4) specifications, (5) container closure system, and (6) labeling, as well as (7) miscellaneous changes and (8) multiple related changes.

Recommendations on reporting categories for changes relating to specified biotechnology and specified synthetic biological products regulated by CDER are found in the guidance for industry

[1] This guidance has been prepared under the direction of the Chemistry, Manufacturing and Controls Coordinating Committee in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA).

Paperwork Reduction Act Public Burden Statement: This guidance contains information collection provisions that are subject to review by the Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (PRA) (44 U.S.C. 3501-3520). The collection(s) of information in this guidance were approved under OMB Control No. 0910-0538 (until August 31, 2005).

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entitled *Changes to an Approved Application for Specified Biotechnology and Specified Synthetic Biological Products* (July 1997).[2]

On November 21, 1997, the President signed the Food and Drug Administration Modernization Act of 1997 (the Modernization Act).[3] Section 116 of the Modernization Act amended the the Act by adding section 506A, which provides requirements for making and reporting manufacturing changes to an approved application and for distributing a drug product made with such changes. The FDA has revised its regulations on supplements and other changes to an approved application (21 CFR 314.70) to conform to section 506A of the Act.

This guidance does not provide recommendations on the specific information that should be developed by an applicant to assess the effect of the change on the identity, strength (e.g., assay, content uniformity), quality (e.g., physical, chemical, and biological properties), purity (e.g., impurities and degradation products), or potency (e.g., biological activity, bioavailability, bioequivalence) of a drug product as these factors may relate to the safety or effectiveness of the drug product. An applicant should consider all relevant CDER guidance documents for recommendations on the information that should be submitted to support a given change.[4]

CDER has published guidances, including the SUPAC (scale-up and postapproval changes) guidances, that provide recommendations on reporting categories. To the extent that the recommendations on ***reporting categories*** in this guidance are found to be inconsistent with guidances published before this guidance was finalized, the recommended reporting categories in such previously published guidances are superseded by this guidance. This guidance does not provide extensive recommendations on reporting categories for components and composition changes (see section V). Therefore, recommended reporting categories for components and composition changes provided in previously published guidances, such as the SUPAC guidances, still apply. Section 506A of the Act and § 314.70(c) provide for two types of changes-being-effected supplements (see section II), while previously there was only one type. It is important for applicants to use this guidance to determine which type of changes-being-effected supplement is recommended. CDER intends to update the previously published guidances to make them consistent with this guidance.

If guidance for either recommended reporting categories or information that should be submitted to support a particular change is not available, the appropriate CDER chemistry or microbiology review staff can be consulted for advice.

FDA's guidance documents, in general, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required. Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect. If you

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[2] FDA is currently revising the 1997 guidance and intends to issue it in draft for public comment.

[3] Public Law 105-115.

[4] A list of CDER guidances is available on the Internet at http://www.fda.gov/cder/guidance/index.htm.

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II. REPORTING CATEGORIES

Section 506A of the Act and § 314.70 provide for four reporting categories that are distinguished in the following paragraphs.

A ***major change*** is a change that has a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product. A major change requires the submission of a supplement and approval by FDA prior to distribution of the drug product made using the change. This type of supplement is called, and should be clearly labeled, a ***Prior Approval Supplement*** (§ 314.70(b)). An applicant may ask FDA to expedite its review of a prior approval supplement for public health reasons (e.g., drug shortage) or if a delay in making the change described in it would impose an extraordinary hardship on the applicant. This type of supplement is called, and should be clearly labeled, a *Prior Approval Supplement - Expedited Review Requested* (§ 314.70(b)(4)).[5] FDA is most likely to grant requests for expedited review based on extraordinary hardship for manufacturing changes made necessary by catastrophic events (e.g., fire) or by events that could not be reasonably foreseen and for which the applicant could not plan.

A ***moderate change*** is a change that has a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product. There are two types of moderate change. One type of moderate change requires the submission of a supplement to FDA at least 30 days before the distribution of the drug product made using the change. This type of supplement is called, and should be clearly labeled, a ***Supplement - Changes Being Effected in 30 Days*** (§ 314.70(c)(3)). The drug product made using a moderate change cannot be distributed if FDA informs the applicant within 30 days of receipt of the supplement that a prior approval supplement is required (§ 314.70(c)(5)(i)). For each change, the supplement must contain information determined by FDA to be appropriate and must include the information developed by the applicant in assessing the effects of the change (§ 314.70(a)(2) and (c)(4)). If FDA informs the applicant within 30 days of receipt of the supplement that information is missing, distribution must be delayed until the supplement has been amended to provide the missing information (§ 314.70(c)(5)(ii)).

FDA may identify certain moderate changes for which distribution can occur when FDA receives the supplement (§ 314.70(c)(6)). This type of supplement is called, and should be clearly labeled, a ***Supplement - Changes Being Effected***. If, after review, FDA disapproves a changes-being-effected-in-30-days supplement or changes-being-effected supplement, FDA may order the

[5] Internal Agency policies and procedures relating to processing requests for expedited review of supplements to approved ANDAs and NDAs are documented in CDER's Manual of Policies and Procedures (MAPP) at 5240.1 and 5310.3, respectively. MAPPs can be located on the Internet at lttp://www.fda.gov/cder/mapp.htm.

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manufacturer to cease distribution of the drug products made using the disapproved change (§ 314.70(c)(7)).

A ***minor change*** is a change that has minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product. The applicant must describe minor changes in its next ***Annual Report*** (§ 314.70(d)).

Under § 314.70(e), an applicant can submit one or more protocols (i.e., comparability protocols) describing tests, studies, and acceptance criteria to be achieved to demonstrate the absence of an adverse effect from specified types of changes. A comparability protocol can be used to reduce the reporting category for specified changes. A proposed comparability protocol that was not approved as part of the original application must be submitted as a prior approval supplement (314.70(e)). On February 25, 2003, FDA issued a draft guidance on comparability protocols entitled *Comparability protocols - Chemistry, Manufacturing, and Controls Information*.

III. GENERAL REQUIREMENTS

Other than for editorial changes in previously submitted information (e.g., correction of spelling or typographical errors, reformatting of batch records), an applicant must notify FDA about each change in each condition established in an approved application beyond the variations already provided for in the application (§ 314.70(a)(1)).

A supplement or annual report must include a list of all changes contained in the supplement or annual report. On the list, FDA recommends that the applicant describe each change in enough detail to allow FDA to quickly determine whether the appropriate reporting category has been used. For supplements, this list must be provided in the cover letter (§ 314.70(a)(6)). In annual reports, the list should be included in the summary section (§ 314.81(b)(2)(i)). The applicant must describe each change fully in the supplement or annual report (§ 314.70(a)(1)).

An applicant making a change to an approved application under section 506A of the Act must also conform to other applicable laws and regulations, including current good manufacturing practice (CGMP) requirements of the Act (21 U.S.C. 351(a)(2)(B)) and applicable regulations in Title 21 of the *Code of Federal Regulations* (e.g., 21 CFR parts 210, 211, 314). For example, manufacturers must comply with relevant CGMP validation and recordkeeping requirements and ensure that relevant records are readily available for examination by authorized FDA personnel during an inspection.

A changes-being-effected supplement providing for labeling changes under § 314.70(c)(6)(iii) must include 12 copies of the final printed labeling (§ 314.70(c)(1)). In accordance with

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§ 314.70(a)(4), an applicant also must promptly revise all promotional labeling and drug advertising to make it consistent with any labeling change implemented in accordance with § 314.70(b) or (c).

Except for supplements providing only for a change in labeling, an applicant must include in each supplement and amendment to a supplement a statement certifying that a field copy has been provided in accordance with 21 CFR 314.440(a)(4)[6] (§ 314.70(a)(5)).

IV. ASSESSING THE EFFECT OF MANUFACTURING CHANGES

A. Assessment of the Effects of the Change

The holder of an approved application under section 505 of the Act ***must assess the effects of the change before distributing a drug product made with a manufacturing change*** (§ 314.70(a)(2)).[7] For each change, the supplement or annual report must contain information determined by FDA to be appropriate and must include the information developed by the applicant in assessing the effects of the change (section 506A(b), (c)(1), (d)(2)(A), and (d)(3)(A) of the Act). The type of information that must be included in a supplemental application or an annual report is specified in § 314.70(b)(3), (c)(4), and (d)(3).

1. Conformance to Specifications

An assessment of the effects of a change on the identity, strength, quality, purity, and potency of the drug product should include a determination that the drug substance intermediates, drug substance, in-process materials, and/or drug product affected by the change conform to the approved specifications.[8] A *specification* is a quality standard (i.e., tests, analytical procedures, and acceptance criteria) provided in an approved application to confirm the quality of drug substances, drug products, intermediates, raw materials, reagents, components, in-process materials, container closure systems, and other materials used in the production of a drug substance or drug product. *Acceptance criteria* are numerical limits, ranges, or other criteria for the tests described (§ 314.3(b)). Conformance to a specification means that the

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[6] Mailing information for field copies is provided in 21 CFR 314.440(a)(4). FDA recommends that the *applicant's home FDA district office* referred to in the regulations be the district office where the applicant's headquarters is located.

[7] *Assess the effects of the change* means to evaluate the effects of a manufacturing change on the identity, strength, quality, purity, and potency of a drug product as these factors relate to the safety or effectiveness of the drug product. The terms *assess* or *assessment* as used in this guidance are not the same as validation. Certain validation information, such as for sterilization processes, is considered information that is needed to assess the effect of the change as specified in § 314.70(a)(2) and should be submitted in an NDA or ANDA. Unless otherwise specified by FDA, validation (e.g., process, equipment) data need not be submitted in the application, but should be retained at the facility and be available for review by FDA at the Agency's discretion under CGMPs.

[8] If a specification needs to be revised as a result of the change, this would be considered a multiple change (see sections VIII and XII).

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material, when tested according to the analytical procedures listed in the specification, will meet the listed acceptance criteria.

2. Additional Testing

In addition to confirming that the material affected by manufacturing changes continues to meet its specification, we recommend that the applicant perform additional testing, when appropriate, to assess whether the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product have been or will be affected. The assessment should include, as appropriate, evaluation of any changes in the chemical, physical, microbiological, biological, bioavailability, and/or stability profiles. This additional assessment could involve testing of the postchange drug product itself or, if appropriate, the material directly affected by the change. The type of additional testing that an applicant should perform would depend on the type of manufacturing change, the type of drug substance and/or drug product, and the effect of the change on the quality of the drug product. For example:

- Evaluation of changes in the impurity or degradant profile could first involve profiling using appropriate chromatographic techniques and then, depending on the observed changes in the impurity profile, toxicology tests to qualify a new impurity or degradant or to qualify an impurity that is above a previously qualified level.[9]

- Evaluation of the hardness or friability of a tablet after certain changes.

- Assessment of the effect of a change on bioequivalence when required under 21 CFR part 320 could include, for example, multipoint and/or multimedia dissolution profiling and/or an in vivo bioequivalence study.

- Evaluation of extractables from new packaging components or moisture permeability of a new container closure system.

An applicant should refer to all relevant CDER guidance documents for recommendations on the information that should be submitted to support a given change. If guidance for information that should be submitted to support a particular change is not available, applicants can consult the appropriate CDER chemistry or microbiology review staff for advice.

B. Equivalence

When testing is performed, the applicant should usually assess the extent to which the manufacturing change has affected the identity, strength, quality, purity, and potency of the

[9] Recommendations on identifying, qualifying, and reporting impurities can be found in relevant guidances (e.g., ICH Q3B *Impurities in New Drug Products* (November 1996)).

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drug product. Typically this is accomplished by comparing test results from pre- and postchange material and determining if the test results are equivalent. Simply stated: Is the drug product made after the change equivalent to the drug product made before the change? An exception to this general approach is that when bioequivalence is redocumented for certain ANDA postapproval changes, FDA recommends that the comparator be the reference listed drug. Equivalence comparisons frequently have a criterion for comparison with calculation of confidence intervals relative to a predetermined equivalence interval. For this, as well as for other reasons, *equivalent* does not necessarily mean *identical*. Equivalence may also relate to maintenance of a quality characteristic (e.g., stability) rather than a single performance of a test.

C. Adverse Effect

Some manufacturing changes have an adverse effect on the identity, strength, quality, purity, or potency of the drug product. In many cases, the applicant chooses not to implement these manufacturing changes, but sometimes the applicant wishes to do so. If an assessment indicates that a change has adversely affected the identity, strength, quality, purity, or potency of the drug product, FDA recommends that ***the change be submitted in a prior approval supplement regardless of the recommended reporting category for the change.*** For example, a process change recommended for a changes-being-effected-in-30-days supplement could cause the formation of a new degradant that requires qualification and/or identification.[10] The applicant's degradation qualification procedures may indicate that there are no safety concerns relating to the new degradant. Even so, we recommend that the applicant submit this change in a prior approval supplement with appropriate information to support the continued safety and effectiveness of the drug product. During the review of the prior approval supplement, the FDA will assess the impact of any adverse effect on the drug product as this change may relate to the safety or effectiveness of the drug product.

Applicants are encouraged to consult with the appropriate CDER chemistry or microbiology review staff if there are any questions on whether a change in a characteristic would be viewed by CDER as adversely affecting the identity, strength, quality, purity, or potency of the drug product.

V. COMPONENTS AND COMPOSITION

Changes in the qualitative or quantitative formulation, including inactive ingredients, as provided in the approved application, are considered major changes requiring a prior approval supplement, unless exempted by regulation or guidance (§ 314.70(b)(2)(i)). The deletion or reduction of an ingredient intended to affect only the color of the drug product may be reported in an annual report (§ 314.70(d)(2)(ii)). Guidance on changes in components and composition that may be submitted in a changes-being-effected supplement or annual report is not included in this document because

[10] Recommendations on identifying, qualifying, and reporting impurities can be found in relevant guidances.

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of the complexity of the recommendations, but may be covered in one or more guidance documents describing postapproval changes (e.g., SUPAC documents).

VI. MANUFACTURING SITES[11]

A. General Considerations

CDER must be notified when a manufacturer changes to a manufacturing site that is different from those specified in the approved application (314.70(a)). Sites can include those used by an applicant to (1) manufacture or process drug products,[12] in-process materials, drug substances, or drug substance intermediates, (2) package drug products, (3) label drug products, and (4) test components, drug product containers, closures, packaging materials, in-process materials, or drug products. Sites include those owned by the applicant or contract sites used by an applicant. Testing sites include those performing physical, chemical, biological, and microbiological testing to monitor, accept, or reject materials, as well as those performing stability testing. Sites used to label drug products are considered those that perform labeling of the drug product's primary or secondary packaging components. Sites performing operations that place identifying information on the dosage form itself (e.g., ink imprint on a filled capsule) are considered to be facilities that manufacture or process the drug product. FDA recommends that the supplement or annual report identify whether the proposed manufacturing site is an alternative to or replacement for the site or sites provided for in the approved application.

FDA recommends that a move to a different manufacturing site, when it is a type of site routinely subject to FDA inspection, be submitted as a prior approval supplement if the site does not have a ***satisfactory CGMP inspection***[13] for the ***type of operation***[14] being moved (see sections VI.B.1 and 2).

For labeling, secondary packaging, and testing site changes, the potential for adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product is considered to be independent of the type of drug product dosage form or specific type of operation being performed. Therefore, the recommended reporting category for any one of these manufacturing site changes will be the same for all types of drug products and operations. For manufacturing sites used to (1) manufacture or process drug products, in-process materials, drug substances, or drug substance intermediates or (2) perform primary packaging operations,

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[11] See Attachment A for a discussion of the definition of *same manufacturing site* and *different manufacturing site.*

[12] Manufacturing or processing drug product would also include the preparation (e.g., sterilization, depyrogenation, irradiation, washing) by the applicant or applicant's contractor of container closure systems or packaging components. Changes in the site used to fabricate packaging components (e.g., bottles) or manufacture packaging materials (e.g., resins) need not be reported to CDER if there are no other changes (e.g., dimensions, compositions, processing aids). If other changes occur, the reporting category should be based on the recommended reporting categories for these changes (i.e., the manufacturing site change does not need to be considered when determining the appropriate reporting category).

[13] See Glossary for a definition of *satisfactory CGMP inspection.*

[14] See Attachment B for a discussion of the term *type of operation.*

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the potential for adverse effect depends on factors such as the type of drug substance or drug product and operation being performed. Therefore, recommended reporting categories may differ depending on the type of drug product and operations.

Except for the situations described in sections VI.B.4, VI.C.1.b, and VI.D.5, construction activities at a manufacturing site or moving production operations within a building or between buildings at the same manufacturing site do not have to be reported to CDER.

We recommend that a move to a manufacturing site that involves other changes (e.g., process, equipment) be evaluated as a multiple related change (see section XII) to determine the appropriate reporting category.

B. Major Changes (Prior Approval Supplement)

The following are examples of changes considered to have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. A move to a different manufacturing site, except one used to manufacture or process a drug substance intermediate, when the new manufacturing site has never been inspected by FDA for the type of operation that is being moved or the move results in a restart at the new manufacturing site of a type of operation that has been discontinued for more than two years.

2. A move to a different manufacturing site, except one used to manufacture or process a drug substance intermediate, when the new manufacturing site does not have a satisfactory CGMP inspection for the type of operation being moved.

3. A move to a different manufacturing site for (1) the manufacture, processing, or primary packaging of drug products when the primary packaging components control the dose delivered to the patient or the formulation modifies the rate or extent of availability of the drug, or (2) the manufacture or processing of in-process materials with modified-release characteristics. Examples of these types of drug products include modified-release solid oral dosage forms,[15] transdermal systems, liposomal drug products, depot drug products, oral and nasal metered-dose inhalers (MDIs), dry powder inhalers (DPIs), and nasal spray pumps.

4. Transfer of the manufacture of an aseptically processed sterile drug substance or aseptically processed sterile drug product to (1) a newly constructed or refurbished aseptic processing facility or area or (2) an existing aseptic processing facility or area that does not manufacture similar (including container types and sizes) approved drug products. An example

[15] Certain operations relating to the manufacture, processing, or primary packaging of modified-release solid oral dosage form drug products need not be reported in a prior approval supplement (see sections VI.C.1.c and VI.D.6).

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would be transferring the manufacture of a lyophilized drug product to an existing aseptic process area where no approved lyophilized drug products are manufactured or where the approved lyophilized drug products being manufactured have different container types and/or sizes than the container of the drug product being transferred. See section VI.C.1.b for recommendations for other manufacturing site changes relating to aseptically processed sterile drug substance or aseptically processed sterile drug product.

5. Transfer of the manufacture of a finished drug product sterilized by terminal processes to a newly constructed facility at a different manufacturing site. Once this change has been approved, subsequent site changes to the facility for similar drug product types and processes may be submitted as a changes-being-effected-in-30-days supplement (see section VI.C.1.a).

C. Moderate Changes (Supplement - Changes Being Effected)

The following are examples of changes considered to have a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product. If the new site does not have a satisfactory CGMP inspection for the type of operation being moved (see sections VI.B.1 and 2), then FDA recommends that the changes listed below (excluding changes relating to drug substance intermediate manufacturing sites) be submitted in a prior approval supplement.

1. Supplement - Changes Being Effected in 30 Days

a. A move to a different manufacturing site for the manufacture or processing of any drug product, in-process material, or drug substance that is not otherwise provided for in this guidance.

b. For aseptically processed sterile drug substance or aseptically processed sterile drug product, a move to an aseptic processing facility or area at the same or different manufacturing site except as provided for in section VI.B.4.

c. A move to a different manufacturing site for the primary packaging of (1) any drug product that is not otherwise listed as a major change and (2) modified-release solid oral dosage form drug products.

d. A move to a different manufacturing site for testing if (1) the test procedures approved in the application or procedures that have been implemented via an annual report are used, (2) all postapproval commitments made by the applicant relating to the test procedures have been fulfilled (e.g., providing methods validation

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samples), and (3) the new testing facility has the capability to perform the intended testing.

2. *Supplement - Changes Being Effected*

A move to a different manufacturing site for the manufacture or processing of the final intermediate.

D. Minor Changes (Annual Report)

The following are examples of changes considered to have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product. If the new site does not have a satisfactory CGMP inspection for the type of operation being moved, then FDA recommends that the changes listed below (excluding changes relating to drug substance intermediate manufacturing sites) be submitted in a prior approval supplement (see sections VI.B.1 and 2).

1. A move to a different manufacturing site for secondary packaging.

2. A move to a different manufacturing site for labeling.

3. A move to a different manufacturing site for the manufacture or processing of drug substance intermediates other than the final intermediate.

4. A change in the contract sterilization site for packaging components when the process is not materially different from that provided for in the approved application

5. A transfer of the manufacture of a finished product sterilized by terminal processes to a newly constructed building or existing building at the same manufacturing site.

6. A move to a different manufacturing site for the ink imprinting of solid oral dosage form drug products.

VII. MANUFACTURING PROCESS

A. General Considerations

The potential for adverse effects on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product depends on the type of manufacturing process and the changes being instituted for the drug substance or drug product. In some cases, there may be a substantial potential for adverse effect regardless of direct testing of the drug substance or drug product for conformance with the approved specification. When there is a substantial potential for adverse effects, a change must be submitted in a prior approval supplement (section 506A(c) of the Act).

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B. Major Changes (Prior Approval Supplement)

The following are examples of changes considered to have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. Changes that may affect the controlled (or modified) release, metering or other characteristics (e.g., particle size) of the dose delivered to the patient, including the addition or deletion of a code imprint by embossing, debossing, or engraving on a modified-release solid oral dosage form.

2. Changes that may affect drug product sterility assurance including, where appropriate, process changes for sterile drug substances and sterile packaging components. These include:

   - Changes in the sterilization method (e.g., gas, dry heat, irradiation). These include changes from sterile filtered or aseptic processing to terminal sterilization, or vice versa.
   - Addition, deletion, or substitution of sterilization steps or procedures for handling sterile materials in an aseptic processing operation.
   - Replacing sterilizers that operate by one set of principles with sterilizers that operate by another principle (e.g., substituting a gravity displacement steam process with a process using superheated water spray).
   - Addition to an aseptic processing line of new equipment made of different materials (e.g., stainless steel versus glass, changes between plastics) that will come in contact with sterilized bulk solution or sterile drug components, or deletion of equipment from an aseptic processing line.
   - Replacing a Class 100 aseptic fill area with a barrier system or isolator for aseptic filling. Once this change has been approved, subsequent process changes for similar product types in the same barrier system or isolator may be submitted as a changes-being-effected-in-30-days supplement.
   - Replacement or addition of lyophilization equipment of a different size that uses different operating parameters or lengthens the overall process time.
   - Changes from bioburden-based terminal sterilization to the use of an overkill process, and vice versa.
   - Changes to aseptic processing methods, including scale, that extend the total processing, including bulk storage time, by more than 50 percent beyond the validated limits in the approved application.
   - Changes in sterilizer load configurations that are outside the range of previously validated loads.

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- Changes in materials or pore size rating of filters used in aseptic processing.

3. The following changes for a natural product:[16]

   - Changes in the virus or adventitious agent removal or inactivation methods. This applies to any material where such procedures are necessary, including drug substance, drug product, reagents, and excipients.
   - For drug substance and drug product, changes in the source material (e.g., microorganism, plant) or cell line.
   - For drug substance and drug product, establishment of a new master cell bank or seed.

4. Any fundamental change in the manufacturing process or technology from that currently used by the applicant. For example:

   a. Drug product

      - Dry to wet granulation or vice versa.
      - Change from one type of drying process to another (e.g., oven tray, fluid bed, microwave).

   b. Drug substance

      - Filtration to centrifugation or vice versa.
      - Change in the route of synthesis of a drug substance.

5. The following changes for drug substance

   - Any process change made after the final intermediate processing step in drug substance manufacture.

   - Changes in the synthesis or manufacture of the drug substance that may affect its impurity profile and/or the physical, chemical, or biological properties.

6. Addition of an ink code imprint or change to or in the ink used for an existing imprint code for a solid oral dosage form drug product when the ink as changed is not currently used on ***CDER-approved drug products***.[17]

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[16] For the purposes of this guidance, *natural product* refers to materials (e.g., drug substance, excipients) that are derived from plants, animals, or microorganisms, and that are subject to approval under section 505 of the Act. The specific recommendations for natural products are not applicable to inorganic compounds (e.g., salts, minerals).

[17] See Attachment C for a discussion of *CDER-approved drug products.*

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

7. Establishing a new procedure for reprocessing a batch of drug substance or drug product that fails to meet the approved specification.

C. Moderate Changes (Supplement - Changes Being Effected)

The following are examples of changes considered to have a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. Supplement - Changes Being Effected in 30 Days

a. For drug products, any change in the process, process parameters, and/or equipment except as otherwise provided for in this guidance.

b. For drug substances, any change in process and/or process parameters except as otherwise provided for in this guidance.

c. For natural protein drug substances and natural protein drug products:

- Any change in the process, process parameters, and/or equipment except as otherwise provided for in this guidance (e.g., section VII.B.5, VII.D.7).
- An increase or decrease in production scale during finishing steps that involves different equipment.
- Replacement of equipment with equipment of different design that does not affect the process methodology or process operating parameters.

d. For sterile drug products, drug substances, and components, as appropriate:

- Changes in dry heat depyrogenation processes for glass container systems for drug substances and drug products that are produced by terminal sterilization processes or aseptic processing.
- Changes to filtration parameters for aseptic processing (including flow rate, pressure, time, or volume, but not filter materials or pore size rating) when additional validation studies for the new parameters should be performed.
- Filtration process changes that provide for a change from single to dual sterilizing filters in series, or for repeated filtration of a bulk.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

- Changes from one qualified sterilization chamber to another for in-process or terminal sterilization that result in changes to validated operating parameters (time, temperature, F_0, and others).
- Changes in scale of manufacturing for terminally sterilized drug products that increase the bulk solution storage time by more than 50 percent beyond the validated limits in the approved application when bioburden limits are unchanged.

e. For drug substances, redefinition of an intermediate, excluding the final intermediate, as a starting material.

2. *Supplement - Changes Being Effected*

a. A change in methods or controls that provides increased assurance that the drug substance or drug product will have the characteristics of identity, strength, quality, purity, or potency that it purports or is represented to possess.

b. For sterile drug products, elimination of in-process filtration performed as part of the manufacture of a terminally sterilized drug product.

D. Minor Changes (Annual Report)

The following are examples of changes considered to have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. For drug products, changes to equipment of the same design and operating principle and/or changes in scale except as otherwise provided for in this guidance (e.g., section VII.C.1.c, VII.D.7).

2. A minor change in an existing code imprint for a dosage form. For example, changing from a numeric to alphanumeric code.

3. Addition of an ink code imprint or a change in the ink used in an existing code imprint for a solid oral dosage form drug product when the ink is currently used on CDER-approved drug products.

4. Addition or deletion of a code imprint by embossing, debossing, or engraving on a solid dosage form drug product other than a modified-release dosage form.

5. A change in the order of addition of ingredients for solution dosage forms or solutions used in unit operations (e.g., granulation solutions).

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

6. Changes in scale of manufacturing for terminally sterilized drug products that increase the bulk solution storage time by no more than 50 percent beyond the validated limits in the approved application when bioburden limits are unchanged.

7. For natural protein drug products and natural protein drug substances:

   - An increase or decrease in production scale during finishing steps that does not involve an equipment change.
   - Replacement of equipment with equipment of the same design, operating principle, and capacity with no change in production scale.

VIII. SPECIFICATIONS

A. General Considerations

All changes in specifications from those in the approved application must be submitted in a prior approval supplement unless otherwise exempted by regulation or guidance (§ 314.70(b)(2)(i)). *Specifications* (i.e., tests, analytical procedures, and acceptance criteria) are the quality standards provided in an approved application to confirm the quality of drug substances, drug products, intermediates, raw materials, reagents, components, in-process materials, container closure systems, and other materials used in the production of a drug substance or drug product. For the purpose of defining specifications, *acceptance criteria* are numerical limits, ranges, or other criteria for the tests described. Examples of a test, an analytical procedure, and an acceptance criterion are, respectively, an assay, a specific, fully described high pressure liquid chromatography (HPLC) procedure, and a range of 98.0–102.0 percent. The recommendations in this section also apply to specifications associated with sterility assurance that are included in NDA and ANDA submissions.[18]

A *regulatory* analytical procedure is the procedure in the approved application that is designated for use in evaluating a defined characteristic of the drug substance or drug product. Section 501(b) of the Act recognizes the analytical procedures in the *U.S. Pharmacopeia/National Formulary* (USP/NF) as the regulatory analytical procedures for compendial items. Tests and associated acceptance criteria and regulatory analytical procedures in addition to those specified in the USP/NF may be required for approving compendial items (section 505 of the Act).

The applicant may include in its application *alternatives* to the approved regulatory analytical procedures for testing the drug substance and drug product. However, for purposes of determining compliance with the Act, regulatory analytical procedures are used.

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[18] See FDA guidance for industry on the *Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products* (November 1994).

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

In sections B through D below, the use of the term *analytical procedure* without a qualifier such as *regulatory* or *alternative* refers to an analytical procedure used to test materials other than the drug substance or drug product.

B. Major Changes (Prior Approval Supplement)

The following are examples of changes in specifications considered to have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. Relaxing an acceptance criterion except as otherwise provided for in this guidance (e.g., section VIII.C.1.b, VIII.C.1.e).

2. Deleting any part of a specification except as otherwise provided for in this guidance (e.g., section VIII.D.2).

3. Establishing a new regulatory analytical procedure including designation of an alternative analytical procedure as a regulatory procedure.

4. A change in a regulatory analytical procedure that does not provide the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the regulatory analytical procedure described in the approved application.

5. A change in an analytical procedure used for testing components, packaging components, the final intermediate, in-process materials after the final intermediate, or starting materials introduced after the final intermediate that does not provide the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the analytical procedure described in the approved application except as otherwise noted. For example, a change from an HPLC procedure that distinguishes impurities to (1) an HPLC procedure that does not, (2) another type of analytical procedure (e.g., titrimetric) that does not, or (3) an HPLC procedure that distinguishes impurities but the limit of detection and/or limit of quantitation is higher.

6. Relating to testing of raw materials for viruses or adventitious agents:[19] (1) relaxing an acceptance criterion, (2) deleting a test, or (3) a change in the analytical procedure that does not provide the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the analytical procedure described in the approved application.

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[19] In this context, testing for adventitious agents is not considered to include tests that are found in an official compendium (e.g., USP <61>).

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

C. Moderate Changes (Supplement - Changes Being Effected)

The following are examples of changes in specifications considered to have a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. Supplement - Changes Being Effected in 30 Days

a. Any change in a regulatory analytical procedure other than those identified as major changes or editorial changes.

b. Relaxing an acceptance criterion or deleting a test for raw materials used in drug substance manufacturing, in-process materials prior to the final intermediate, starting materials introduced prior to the final drug substance intermediate, or drug substance intermediates (excluding final intermediate) except as provided for in section VIII.B.6.

c. A change in an analytical procedure used for testing raw materials used in drug substance manufacturing, in-process materials prior to the intermediate, starting materials introduced prior to the final drug substance intermediate, or drug substance intermediates (excluding final intermediate) that does not provide the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the analytical procedure described in the approved application except as provided for in section VIII.B.6.

d. Relaxing an in-process acceptance criterion associated with microbiological monitoring of the production environment, materials, and components that are included in NDA and ANDA submissions. For example, increasing the microbiological alert or action limits for critical processing environments in an aseptic fill facility or increasing the acceptance limit for bioburden in bulk solution intended for filtration and aseptic filling.

e. Relaxing an acceptance criterion or deleting a test to comply with an official compendium that is consistent with FDA statutory and regulatory requirements (§ 314.70(c)(2)(iii)).

2. Supplement - Changes Being Effected

a. An addition to a specification that provides increased assurance that the drug substance or drug product will have the characteristics of identity, strength, quality, purity, or potency that it purports or is represented to possess. For example, adding a new test and associated analytical procedure and acceptance criterion.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

b. A change in an analytical procedure used for testing components, packaging components, the final intermediate, in-process materials after the final intermediate, or starting materials introduced after the final intermediate that provides the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the analytical procedure described in the approved application.

D. Minor Changes (Annual Report)

The following are examples of changes in specifications considered to have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. Any change in a specification made to comply with an official compendium, except the changes described in section VIII.C.1.e, that is consistent with FDA statutory and regulatory requirements (§ 314.70(d)(2)(i)).

2. For drug substance and drug product, the addition or revision of an alternative analytical procedure that provides the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the analytical procedure described in the approved application or deletion of an alternative analytical procedure.

3. Tightening of acceptance criteria.

4. A change in an analytical procedure used for testing raw materials used in drug substance synthesis, starting materials introduced prior to the final drug substance intermediate, in-process materials prior to the final intermediate, or drug substance intermediates (excluding final intermediate) that provides the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the analytical procedure described in the approved application.

IX. CONTAINER CLOSURE SYSTEM

A. General Considerations

The potential for adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product when making a change to or in the container closure system is generally dependent on the route of administration of the drug product, performance of the container closure system, and the likelihood of interaction between the packaging component and the dosage form. In some cases there may be a substantial potential for adverse effect, regardless of direct drug product testing for conformance with the approved specification.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

A change to or in a packaging component will often result in a new or revised specification for the packaging component. This situation does not have to be considered a multiple related change. Only the reporting category for the packaging change needs to be considered.

B. Major Changes (Prior Approval Supplement)

The following are examples of changes considered to have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. For liquid (e.g., solution, suspension, elixir) and semisolid (e.g., creams, ointments) dosage forms, a change to or in polymeric materials (e.g., plastic, rubber) of primary packaging components, when the composition of the component as changed has never been used in a CDER-approved drug product of the same dosage form and same route of administration. For example, a polymeric material that has been used in a CDER-approved topical ointment would not be considered CDER-approved for an ophthalmic ointment.

2. For liquid (e.g., solution, suspension, elixir) and semisolid (e.g., creams, ointments) dosage forms in permeable or semipermeable container closure systems, a change from an ink and/or adhesive used on the permeable or semipermeable packaging component to an ink or adhesive that has never been used in a CDER-approved drug product of the same dosage form and same route of administration ***and*** with the same type of permeable or semipermeable packaging component (e.g., low density polyethylene, polyvinyl chloride).

3. A change in the primary packaging components for any drug product when the primary packaging components control[20] the dose delivered to the patient (e.g., the valve or actuator of a metered-dose inhaler).

4. For sterile drug products, any change that may affect drug product sterility assurance, such as:[21]

    - A change from a glass ampule to a glass vial with an elastomeric closure.

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[20] A container closure system that is considered to control the dose delivered to the patient is a container closure system where the system itself, rather than a person, regulates the amount of drug product ultimately delivered to a patient. A container closure system where a person controls the amount of drug product administered or that allows verification that the appropriate amount has been administered (e.g., number of tablets, milliliters of liquid) is not considered a container closure system that controls the dose delivered to the patient.

[21] Some of these identified changes, depending on the circumstances, may have to be submitted as original NDAs or ANDAs instead of as supplements. Applicants can consult the appropriate CDER chemistry division/office if there are questions.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

- A change to a flexible container system (bag) from another container system.
- A change to a prefilled syringe dosage form from another container system.
- A change from a single unit dose container to a multiple dose container system.
- Changes that add or delete silicone treatments to container closure systems (such as elastomeric closures or syringe barrels).
- Changes in the size and/or shape of a container for a sterile drug product.

5. Deletion of a secondary packaging component intended to provide additional protection to the drug product (e.g., carton to protect from light, overwrap to limit transmission of moisture or gases) or a change in the composition of, or the addition of, a secondary packaging component that may affect the impurity profile of the drug product.

6. A change to a new container closure system if the new container closure system does not provide the same or better protective properties than the approved container closure system.

C. Moderate Changes (Supplement - Changes Being Effected)

The following are examples of changes considered to have a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. Supplement - Changes Being Effected in 30 Days

a. A change to or in a container closure system, except as otherwise provided for in this guidance, that does not affect the quality of the drug product.

b. Changes in the size or shape of a container for a sterile drug substance.

c. A change in the number of units (e.g., tablets, capsules) or labeled amount (e.g., grams, milliliters) of a nonsterile drug product in a unit-of-use container.[22]

2. Supplement - Changes Being Effected

[22] A unit-of-use container is one that contains a specific quantity of a drug product and is intended to be dispensed to the patient without further modification except for the addition of appropriate labeling.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

a. A change in the size and/or shape of a container for a nonsterile drug product, except for solid dosage forms (see section IX.D.2), without a change from one container closure system to another (§ 314.70(c)(6)(ii)).

b. A change in the labeled amount (e.g., grams, milliliters) of drug product for a nonsterile drug product in a multiple-unit container,[23] except for solid dosage forms (see section IX.D.3) .

c. A change in or addition or deletion of a desiccant.

D. Minor Changes (Annual Report)

The following are examples of changes considered to have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. A change in the container closure system for a nonsterile drug product, based on a showing of equivalency to the approved system under a protocol approved in the application or published in an official compendium (§ 314.70(d)(2)(v)).

2. A change in the size and/or shape of a container for a nonsterile solid dosage form (§ 314.70(d)(2)(iv)).

3. A change in the number of units (e.g., tablets, capsules) or labeled amount (e.g., grams) of nonsterile solid dosage form in a multiple-unit container.

4. The following changes in the container closure system of solid oral dosage form drug products as long as the new package provides the same or better protective properties (e.g., light, moisture) and any new primary packaging component materials have been used in and been in contact with CDER-approved solid oral dosage form drug products:[24]

   - Adding or changing a child-resistant closure, changing from a metal to plastic screw cap, or changing from a plastic to metal screw cap.

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[23] A multiple-unit container is a container that permits withdrawal of successive portions of the contents without changing the strength, quality, or purity of the remaining portion. This type of container is not distributed directly to patients but is used by health care practitioners who dispense the drug product in smaller amounts to a patient in accordance with a physician's instructions.

[24] For sections IX.D.4 to IX.D.7, changes in the container closure system that result in drug product contact with a component material that has never been used in any CDER-approved drug product of the same type should be submitted as a changes-being-effected-in-30-days supplement (section IX.C.1) or prior approval supplement (section IX.B.1).

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

- Changing from one plastic container to another of the same type of plastic (e.g., high density polyethylene (HDPE) container to another HDPE container).
- Changes in packaging materials used to control odor (e.g., charcoal packets).
- Changes in bottle filler (e.g., change in weight of cotton or amount used) without changes in the type of filler (e.g., cotton to rayon).
- Increasing the wall thickness of the container.
- A change in or addition of a cap liner.
- A change in or addition of a seal (e.g., heat induction seal).
- A change in an antioxidant, colorant, stabilizer, or mold releasing agent for production of the container and/or closure to one that is used at similar levels in the packaging of CDER-approved solid oral dosage form drug products.
- A change to a new container closure system when the container closure system is already approved in the NDA or ANDA for other strengths of the drug product.

5. The following changes in the container closure system of nonsterile liquid drug products as long as the new package provides the same or better protective properties and any new primary packaging component materials have been used in and been in contact with CDER-approved liquid drug products with the same route of administration (i.e., the material in contact with a liquid topical should already have been used with other CDER-approved liquid topical drug products):

   - Adding or changing a child-resistant closure, changing from a metal to plastic screw cap, or changing from a plastic to metal screw cap.
   - Increasing the wall thickness of the container.
   - A change in or addition of a cap liner.
   - A change in or addition of a seal (e.g., heat induction seal).

6. A change in the container closure system of unit dose packaging (e.g., blister packs) for nonsterile solid dosage form drug products as long as the new package provides the same or better protective properties and any new primary packaging component materials have been used in and been in contact with CDER-approved drug products of the same type (e.g., solid oral dosage form, rectal suppository).

7. The following changes in the container closure system of nonsterile semisolid drug products as long as the new package provides the same or

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better protective properties and any new primary packaging component materials have been used in and been in contact with CDER-approved semisolid drug products:

- Changes in the closure or cap.
- Increasing the wall thickness of the container.
- A change in or addition of a cap liner.
- A change in or addition of a seal.
- A change in the crimp sealant.

8. A change in the flip seal cap color as long as the cap color is consistent with any established color coding system for that class of drug products.

X. LABELING

A. General Considerations

A drug product labeling change includes changes in the package insert, package labeling, or container label. In accordance with § 314.70(a)(4), an applicant must promptly revise all promotional labeling and drug advertising to make it consistent with any labeling change implemented in accordance with paragraphs (b) or (c) of § 314.70. All labeling changes for ANDA drug products must be consistent with section 505(j) of the Act.

B. Major Changes (Prior Approval Supplement)

Any proposed change in the labeling, except changes designated as moderate or minor by regulation or guidance, must be submitted as a prior approval supplement (§ 314.70(b)(2)(v)(A)). If applicable, any change to a Medication Guide required under 21 CFR part 208, except for changes in the information specified in § 208.20(b)(8)(iii) and (b)(8)(iv), must be submitted in a prior approval supplement (§ 314.70(b)(v)(B)). The following list contains some examples of changes currently considered by CDER to fall into this reporting category.

1. Changes based on postmarketing study results, including, but not limited to, labeling changes associated with new indications and usage.

2. Change in, or addition of, pharmacoeconomic claims based on clinical studies.

3. Changes to the clinical pharmacology or the clinical study section reflecting new or modified data.

4. Changes based on data from preclinical studies.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

5. Revision (expansion or contraction) of population based on data.

6. Claims of superiority to another drug product.

7. Change in the labeled storage conditions, unless exempted by regulation or guidance.

C. Moderate Changes (Supplement - Changes Being Effected)

Under § 314.70(c)(6)(iii), a changes-being-effected supplement must be submitted for any labeling change that (1) adds or strengthens a contraindication, warning, precaution, or adverse reaction, (2) adds or strengthens a statement about drug abuse, dependence,

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

psychological effect, or overdosage, (3) adds or strengthens an instruction about dosage and administration that is intended to increase the safe use of the drug product, (4) deletes false, misleading, or unsupported indications for use or claims for effectiveness, or (5) normally requires a supplement submission and approval prior to distribution of the drug product that FDA specifically requests be submitted under this provision. A changes-being-effected supplement that provides for a labeling change under §§ 314.70(c)(6)(iii) must include 12 copies of final printed labeling (§ 314.70(c)(1)). The following list includes some examples of changes currently considered by CDER to fall into this reporting category.

1. Addition of an adverse event due to information reported to the applicant or Agency.

2. Addition of a precaution arising out of a postmarketing study.

3. Clarification of the administration statement to ensure proper administration of the drug product.

D. Minor Changes (Annual Report)

Labeling with editorial or similar minor changes or with a change in the information concerning the description of the drug product or information about how the drug is supplied that does not involve a change in the dosage strength or dosage form should be described in an annual report (§ 314.70(d)(2)(ix) and (d)((2)(x)) . The following list includes some examples currently considered by CDER to fall into this reporting category.

1. Changes in the layout of the package or container label that are consistent with FDA regulations (e.g., 21 CFR part 201) without a change in the content of the labeling.

2. Editorial changes, such as adding a distributor's name.

3. Foreign language versions of the labeling if no change is made to the content of the approved labeling and a certified translation is included.

4. Labeling changes made to comply with an official compendium.

XI. MISCELLANEOUS CHANGES

A. Major Changes (Prior Approval Supplement)

The following are examples of changes considered to have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

1. Changes requiring completion of studies in accordance with 21 CFR part 320 to demonstrate equivalence of the drug product to the drug product as manufactured without the change or to the reference listed drug (§ 314.70(b)(2)(ii)).

2. Addition of a stability protocol or comparability protocol.

3. Changes to an approved stability protocol or comparability protocol unless otherwise provided for in this guidance (e.g., VIII.C, VIII.D, XI.C.2).

4. An extension of an expiration dating period based on (1) data obtained under a new or revised stability testing protocol that has not been approved in the application or (2) full shelf life data on pilot scale batches using an approved protocol.

5. Changes to a drug product under an application that is subject to a validity assessment because of significant questions regarding the integrity of the data supporting that application (§ 314.70(b)(2)(viii)).

B. Moderate Changes (Supplement - Changes Being Effected)

The following are examples of changes considered to have a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. Supplement - Changes Being Effected in 30 Days

Reduction of an expiration dating period to provide increased assurance of the identity, strength, quality, purity, or potency of the drug product. Extension of an expiration date that has previously been reduced under this provision should be submitted in a changes-being-effected-in-30-days supplement even if the extension is based on data obtained under a protocol approved in the application.

2. Supplement - Changes Being Effected

No changes have been identified.

C. Minor Changes (Annual Report)

The following are examples of changes considered to have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product.

1. An extension of an expiration dating period based on full shelf life data on production batches obtained under a protocol approved in the application (§ 314.70(d)(2)(vi)).

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

2. Addition of time points to the stability protocol or deletion of time points beyond the approved expiration dating period.

3. A change from previously approved stability storage conditions to storage conditions recommended in International Conference on Harmonisation (ICH) guidances.

4. Non-USP reference standards:

   - Replacement of an in-house reference standard or reference panel (or panel member) according to procedures in an approved application.
   - Tightening of acceptance criteria for existing reference standards to provide greater assurance of drug product purity and potency.

XII. MULTIPLE RELATED CHANGES

Multiple related changes involve various combinations of individual changes. For example, a site change may also involve equipment and manufacturing process changes or a components and composition change may necessitate a change in a specification. For multiple related changes where the recommended reporting categories for the individual changes differ, CDER recommends that the submission be in accordance with the most restrictive of the categories recommended for the individual changes. When the multiple related changes all have the same recommended reporting category, CDER recommends that the submission be in accordance with the reporting category for the individual changes.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

ATTACHMENT A: MANUFACTURING SITES

All owners or operators of all drug establishments (not exempt by regulation) that engage in the manufacture, preparation, propagation, compounding, or processing of a drug or drugs are required to register with the FDA (21 CFR 207.20). An *establishment* means a place of business under one management at one general physical location (§ 207.3(a)(7)). A *general physical location* is reasonably construed to include separate buildings within the same city ***if*** the activities in the buildings are closely related to the same business enterprise, are under the supervision of the same local management, and are all inspected at the same time (ORA Field Management Directive No. 132).

For the purposes of determining the reporting category for moves between buildings, the terms *same manufacturing site* and *different manufacturing site* mean:

Domestic Establishments

Same manufacturing site:

- The new and old buildings are included under the same drug establishment registration number[25]

and

- The same FDA district office is responsible for inspecting the operations in both the new and old buildings.

Different manufacturing site:

- The new and old buildings have different drug establishment registration numbers

or

- Different FDA district offices are responsible for inspecting operations in the new and old buildings.

For domestic establishments, the terms *same manufacturing site* and *different manufacturing site* supersede the terms *contiguous campus*, *same campus*, and *different campus* as used in the SUPAC guidances.

Foreign Establishments

[25] The registration number is the number assigned to the establishment as part of the registration process (e.g., ORA Field Management Directive No. 92).

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

Foreign establishments are not currently required to register with the FDA. On May 14, 1999, FDA published a proposed rule to require registration of foreign establishments (64 FR 26330). Until registration of foreign establishments is required, same and different manufacturing sites mean:

Same manufacturing site:

- A contiguous or unbroken site or a set of buildings in adjacent city blocks.

Different manufacturing site:

- The new and old buildings are not on a contiguous site or not in adjacent city blocks.

* Insofar as this guidance adjusts reporting categories pursuant to section 506A of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.70, it does have binding effect.

ATTACHMENT B: TYPE OF OPERATION AND CGMP INSPECTIONS

Section VI states that a change to a different manufacturing site should be submitted in a prior approval supplement when (1) the new manufacturing site has never been inspected by FDA for the type of operation being moved, (2) the move results in a restart at the new manufacturing site of a type of operation that has been discontinued for more than two years, or (3) the new manufacturing site does not have a satisfactory current good manufacturing practice (CGMP) inspection for the type of operation being moved.

A *profile class system* is used by FDA to assist in (1) managing the CGMP inspection process, (2) evaluating the findings and the compliance follow-up needed, and (3) communicating the results of inspections. A profile class can relate to the manufacture of a particular dosage form (e.g., large volume parenterals, oral liquids), type of drug substance (e.g., sterile bulk by chemical synthesis), or specific function performed at a site (e.g., control testing laboratory). There are profile class codes for major categories of drug substance processes, dosage forms, and manufacturing functions (see table below). However, the system is not comprehensive for all operations performed in the pharmaceutical industry (see not elsewhere classified (NEC) profile class code).

The term *type of operation* refers to the specialized or even unique conditions and practices that are employed to manufacture a class or category of drug substance or drug product or to perform a limited segment of the manufacturing process. These conditions and practices exist and are performed within the framework of CGMPs, along with general conditions and practices that contribute to the manufacture of all drug products at a given manufacturing site. The conditions and practices, both general and specific, are inspected to evaluate the CGMP acceptability of a manufacturing site. A wide variety of classes or categories of drug substances and drug products may be produced at a manufacturing site, or the manufacturing site may only produce a single class of drug substance and/or drug product or perform a limited segment of a manufacturing process. Each type of operation is represented by a ***profile class code.***

Generally, a satisfactory CGMP status for a profile class code is used to communicate a satisfactory CGMP clearance for all of the products and for all of the operations included within the category that code represents. Thus the profile class code for a particular dosage form or type of drug substance is used to communicate the CGMP status for all aspects of manufacturing, processing, packing, or holding that are performed at the specific manufacturing site relating to that particular dosage form or type of drug substance, including packaging and labeling operations, testing, and quality control. The profile class code for a particular dosage form or type of drug substance is also used to communicate the CGMP status for manufacturing sites that produce in-process material (e.g., controlled-release beads), package drug products, or label drug products, even if these are stand-alone (e.g., contractor) operations.

A few profile class codes that describe certain types of operations (see items in boldface in table) are provided to report the CGMP status for contractor firms whose only function in the manufacturing process is to perform this operation. If one of these operations (e.g., steam sterilization process) is performed at the manufacturing site involved in producing the drug product/drug substance, the CGMP status for that operation is reported as part of the profile class code for the particular dosage form or type of drug substance. For example, a manufacturing site

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producing a terminally sterilized small volume parenteral drug product would be reported with the profile class code for the dosage form (SVT), not by the profile code for the sterilization process (SSP).

Certain inspections may be required by program priorities even if the rating for a profile class code indicates an acceptable CGMP status. The current profile codes/classes for human drugs are:

ADM	Aerosol dispensed medication	NEC	Not elsewhere classified (when using this class, specific drug products are noted)
CBI	Biotechnology crude drug	OIN	Ointment, nonsterile (includes cream, jelly, paste)
CEX	Plant/animal extraction crude drug	POW	Powders (includes oral and topical)
CFS	Sterile bulk by fermentation crude drug	RAD	Radiopharmaceutical
CFN	Nonsterile bulk by fermentation crude drug	**RSP**	**Radiation sterilization process**
CHG	Capsule, prompt release	SNI	Sterile noninjectable
CRU	Crude bulk drugs-nonsynthesized	SOP	Soap
CSG	Capsules, soft gelatin	**SSP**	**Steam sterilization process**
CSN	Nonsterile bulk by chemical synthesis	SUP	Suppositories
CSP	**Chemical sterilization process**	SVL	Small volume parenterals (lyophilized)
CSS	Sterile bulk by chemical synthesis	SVS	Sterile-filled small volume parenterals
CTL	**Control testing laboratories**	SVT	Terminally sterilized small volume parenteral
CTR	Capsules, modified-release	TCM	Tablets, prompt-release
GAS	Medical gas (includes liquid oxygen and other)	TCT	Tablets, delayed-release
GSP	**Gas sterilization process**	TDP	Transdermal patches
HSP	**Dry heat sterilization process**	**TSP**	**Fractional (tyndallization) sterilization process**
LIQ	Liquid (includes solutions, suspension, elixirs, and tinctures)	TTR	Tablets, extended-release
LVP	Large volume parenterals	**WSP**	**Water sterilization process**

CGMP inspectional status, based on the profile class, is available through FDA's Freedom of Information (FOI) Office. (See Glossary under Satisfactory Current Good Manufacturing Practice (CGMP) Inspection for more information regarding FOI requests.)

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Examples of postapproval manufacturing site changes and recommended reporting categories:

- An applicant wants to move the manufacture of an immediate-release tablet (TCM) to a different manufacturing site that currently manufactures, and has satisfactory CGMP status for, capsules (CHG) and powders for oral solution (POW). This manufacturing site change should be submitted in a prior approval supplement because the new manufacturing site does not have a satisfactory CGMP inspection for immediate-release tablets.
- An applicant wants to contract out packaging operations for immediate-release tablets (TCM) and capsules (CHG) and modified-release capsules (CTR). The potential contract packager has a satisfactory CGMP status for immediate-release and modified-release capsules but has never packaged immediate-release tablets. The packaging site change for the immediate-release tablet drug products should be submitted in a prior approval supplement. The packaging site change for the capsule drug products should be submitted as recommended in section VI of this guidance for packaging sites with a satisfactory CGMP inspection.
- An applicant wishes to consolidate product testing to a single analytical laboratory at a manufacturing site. This manufacturing site produces various solid oral dosage form drug products, has an operational analytical laboratory currently at the site, and satisfactory CGMP inspections for the manufacturing occurring at the facility. Some of the drug products that will be tested at the analytical laboratory when the consolidation occurs are not solid oral dosage form products. Unlike most other production operations, testing laboratories (and other operations in boldface in the table) are not inspected on a dosage form/type of drug substance specific basis. The satisfactory CGMP inspection of the analytical laboratory, which was performed as part of the CGMP inspection for manufacture of the solid oral dosage form drug products, is considered to apply to all dosage forms, including those not actually produced at the site. The consolidation can be submitted in a changes-being-effected-in-30-days supplement if the change is consistent with the recommendations in section VI.C.1.d.

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ATTACHMENT C: CDER-APPROVED DRUG PRODUCTS

In several places throughout the guidance, different reporting categories are proposed for changes to or the addition of certain components based on whether the component/material has been used in and has been in contact with CDER-approved drug products. Different reporting categories are recommended once CDER has reviewed certain components/materials in association with a drug product approval because similar subsequent changes then have a reduced potential to have an adverse effect on the identity, strength, quality, purity, or potency of a drug product as these factors may relate to the safety or effectiveness of the drug product. For example, certain changes in the container closure systems of solid oral dosage form drug products may be included in an annual report as long as the new package provides the same or better protective properties and any new primary packaging component materials have been used in and been in contact with CDER-approved solid oral dosage form drug products (see section IX.D.4). If the new primary packaging component material has not been used in or has not been in contact with CDER-approved solid oral dosage form drug products, then submission of the change in an annual report is not recommended.

CDER-approved drug products are considered those drug products subject to an approved NDA or ANDA. Some information on which components/materials are used in CDER-approved products is available from the Agency (e.g., FDA, CDER, *Inactive Ingredient Guide*, 1996, Division of Drug Information Resources). When information is not available, an applicant should use reliable sources of information to determine that the component or material has been used in and has been in contact with a CDER-approved drug product of the same dosage form and route of administration, as appropriate. The applicant should identify in the supplement or annual report the basis for the conclusion that the component or material is used in a CDER-approved drug product.

If an applicant cannot confirm that a component or material has been used in and has been in contact with a CDER-approved drug product of the same dosage form and route of administration, the applicant has the option of submitting the change for a single NDA or ANDA using the higher recommended reporting category and, after approval, submitting similar changes for other NDAs and ANDAs using the lower recommended reporting category.

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GLOSSARY

Acceptance Criteria: Numerical limits, ranges, or other criteria for the tests described (21 CFR 314.3(b)).

Active Ingredient/Drug Substance: Any component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of a disease, or to affect the structure or any function of the human body, but does not include intermediates used in the synthesis of such ingredient. The term includes those components that may undergo chemical change in the manufacture of the drug product and are present in the drug product in a modified form intended to furnish the specified activity or effect (21 CFR 210.3(b)(7) and 314.3(b)).

Assess the Effects of the Change: To evaluate the effects of a manufacturing change on the identity, strength, quality, purity, and potency of a drug product as these factors may relate to the safety or effectiveness of the drug product (21 CFR 314.3(b)).

Container Closure System: The sum of packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components if the latter are intended to provide additional protection to the drug product.

Component: Any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product (21 CFR 210.3(b)(3)).

Drug Product: A finished dosage form, for example, tablet, capsule, or solution, that contains an active ingredient generally, but not necessarily, in association with inactive ingredients (21 CFR 210.3(b)(4)).

Final Intermediate: The last compound synthesized before the reaction that produces the drug substance. The final step forming the drug substance involves covalent bond formation or breakage; ionic bond formation (i.e., making the salt of a compound) does not qualify. Consequently, when the drug substance is a salt, the precursors to the organic acid or base, rather than the acid or base itself, should be considered the final intermediate.

Inactive Ingredient: Any intended component of the drug product other than an active ingredient.

In-process Material: Any material fabricated, compounded, blended, or derived by chemical reaction that is produced for, and used in, the preparation of the drug product (21 CFR 210.3(b)(9)). For drug substance, in-process materials are considered those materials that are undergoing change (e.g., molecular, physical).

Intermediate: A material that is produced during steps of the synthesis of a drug substance and undergoes further molecular change before it becomes a drug substance.

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Package: The container closure system and labeling, associated components (e.g., dosing cups, droppers, spoons), and external packaging (e.g., cartons, shrink wrap).

Packaging Component: Any single part of a container closure system.

Primary Packaging Component: A packaging component that is or may be in direct contact with the dosage form.

Reference Listed Drug: The listed drug identified by FDA as the drug product on which an applicant relies in seeking approval of its abbreviated application (21 CFR 314.3(b)).

Satisfactory Current Good Manufacturing Practice (CGMP) Inspection: A satisfactory CGMP inspection is an FDA inspection during which (1) no objectionable conditions or practices were found (No Action Indicated (NAI)) or (2) objectionable conditions were found, but voluntary corrective action is left to the firm and the objectionable conditions will not be the subject of further administrative or regulatory actions (Voluntary Action Indicated (VAI)).

Information about the CGMP status of a firm may be obtained by requesting a copy of the Quality Assurance Profile (QAP) from the FDA's Freedom of Information (FOI) Office. The QAP contains information on the CGMP compliance status of firms that manufacture, package, assemble, repack, relabel, or test human drugs, devices, biologics, and veterinary drugs. All FOI requests must be in writing (21 CFR 20.40(a)) and should be prepared following the instructions found in the reference entitled *A Handbook for Requesting Information and Records from FDA*. An electronic version of this reference is available on the Internet at http://www.fda.gov/opacom/backgrounders/foiahand.html.

Secondary Packaging Component: A packaging component that is not and will not be in direct contact with the dosage form.

Specification: The quality standard (i.e., tests, analytical procedures, and acceptance criteria) provided in an approved application to confirm the quality of drug substances, drug products, intermediates, raw materials, reagents, components, in-process materials, container closure systems, and other materials used in the production of a drug substance or drug product (21 CFR 314.3(b)).

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

----------x
STEADYMED LTD.,

Petitioner,

vs.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.
----------x

VIDEOTAPED DEPOSITION OF

JEFFREY D. WINKLER, Ph.D.

New York, New York

June 14, 2016

9:33 a.m.

Reported by:
Jennifer Ocampo-Guzman, CRR, CLR
JOB NO. 44975

June 14, 2016

9:33 a.m.

Videotaped Deposition of JEFFREY D. WINKLER, Ph.D., held at the offices of DLA Piper LLP (US), 1251 Avenue of the Americas, New York, New York, pursuant to notice, before Jennifer Ocampo-Guzman, a Certified Real-Time Shorthand Reporter and a Notary Public of the State of New York.

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ALSO PRESENT:

JOSE RIVERA, VIDEOGRAPHER

SHAUN SNADER, ESQ. (UNITED THERAPEUTICS)

(Winkler Exhibit 1, Curriculum Vitae of Jeffrey David Winkler, [SteadyMed-Exhibit 1010], marked for identification, this date.)

(Winkler Exhibit 2, Declaration of Jeffrey D. Winkler in Support of Petition for Inter Partes Review of Claims 1-22 of U.S. Patent No. 8,497,393, [SteadyMed-Exhibit 1009], marked for identification, this date.)

(Winkler Exhibit 3, Copy of U.S. Patent No. 8,497,393, [SteadyMed-Exhibit 1001], marked for identification, this date.)

THE VIDEOGRAPHER: This is media unit number 1 in the video deposition of Jeffrey D. Winkler in the matter of SteadyMed Limited, petitioner, versus United Therapeutics Corporation, patent owner.

This deposition is being held at DLA Piper LLP, 1251 Avenue of

the Americas, New York, New York on June 14, 2016, at approximately 9:33 a.m.

My name is Jose Rivera from the firm of David Feldman Worldwide and I am the legal video specialist. The court reporter is Jennifer Ocampo-Guzman in association with David Feldman Worldwide, located at 450 Seventh Avenue, New York, New York.

For the record, will counsels please introduce themselves.

MR. DELAFIELD: Bobby Delafield of Wilson Sonsini Goodrich & Rosati representing patent owner, United Therapeutics Corporation.

MR. MAEBIUS: Stephen Maebius, Foley & Lardner, representing patent owner, United Therapeutics Corporation.

MR. SNADER: Shaun Snader, United Therapeutics, Washington,

DC, for patent owner, United Therapeutics.

MR. POLLACK: Stuart E. Pollack from DLA Piper LLP (US) on behalf of Professor Winkler and on behalf of SteadyMed Limited.

THE VIDEOGRAPHER: Now will the court reporter please swear in the witness.

J E F F R E Y D. W I N K L E R, called as a witness, having been duly sworn, was examined and testified as follows:

EXAMINATION BY

MR. DELAFIELD:

Q. Good morning, Dr. Winkler.

A. Good morning.

Q. Could you please state and spell your full name for the record?

A. Jeffrey David Winkler, J-E-F-F-R-E-Y, D-A-V-I-D, W-I-N-K-L-E-R.

Q. Have you been deposed before?

A. Yes, I have.

Q. About how many times?

Winkler

A. About ten or 12 times.

Q. Okay. Well, you probably know all the ground rules, but I just want to go over a few just to refresh your memory. I'll be asking a series of questions and you need to provide an answer, unless your counsel instructs you not to do so.

Because this is being recorded, the answers need to be in verbal form, and so no head shakes or nods, because it won't be recorded by the stenographer.

You are reminded your testimony is under oath, so your answers need to be truthful and full to the best of your knowledge.

I might ask a confusing question, and if so, feel free to ask me to clarify, if you have any issues with my question.

Also, the stenographer has to record everything we say, so I'm asking if you could not speak over me or vice

Winkler

versa, so that she can record both of what we're saying.

Also, if you need a break at any time, feel free to just tell me, and we can take a break, as long as a question isn't pending.

Is there any reason you can think of why you will not be able to answer my questions today fully and accurately?

A. No.

Q. Are you taking any medication or drugs of any kind that might make it difficult for you to understand and answer my questions?

A. No.

Q. Okay. So you've provided a declaration regarding the '393 patent in this case; is that correct?

A. Yes, it is.

Q. So I want to go ahead and hand you three exhibits.

(Discussion off the record.)

MR. POLLACK: Thank you.

Winkler

Q. So if you turn to Exhibit 1, can you tell me what that exhibit is?

A. Exhibit 1 is my curriculum vitae.

Q. Is that a true and accurate copy of your CV?

A. Yes, it is.

Q. Can you briefly summarize your educational background?

A. So I was an undergraduate at Harvard College. I graduated with honors in 1977, and I then pursued graduate studies at Columbia University, under the direction of the Professor Gilbert Stork, where I received an MA and MPhil and finally a Ph.D. degree in 1981. I stayed at Columbia as an American Cancer Society post-doctoral fellow in the laboratory of Professor Ronald Breslow from 1982 to 1983.

And that was the end of my formal education.

Q. Okay. So looking at page 1 of your CV, it lists your professional

Winkler

experience. Is that a complete listing of your professional experience since getting your Ph.D.?

A. Yes, it is.

Q. So you've never worked as a chemist outside of academia; is that correct?

A. Well, actually I spent a year long sabbatical at Bristol-Myers Squibb in Lawrenceville, New Jersey, in about 2000 or 2001. And I've consulted with a number of pharmaceutical and chemical companies over the course of my career.

Q. Okay. But other than the sabbatical, you've never been employed full time at a chemical company apart from your job as a --

MR. DELAFIELD: Strike that.

Q. Apart from the year long sabbatical, your full-time employment has been with universities; is that correct?

MR. POLLACK: Objection to form.

You can answer.

Winkler

A. Well, as I stated, my -- I've been involved with companies over the course of my career full time, only during that year at BMS.

Q. And what did you do during that year at BMS?

MR. POLLACK: I'm just, don't reveal any confidential information that belongs to BMS, but if it's not confidential, you can reveal that now.

A. So during the year at BMS, I was part of I think two different research teams investigating various aspects of drug development, and then I also taught a course to BMS scientists that was ongoing throughout the course of the year.

Q. Have you ever formulated a drug product?

MR. POLLACK: Objection, objection to form, vague.

A. I'm sorry. What do you mean by "formulated a drug product"?

Winkler

Q. Have you ever worked in a lab for a pharmaceutical company to make a drug product?

A. Well, I guess I would say that in my time during the sabbatical year, I was working with two teams on the development of drug products.

Q. Have you ever synthesized a drug substance?

A. I'm sorry, I don't understand what you mean by that.

Q. Well, have you ever personally or directed others to actually synthesize a drug substance that was used in a commercially available drug product?

A. Yes, I have.

Q. And what was that?

A. For a while, in the 1990s, my laboratory was involved in the synthesis of Ritalin, of -- of 30, P-H-R-E-O, Phenidate, P-H-E-N-I-D-A-T-E, which is the API in Ritalin, R-I-T-A-L-I-N.

Q. And so was that API actually used in a commercial product?

Winkler

MR. POLLACK: Objection to form.

You can answer.

A. I don't know the answer to that question.

Q. Have you ever submitted a filing to the FDA regarding any drug product or drug substance?

A. No, I have not.

Q. Have you ever corresponded with the FDA at all about any drug?

A. No, I have not.

Q. Have you ever developed a protocol for evaluating the impurity profile of a drug?

A. Well, my laboratory is routinely involved in the purification and the assay of the substances that we create in our laboratory.

Q. Sorry. Maybe you misunderstood my question.

Have you ever developed an impurity profile for a drug product?

MR. POLLACK: Objection to

form.

A. I'm afraid I don't understand your question.

Q. Well, you understand that to have a drug substance or a drug product you must submit an impurity profile to the FDA. Do you understand that?

A. I have never submitted a drug impurity profile to the FDA.

Q. Okay. Have you ever developed the impurity profile for someone else to submit to the FDA?

A. Not that I can think of sitting here now.

Q. Do you have any experience synthesizing prostaglandins?

A. I have certainly studied the synthesis of prostaglandins and taught the synthesis of prostaglandins.

Q. And you have or people working for you ever synthesized prostaglandins?

A. My laboratory has worked on the development of the methodology, synthetic methodology that could be

Winkler

applicable to the synthesis of prostaglandins.

Q. But you or your lab haven't actually synthesized prostaglandins; is that correct?

A. Sitting here I can't think of an example where we have synthesized prostaglandins.

Q. Do you have any experience manufacturing --

A. Excuse me. Although, I should add that my laboratory has synthesized compounds that are certainly related to the prostaglandins.

Q. Okay. Do you have any prior experience synthesizing or analyzing treprostinil or any of its derivatives? Prior to this case?

MR. POLLACK: Objection to form, compound.

A. Prior to this case, I have not had -- I'm sorry, could you repeat the question, please?

Q. Prior to this case, did you

have any experience synthesizing or analyzing treprostinil?

A. Prior to this case, I have not had experience with treprostinil, specifically.

Q. Do you have any experience scaling up drug substances from lab scale to industrial scale?

A. In my role as a consultant in the pharmaceutical industry, I certainly have had experience with the scale up of reactions in the pharmaceutical industry.

Q. When you say you have experience with reactions, what do you mean by that?

A. Excuse me. In saying that I have experience with scale up, that means that as part of my association with pharmaceutical companies, I've had the opportunity to consult on and discuss with pharmaceutical scientists and advise pharmaceutical scientists on large scale reactions.

Q. Have you or your lab performed

Winkler

any large scale reactions?

A. I'm afraid I don't understand exactly what you mean by "large scale reactions."

Q. Well, you just mentioned that you had advised and mentioned the term "large scale reactions," and so I guess to put a number on it, have you or your lab performed any syntheses on a kilogram scale?

A. I would have to go back to the lab notebooks in my research group to know whether we had done reactions on that scale.

So sitting here, I can't really answer that.

Q. But sitting here today, you can't remember anything specific on that scale, or larger; is that correct?

MR. POLLACK: Objection to form.

A. I can't remember having done reactions on kilogram scale, but I certainly can't remember not having done

them on kilogram scale.

Q. Are you familiar with the FDA guidelines regarding impurity profiles for a drug?

A. No, I am not.

Q. Do you know what is required in order to change a drug specification with the FDA?

A. No, I do not.

Q. Are you familiar with published guidances from the FDA regarding changes to new drug applications or abbreviated new drug applications?

A. I'm sorry, could you repeat the question?

Q. Are you familiar with published guidances from the FDA regarding changes to new drug applications or abbreviated new drug applications?

A. No, I am not familiar with that.

Q. You had mentioned you had been

Winkler

deposed several times. Do you recall how many patent litigations you've worked on?

A. I don't remember exactly, no.

Q. So let's look at Exhibit 3, which is the '393 patent. Do you recognize this document?

A. Yes, I do.

Q. And this is the '393 patent that is at issue in this case, correct?

A. That's my understanding, yes.

Q. If you could turn to column 2 -- actually, column 3, I'm sorry.

And do you see in column 3 structure Roman numeral (IV)?

A. Yes, I do.

Q. Do you recognize that structure?

A. I do, yes.

Q. And what is that structure?

A. That is the chemical structure of treprostinil.

Q. Would you agree that treprostinil has five chiral centers?

A. Yes, I would, five chiral

Winkler

centers or 5 stereo centers, yes.

Q. So if a molecule has five chiral centers, that means that it has 32 possible stereoisomers; is that right?

A. Two to the five, that's correct.

Q. Is it fair to say that treprostinil is a complex molecule?

MR. POLLACK: Objection to form.

A. I think that's a difficult question for me, because the question would be, complex relative to what?

Q. Well, just your experience as a chemist, would you consider treprostinil to be complex compared to other chemicals that you've since synthesized?

MR. POLLACK: Objection to form.

A. We've synthesized compounds in my laboratory that are much more complex than treprostinil, and we've synthesized some molecules that are less complex than

Winkler

treprostinil.

Q. You've reviewed the synthesis for treprostinil for the '393 patent, correct?

A. Yes, I have.

Q. And you've reviewed some of the prior art that had other syntheses for treprostinil; is that right?

A. Yes, I have.

Q. And the total synthesis is, I believe, roughly 20 steps, depending on which synthesis, but it's a multi-step process; is that correct?

MR. POLLACK: Objection to form.

A. I'm sorry, I don't understand the question.

Q. You would agree that the synthesis for treprostinil is approximately 20 steps?

A. I actually haven't counted the number of steps in the synthesis.

Q. Would you consider the synthesis of treprostinil to be complex?

Winkler

MR. POLLACK: Objection to form.

A. Again, the problem that I would have in answering that question is: Complex to relative to what? There are things that are much more complex than treprostinil, and there are things that are decidedly less complex.

Q. Well, would you expect, let's say, undergraduate students to be synthesizing treprostinil or structures similar to treprostinil in their lab?

A. That's a very difficult question for me to answer, because it would depend on the level and skill of the undergraduate student.

Q. Well, just as a matter of course, within the courses you teach, for example, are you aware of any syntheses that are multiple steps that have --

MR. DELAFIELD: Strike that.

Q. Do you teach organic chemistry?

A. Yes, I do.

Winkler

Q. And in your experience in teaching organic chemistry, do students typically synthesize structures that have five or more chiral centers from commercially available starting materials?

A. I don't know the answer to that.

Q. But sitting here today, you can't think of any?

A. I'm sorry, I don't understand the question.

Q. Sitting here today, you are not aware of any syntheses that your students perform synthesizing molecules with five or more chiral centers?

A. Sitting here today, I can't think of any examples.

Q. Do your undergraduate students typically perform kilogram scale reactions?

A. I'm afraid I don't understand the question.

Q. Well, you teach undergraduate

chemistry, correct?

A. That is correct.

Q. And in those classes and labs, they perform experiments, right?

A. The students in the laboratories, in the teaching laboratories certainly do perform experiments, yes.

Q. And are you aware if those students perform syntheses on a kilogram scale?

A. I am not --

MR. POLLACK: Objection to form.

A. I am not aware.

Q. Do you know if the lab equipment in undergraduate labs is even capable of synthesizing kilogram scale reactions?

A. I do not know.

Q. Would it surprise you if they didn't?

MR. POLLACK: Objection to form.

Winkler

A. I'm sorry, I don't understand your question.

Q. Well, you're aware of the equipment used in undergraduate laboratories, correct?

A. No, I am not.

Q. So you're not aware of what laboratory equipment is used in the undergraduate courses that you teach?

A. Well, the undergraduate courses that I teach are lecture courses, so they don't have laboratory components to them.

Q. So you don't teach the lab courses?

A. I do not.

Q. If you could look at Exhibit 2, which is a copy of your declaration -- well, first, is that a true and correct copy of your declaration?

A. Yes, it is.

Q. Are you aware of any errors in your declaration?

A. I am.

Q. And what are those errors?

A. There's a citation to Phares, Exhibit 1005, at the bottom of page 14 that continues on to page 15, and it cites Exhibit 1005, page 24, bottom paragraph, and that number should actually be page 22.

Q. Okay. Are you aware of any other errors?

A. No, I am not.

Oh, excuse me. There is one other error, I guess. There appears to be a duplicate signature page at the end of the report. I'm not sure what the reason is for that.

Q. So if we could take a look at paragraph 14 in your declaration, do you see that?

A. Yes, I do.

Q. And paragraph 14 says, "Given the high education level of the scientists actually working in this field, a person of ordinary skill in the

art ('POSA') of chemistry at the time of the alleged invention would have a master's degree or a Ph.D. in medicinal or organic chemistry, or a closely related field. Alternatively a person of ordinary skill would include a bachelor's degree and at least five years of practical experience in medicinal or organic chemistry." Do you see that?

A. Yes, I do.

Q. And you agree with that definition of person of ordinary skill with regard to the '393 patent?

A. Yes, I do.

Q. So do you recall how you came up with that definition?

A. I came up with that definition as a function in large measure of looking at the inventors of the patent and what their level of expertise and training was.

Q. Did you do anything else to determine what the level of ordinary skill would be?

Winkler

A. I think my opinion was formed based on the background of the inventors and on my own reading of the patent.

Q. So when you say your own reading of the patent, what informed your decision to choose that level of skill based on your reading of the patent?

A. I formed that opinion based on the science, the chemistry that was in the patent.

Q. And so you would agree that to understand the science and chemistry of the patent, you would need this level of skill in the art?

A. Yes, that is my opinion.

Q. Okay. So let's turn back to paragraph 3 in your report.

And the last full sentence says, "The technology of the '393" --

MR. DELAFIELD: Strike that.

Q. -- "The technology of the '393 patent involves nothing more than basic organic chemistry techniques-in my view, 'organic chemistry 101'-all of which were

Winkler

well-known in the art prior to December 17, 2007."

Do you see that?

A. Yes, I do.

Q. So do you disagree with that statement then?

A. No, I do not.

Q. Well, I believe you just said that you agree that to understand the science and chemistry of the '393 patent you would need that level of skill in the art being a Ph.D. or master's with experience in medicinal or organic chemistry, correct?

A. I'm sorry, could you repeat that, please?

Q. I believe you just previously answered that you would need the level of skill in the art that you list in paragraph 14 to understand the chemistry of the '393 patent, correct?

A. I don't think that's really what I said. I think what I said was that a person of ordinary skill in the

art at the time of the invention would have a master's degree or a Ph.D. Alternatively, the person of ordinary skill would include an individual with a bachelor's degree and at least five years of practical experience.

Q. Yes, but I believe your testimony was that to understand the chemistry, you would need the level of ordinary skill in the art described in paragraph 14, whether it's a Ph.D. and master's with less experience or a bachelor's with more experience?

A. I'm sorry, I must have misspoken.

What I meant to say was that my definition of a person of ordinary skill in the art is as listed here in paragraph 14.

I'm afraid I may not have understood the question that you asked me.

Q. So the technology of the '393 patent involves more than just organic

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chemistry 101, if the ordinary level of skill in the art is a Ph.D. with years of experience in medicinal and organic chemistry, correct?

MR. POLLACK: Objection to form.

A. No, I don't think that's correct at all. I think the statement that I'm making in paragraph 3 is that the technology of the patent involves basic organic chemistry techniques. That is my opinion.

I think that the definition that I gave of a person of ordinary skill in the art is one who would have a master's degree or a Ph.D. in organic chemistry or a person of ordinary skill with at least five years of practical experience.

Q. But if the chemistry involved in the '393 patent involves no more than organic chemistry 101, why would you need a master's degree or Ph.D. in medicinal chemistry or closely related field or a

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bachelor's degree with at least five years of experience in medicinal and organic chemistry?

MR. POLLACK: Objection to form.

A. Well, my opinion is that a person of ordinary skill would have the qualifications that I listed in paragraph 14 based on the expertise of the patent authors.

But the technology that we're discussing here in my opinion is basic, and as I stated in paragraph 3, it involves nothing more than basic organic chemistry techniques, in my view, as I state here, "organic chemistry 101."

Q. So if a person took organic chemistry 101, would they be a person of ordinary skill in the art with regard to the '393 patent?

MR. POLLACK: Objection to form.

A. If a person took organic chemistry 101, they would certainly have

Winkler

been exposed to the technology of the '393 patent, and I would say, understand the technology of the '393 patent.

Q. So do you want to change your level of ordinary skill in the art?

A. No, I do not.

Q. So I guess I just am not understanding how someone would need a Ph.D., if your opinion is that there is nothing in the '393 patent beyond basic organic chemistry.

So you would agree that a person who had taken organic chemistry 101 would not be a person of ordinary skill in the art with respect to the '393 patent, correct?

A. A person who had taken only the first year of organic chemistry would not qualify as a person of ordinary skill in the art, per the definition that I've offered in paragraph 14, that is correct.

Q. And so your definition does require a lot more than taking one year of organic chemistry to be a person of

Winkler

ordinary skill in the art, correct?

A. To be a person of ordinary skill in the art, according to the definition that I've offered in paragraph 14, would require a master's or Ph.D. degree or bachelor's degree with at least five years of practical experience.

Q. In the courses you teach in chemistry, is organic chemistry a 100 level course?

MR. POLLACK: Objection to form.

A. The number of the undergraduate course that I teach at the University of Pennsylvania in introductory organic chemistry is chemistry 241, it's not a 100 level course. It's a 200 level course.

Q. So there is not actually an organic chemistry 101; is that right?

A. Not precisely. My view of the organic chemistry 101 in paragraph 3 was just to indicate a descriptor of basic organic chemistry, or introductory

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organic chemistry.

Q. Earlier we briefly discussed impurity profiles for drug substances, and I believe you testified that you had not prepared an impurity profile for a drug substance; is that correct?

MR. POLLACK: Objection to form.

A. Again, I'm not actually sure what you mean by that question.

Q. Have you prepared any impurity profile for any drug substance?

A. I guess I don't understand what you mean by "impurity profile," and I don't understand what you mean by "drug substance" in the context of this question.

Q. Well, do you know what an impurity profile is?

A. Well, I would interpret an impurity profile as the profile of impurities in a substance.

Q. And do you understand the FDA requires that impurity profiles be

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submitted with drug substances?

A. I don't think I am aware of that.

Q. Do you know what the purpose of an impurity profile is?

A. I'm afraid I don't understand the question.

Q. What is the purpose of an impurity profile?

A. Well, again, I don't understand in the context to which you're referring.

To me an impurity profile would simply be the profile of the impurities of the substance, what the impurities were.

Q. And why would someone be concerned about what impurities are present in a drug substance, for example?

A. I'm not sure that I understand the question.

Q. I'm just asking why would a person be --

MR. DELAFIELD: Strike that.

Winkler

Q. Can you think of any reason why someone would want to analyze the impurities of a drug substance?

A. I'm sorry, what do you mean by "drug substance"?

Q. Are you not familiar with that term?

A. Not formally, no.

Q. When I say "drug substance," I mean the active pharmaceutical ingredient in a drug product. Does that help your understanding?

A. Yes.

Q. So can you think of any reason why someone would want to analyze the impurities of an active pharmaceutical ingredient in a drug product?

A. Well, I would -- I would think that one would want to know what the impurities were in the API.

Q. Why is that?

A. To know what other compounds are in the API, or what their proportions are.

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Q. Do you know why they would want to know that?

A. Well, it's possible that the, that the impurities could negatively impact the API material, and so it would be important to know what the amounts were of these compounds or what the purity was of the API.

Q. Have you ever studied the negative impact of impurities on any API?

A. I think that I have, but sitting here, I can't think of concrete examples.

Q. Okay. So sitting here today, you can't think of any specific examples, where you studied the negative impacts of impurities on any API; is that correct?

A. Well, I'm pretty sure that I have, but I can't think of specific examples, sitting here now.

Q. Are you familiar with the use of treprostinil?

MR. POLLACK: Objection to form, vague.

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A. I'm afraid I don't know what you mean by that.

Q. Do you know what treprostinil is used for?

A. My understanding is that treprostinil is used as a therapy for pulmonary arterial hypertension.

Q. Would you agree that the treprostinil is a very potent drug?

MR. POLLACK: Objection to form.

A. I have -- I was not asked to form an opinion on that and I do not have one.

Q. So you don't know whether it's potent or not, correct?

MR. POLLACK: Objection to form.

A. As I said, I had not formed an opinion on that.

(Winkler Exhibit 4, Excerpt of the prosecution history for the '393 patent, [SteadyMed-Exhibit 1002], marked for identification,

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this date.)

THE WITNESS: Thank you.

Q. You've been handed what's been marked Exhibit 4, which is a copy of the file history for the '393 patent.

Do you recognize that document?

MR. POLLACK: Just for the record, counsel, this is pages 235 through 405 of Exhibit 1002?

MR. DELAFIELD: I am not sure why that starts there, but I will just call it an excerpt then.

MR. POLLACK: An excerpt from the prosecution?

MR. DELAFIELD: Yes.

Q. Do you recognize this document?

A. Yes, I do.

Q. And did you review this document in forming your opinions in your declaration?

A. Yes, I did.

Q. So if you could turn to --

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A. I'm sorry, if I could just return to a previous answer that I gave.

I have my declaration still here, and on paragraph 3, when I talk about the technology of the '393 patent, what I'm referring to is the idea of taking a carboxylic acid, making a carboxylic acid amine salt, and regenerating the free acid. Those are the techniques that would have been obvious, or the technology that would not have been anything more than basic organic chemistry. In other words, that's what I was referring to as "organic chemistry 101" in paragraph 3 of my declaration.

Q. So just to go back then, so then is it your opinion that you would need the level of education that you give in paragraph 14 to perform those reactions that you just mentioned?

MR. POLLACK: Objection to form.

A. My opinion is that the

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technology of the '393 patent as it involves the formation of a carboxylate salt and the regeneration of the carboxylic acid, those steps represent nothing more than basic organic chemistry techniques, and that's what I was referring to when I described it as "organic chemistry 101," when I described it figuratively, if you will, as organic chemistry 101.

Q. So do you mean a master's degree or Ph.D. in the medicinal or organic chemistry or in a closely related field to perform those steps?

A. I think to perform a carboxylate salt and to regenerate the carboxylic acid, that's something that I would expect a student in basic organic chemistry to be able to do.

Q. So you don't agree that your definition of a person of ordinary skill in the art should be that high level of education?

MR. POLLACK: Objection to

form.

A. I'm sorry, I don't understand your question.

Q. Well, in paragraph 14 you say, "Given the high level of" --

MR. DELAFIELD: Strike that.

Q. "Given the high education level of the scientists actually working in this field, a person of ordinary skill in the art of chemistry at the time of the alleged invention would have a master's degree or a Ph.D. in medicinal or organic chemistry or closely related field." And then you say, or a bachelor's with more experience.

Do you see that?

A. I do.

Q. But you're saying that to perform the steps of the '393, you actually don't need that level of ordinary skill; is that correct?

A. I think to perform the steps of the salt formation and regeneration of the carboxylic acid, those are steps that

are taught in the equivalent of organic chemistry 101. That's the statement that I'm making, or that's the point that I am trying to make in paragraph 3.

Q. And is that your understanding of the entire technology of the '393 patent?

A. No, it is not.

Q. So in paragraph 3 when you say the technology of the '393 patent involves nothing more than organic chemistry techniques, you're only referring to the last few steps and not the entire technology as it's stated there?

A. My particular focus in paragraph 3 when I wrote this sentence was referring to the formation of carboxylate salt and then regeneration of the free acid.

Q. But paragraph 3 doesn't say that, right?

A. It does not say that. Well, I mean it says that in that the technology

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of the '393 involves nothing more than basic organic chemistry techniques, but the technology that I was referring to here was the formation of the carboxylate salt and the regeneration of the free acid.

Q. If you could turn back to Exhibit 4, which an excerpt of the file history of the '393 patent. If you could turn to page 346 in the exhibit.

And this is the first page of the declaration of David Walsh under 37 C.F.R. 1.132. Do you see that?

A. Yes, I do.

Q. And have you reviewed this declaration?

A. Yes, I have.

Q. So if you look at the next page, 347 in paragraph 6, there Walsh states, "In my opinion, each of treprostinil as the free acid and treprostinil diethanolamine prepared according to the process specified in claim 1 or 10 the present application is

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physically different from treprostinil prepared according to the proces of 'Moriarty.'"

Do you see that?

A. Yes, I do.

Q. And he based that analysis on the fact that the impurity profiles were different, correct?

MR. POLLACK: Objection to form.

A. The Walsh report states that each of treprostinil as the free acid and treprostinil diethanolamine prepared according to the process specified in claim 1 or 10 differed from treprostinil prepared according to the process in Moriarty in their respective impurity profiles.

Q. So you agree that Dr. Walsh based his analysis on the fact that the impurity profiles are different, correct?

MR. POLLACK: Objection to form.

A. Could you repeat the question,

please?

Q. There Walsh concluded that the treprostinil made by the '393 process was different than the treprostinil made by the Moriarty process based on differences in impurity profiles, correct?

A. Well, what Walsh states here is that they differ according -- they differ in their respective impurity profile.

Q. So is that a yes to my question?

A. What was your question? I'm sorry.

Q. Dr. Walsh concluded that the treprostinil made by the '393 patent process is different from the treprostinil made by the Moriarty process because their impurity profiles were different, correct?

A. Walsh states that each of the treprostinil is the free acid and the treprostinil diethanolamine prepared according to claim 1 differ from the

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treprostinil prepared according to the process in Moriarty in their respective impurity profiles. That's what it says here.

Q. I don't believe you answered my question. It's just a yes or no question.

It's just saying that he believes that they're different because they have different impurity profiles. Is that correct?

MR. POLLACK: Objection to form.

A. That they differ in their respective impurity profiles, that's what it says here.

Q. And you understand this was submitted during prosecution of the '393 patent, correct?

A. That is correct.

Q. Now, are you generally familiar with the process of prosecuting a patent with the patent office?

MR. POLLACK: Objection to

Winkler

form.

A. I'm sorry, I don't understand exactly what you mean.

Q. Do you understand that Dr. Walsh submitted this declaration to point out differences between the '393 patent and the prior art, correct?

A. I think that's correct.

Q. Sir, if you turn to page 350, Dr. Walsh signed his declaration on June 4, 2013; is that correct?

MR. POLLACK: Objection to form.

A. That's what it says here, yes.

Q. And turn to page 354, do you see at the top of the page it says, "Notice of Allowance and Fees Due"?

A. Yes.

Q. And at the top right it says "date mailed June 12th, 2013." Do you see that?

A. Yes, I do.

Q. So the '393 patent was allowed a week after Dr. Walsh submitted his

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declaration; is that right?

A. Eight days later I would guess.

Q. Yes.

And if you look at the pages in between, nothing was filed from the time Dr. Walsh submitted his declaration until the time the notice of allowance was submitted, correct?

A. Not that I can see here, no.

Q. So the patent office during prosecution considered impurity profiles to be important as to how the claims are interpreted, correct?

MR. POLLACK: Objection to form.

A. I really can't speak to how the patent office interpreted this.

Q. Well, you understand that the patent office allowed it eight days after the submission of the declaration, correct?

A. It appears that there was a notice of allowance from the patent

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office eight days after the submission of the Walsh declaration.

Q. So is it fair to say that the patent was allowed as a result of Dr. Walsh filing his declaration?

MR. POLLACK: Objection to form.

A. Again, I don't think I can really speak to that question.

Q. But you submit that Dr. Walsh submitted his declaration in order to show differences between the '393 patent and the prior art, correct?

A. I think what Dr. Walsh states on page 347 is that each of treprostinil as the free acid and as the salt, prepared according to the process specified in claim 1 or 10, differ from the treprostinil prepared according to Moriarty in their respective impurity profile.

Q. But you don't know if the patent office allowed the patent based on that declaration?

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MR. POLLACK: Objection to form, asked and answered.

A. I do not, no.

Q. Do you think it's possible the patent office allowed it because of the declaration?

MR. POLLACK: Objection to form.

A. I don't know the answer to that question.

Q. Are you aware of any other reason the patent office would have allowed the patent?

A. I don't know.

Q. So looking back at his declaration at page 347, do you see that?

A. Yes, I do.

Q. And there is a chart at the bottom of the page that shows the impurity profile that says, "Treprostinil free acid prepared according to 'Moriarty.'" Do you see that?

A. Yes, I do.

Q. And at the bottom it says,

bottom left says, "Total Related Substances." Do you see that?

A. Yes, I do.

Q. And in the far right it says, "0.6 percent," correct?

A. Yes, it does.

Q. So that is the total amount of impurities in that sample added up; is that correct?

A. Well, all I can read from this is that that's the total of related substances in this sample, of this material that was sampled.

Q. And that's a measure of the purity of the substance, right?

A. I don't know the answer to that question.

Q. So you didn't use the numbers from the Walsh declaration in determining the purity of the '393 patent batches or the Moriarty batches; is that right?

A. Well, actually in my declaration, I actually discuss why the Walsh declaration cannot be used to

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support the question of the purity that the patent owner claims.

Q. So if you keep open the Walsh declaration and then if you also look at your declaration at paragraph 65.

A. Yes.

Q. So paragraph 65, second sentence, you state, "Patent Owner contended based upon Dr. Walsh's measurement that his purification method achieved 99.8 percent purity, while the prior art Moriarty reference achieved 'only' 99.4 percent," and then you cite Exhibit 1002 to page 347. Do you see that?

A. I do.

Q. So if you look at page 347, can you tell me where you got 99.4 percent purity reference from paragraph 65?

A. I obtained that by subtracting .6 percent from 100 percent.

Q. So earlier you said you didn't know if "Total Related Substances" was a

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measure of purity, correct?

MR. POLLACK: Objection to form.

A. My confusion sitting here is that this gives a number for "Total Related Substances" but no number for total unrelated substances, which I've seen in other of the documents, so I made the assumption in my declaration that the purity of the sample was indicated from these data to be only 99.4 percent on that basis.

Q. So you used the "Total Related Substances" number as a measure for purity, correct?

MR. POLLACK: Objection to form.

A. So the patent owner, if I'm not mistaken, claims that Moriarty here was 99.4 percent, pure. Was only 99.4 percent pure, based on the "Total Related Substances" value obtained here of .6 percent.

The point that I was trying to

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make was that the upper limit for this lot would have been 99.4, but could have been lower if there were unrelated substances in the preparation.

I also pointed out in my report, several examples of why these data would be, would be difficult to interpret.

Q. But at least one way to measure the purity is to look at the "Total Related Substances" in a batch, correct?

MR. POLLACK: Objection to form.

A. Well, again, as I mentioned, one way would be to look at "Total Related Substances," but there were other purity profiles that I looked at in the course of my review of the documents that showed related substances as well as unrelated substances.

Q. But looking at "Total Related Substances" is one, possibly many ways, to determine the purity, correct?

Winkler

MR. POLLACK: Objection to form.

A. I think without knowing the percent of unrelated substances, it would be less than accurate to describe the purity of this sample as 99.4.

Q. And when you say "unrelated substances," what do you mean by that?

A. I think, as I said, in the course of my review of the documents some of these purity profiles indicated a percent of related substances and then also a percent of unrelated substances. I'm not sure exactly what they would have been referring to.

Q. So looking back at the Walsh declaration at page 347, above the "Total Related Substances" it lists several different impurities. Do you see that?

A. I do.

Q. And then in the far right column there are numbers and the letters ND. Do you know what "ND" stands for?

A. I think that "ND" stands for

not determined, or I'm sorry, not determined or not detected. That's the typical context in which I've seen those numbers.

Q. So you've seen "ND" referred to meaning not detected, correct?

A. Not detected and not determined, yes.

Q. So you see that, for example, the impurity 2A90 was reported as less than 0.05 percent. Do you see that?

A. Yes, I do.

Q. And 97W86 impurity which is Benzidene trial, which was reported as 0.7 percent. Do you see that?

A. Yes, I do.

Q. So the instrument used to analyze these impurities could detect down to at least 0.07 percent, correct?

A. That's what this would, these numbers would suggest, yes. Although there is no indication of what the error is in these measurements.

Q. In the middle column you see

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that it lists specification for each of these impurities. Do you see that?

A. Yes, I do.

Q. And for 2AU90 it says not more than 0.1 percent, correct?

A. That is correct.

Q. So even if this doesn't report the error, is it fair to say that the error would have to be less than 0.1 percent in order for 0.1 percent to be a meaningful number?

MR. POLLACK: Objection to form.

A. I'm sorry, could you repeat that question, please?

Q. So the specification says not more than 0.1 percent, correct?

A. That is correct.

Q. And although this does not report the amount of error associated with that number in order for a 0.1 percent to be relevant, the error would have to be lower, correct?

A. I'm sorry, could you repeat

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the question, please.

MR. DELAFIELD: Could you read back the question.

(A portion of the record was read.)

A. I think for the measurement of the 2AU90, if the error in the measurement was more than .1 percent, it would be difficult to claim not more than .1 percent.

Q. Okay. So you agree that if you're reporting a number not more than a certain percentage, the error associated with that number would necessarily need to be lower than that, right?

A. I would think that it should be, but I'm just not sure that it is in this case.

MR. DELAFIELD: We've been going about an hour and a half, would you like to take a break, Dr. Winkler?

THE WITNESS: That would be fine.

Winkler

THE VIDEOGRAPHER: The time is 10:53 a.m., and we're going off the record.

(A brief recess was taken.)

THE VIDEOGRAPHER: This begins media unit number 2. The time is 11:09 a.m. and we're back on record.

Q. Hello, Dr. Winkler. During the break, did you discuss with your attorney any of the substance of your testimony today or anything about the case?

A. No, I did not.

Q. So if you could turn back to Exhibit 4, which is the excerpt of the file history we were talking about, Dr. Walsh's declaration. And on page 347, we were discussing the amounts of impurities for --

MR. DELAFIELD: Strike that.

Q. We were discussing the limitations on the impurities in that chart. Do you recall that?

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A. Yes, I do.

Q. And we were discussing that for the impurity 2AU90 the limit for the impurity was no more than 0.1 percent, correct?

A. That's what it says here, yes.

Q. And I believe you testified that you didn't know what the experimental error associated with that number is, because there is not enough information here to determine that; is that right?

A. That's correct.

Q. But it would be reasonable to think that the error would need to be lower than 0.1 percent in order for the limit to be 0.1 percent; is that fair?

MR. POLLACK: Objection to form.

A. I think that actually depends on whether you're referring to a relative error or the absolute error in measuring the .1 percent.

Q. Well, in either case, in order

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for the limit to be 0.1 percent, the error would likely be less than 0.1 percent, correct?

MR. POLLACK: Objection to form.

A. I would think that the error in the measurement for the 2AU90 would be, should be less than .1 percent.

Q. And generally for all the numbers, the error should be less than the maximum number reported, correct?

A. The error should be less than the maximum number reported, that's correct, for the measurement of the materials that are described here.

Q. Sir, if you turn to page 348, on the next page it shows two similar impurity profiles to the treprostinil diethanolamine prepared according to claims 1 and 10, and the treprostinil as the free acid prepared according to claims 1 or 10.

Do you see that?

A. Yes, I do.

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Q. And the top chart, the bottom row says, "Impurities (HPLC) (Total Related Substances.)" Do you see that?

A. "Total related substances," yes, I see that.

Q. And on the far right it says 0.1 percent, correct?

A. It does say that, yes.

Q. And likewise, on the chart below that, it says "Total Related Substances," and the corresponding number 0.2 percent, correct?

A. That's what it says here, yes.

Q. So like the 2AU90 number we discussed before, the error associated with those numbers would also need to be less than the number reported, correct?

MR. POLLACK: Objection to form.

A. Well, again, the issue would be whether we're dealing with relative or absolute errors, for these numbers.

Q. But whether it's relative or absolute error, in order to report a

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number, the error should be less than the number reported, correct?

MR. POLLACK: Objection to form.

A. The error should be less than the number reported.

Q. So if you could turn back to your declaration, which is Exhibit 2, and if you look at paragraph 68, are you there?

A. I am at paragraph 68.

Q. You say, "Third, a 0.1 percentage difference in purity between Walsh's measurement of Moriarty's purity and Claim 2 and Claim 10's 99.5 purity is well within experimental error for measuring impurities, and would not represent a significant deviation from the processes of the prior art."

Do you see that?

A. Yes, I do.

Q. But you don't know what the experimental error is associated with these measurements, correct?

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MR. POLLACK: Objection to form.

A. I don't know precisely what the errors are, but I know that there clearly must be error in these measurements. For example, I know that the Moriarty purity is reported here as 99.4, but in fact in Moriarty, it's reported as 99.7. So that represents a spread of .3 percent in terms of the purity of the products.

Q. But as we just discussed, the numbers recorded in Walsh's declaration, which you're relying on, the errors should be less than the numbers reported, and therefore, less than .1, correct?

MR. POLLACK: Objection to form.

A. Well, I don't -- I don't think that's what I said. And, for example, if you look at the Moriarty data on page 347, that number is .6, so that would suggest a larger possible range.

For this particular lot that

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was tested, they claim an impurity level of .1 percent. The impurity level for the free acid is claimed as .2 percent.

So, again, these are specific lots, and I'm not sure what I could conclude based on this limited data set.

Q. So based on this information you have no idea what the experimental error would be, correct?

MR. POLLACK: Objection to form.

A. Well, again, I assume that the error, at least in the case of Moriarty, is going to be somewhere on the range of plus or minus .2 percent at a minimum. There is other data that I saw in materials that were supplied after my declaration was produced that suggest that the HPLC purities are off by as much as 1 or 2 percent.

Q. But do you agree the HPLC purities given in the Walsh declaration should have experimental errors below that of .1 percent, correct?

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MR. POLLACK: Objection to form.

A. Well, I think in my reading of the data on page 348, I certainly agree that these measurements for these two particular lots should have errors that are less than .1, .2 respectively, but I don't know that that's the case, and given the other data that I've seen, I am imagining that these sorts of differences would all be within the range of experimental.

Q. But in order to know what the experimental error range is you need to know what HPLC was used and whether the same HPLC was used for one analysis in another analysis, correct?

A. I think there are multiple factors that would be required to determine what the experimental error would be.

Q. And because there are multiple factors that would be required to determine what the experimental error

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would be, you don't actually know what would be within or outside experimental error; it's just an assumption, correct?

MR. POLLACK: Objection to form.

A. Looking at these numbers, I can be certain that there is experimental error, but I can't say exactly how large that error is.

Q. So if you look at paragraph 69 of your report, you say, "even a difference of 0.4 percent as discussed below between the claim processes of '393 patent and prior art such as Moriarty would be attributable to experimental error, and that the claimed degree of purity under the claimed processes of the '393 patent presents no distinction from prior art."

Do you see that?

A. Yes, I do.

Q. So based on the multiple factors, you would need to know what the limit of the experimental error is; you

don't in fact know that 0.4 percent would be within the experimental error, correct?

MR. POLLACK: Objection to form.

A. Well, actually, I think I do know that the experimental error could be as high as .4 percent, because of the data that's included in the '393 patent.

Q. And what data are you referring to?

A. I'm referring to the data that's described in paragraph 70 of my report, in which I state in the second sentence, that "in the present case we can estimate the precision of the equipment the inventors actually used since the inventors found that Example 4's Batch 1 had an HPLC Assay of 100.4 percent, which is obviously greater than the 100 percent value theoretically achievable," and that would suggest to me there that is a variation of at least a .4 percent in these measurements.

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Q. And so because the number is over 100 percent, that is your basis for saying that at least .4 percent is due to experimental error; is that correct?

A. That's one of the bases for my opinion.

Q. And in paragraph 70, you mention that the HPLC was an assay of 100.4 percent, right?

A. That's correct.

Q. What is an assay?

A. An assay is a test.

Q. What does it mean in this context?

A. What I interpret this to mean is that the HPLC is a determination of the compound.

Q. Now, earlier in the Walsh declaration we were looking at the "Total Related Substances" as a measure of the purity. Do you remember that?

A. Yes, I do.

Q. Is that the same as an assay?

A. Well, my experimentation --

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could I refer back to the '393?

Q. Sure. It's Exhibit 3.

A. So I would interpret this HPLC assay to be the same purity assay that is described at the bottom of column 14 of the patent; in other words, the area under the curve after the treprostinil, or treprostinil diethanolamine salt that was being assayed.

Q. But you don't know if that's the same purity referred to as the "Total Related Substances" in the Walsh declaration, correct?

A. I'm afraid I don't understand your question.

Q. Well, you referred to the bottom of column 14 in the '393 patent, and that is the number you were referring to with regard to purity in paragraph 70 of your declaration, correct?

A. No, I don't think that's true. What I was referring to in 70 is in the table that sits at the bottom of column 13, at the bottom of the column 13 for

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Batch 1, the HPLC assay indicates a purity, I'm assuming that the HPLC assay there refers to the same purity as described at the bottom of 14, and it gives a purity of 100.4 percent.

From that, I conclude that these numbers can't be better than .4 percent, because it obviously would not be possible to have a material that is more than 100 percent pure.

Q. Are you familiar with the term "reference standard"?

A. I'm sorry?

Q. Are you familiar with the term "reference standard"?

A. Yes, I am.

Q. What is your understanding of what a "reference standard" is with regard to a drug substance?

A. Well, a reference standard is typically authentic sample of a substance.

Q. And what are they used for?

A. Well, they can be used for

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many different things.

Q. Do you have any samples of what they might be used for?

A. They could be used, for example, to calibrate retention times on HPLC.

Q. Are you aware of reference standards being used as the standard in which other samples are compared to?

A. Yes, that would certainly be possible as well.

Q. And so a reference standard is usually a very high purity sample, correct?

MR. POLLACK: Objection to form.

A. It can be.

Q. Now, earlier you said you had reviewed some documents that were filed after you submitted your declaration; is that right?

A. That is correct.

Q. Did you review all the exhibits that were attached to United

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Therapeutics' brief?

A. I am pretty sure that I did.

(Winkler Exhibit 5, Letter dated 1/2/09, [UT Exhibit 2006], marked for identification, this date.)

THE WITNESS: Thank you.

Q. I've handed you what's been marked as Exhibit 5, which is a letter to the FDA from United Therapeutics.

MR. DELAFIELD: I would like to also note for the record that parts of the deposition will be confidential.

MR. POLLACK: Do you want to make this section confidential?

MR. DELAFIELD: Yes.

(The following portion has been deemed confidential and bound under separate cover.)

Q. Do you recognize this document?

A. Yes, I do.

Q. If you turn to page 5, this table shows drug substance specification comparison. Do you see that?

A. Yes, I do.

Q. And it continues on to page 6, if you would turn to the next page. And do you generally understand what this chart shows?

A. I think so, yes.

Q. So on page 6, the second row, it says, "Chromatographic Purity (HPLC)." Do you see that?

A. I do.

Q. And then there are various impurities listed and next to that are the limits for those impurities. Do you see that?

A. Yes, I do.

Q. And again, for example, for [redacted], it says, not more than [redacted] percent, correct?

A. That is correct.

Q. And then below that row is another row that says "Assay (HPLC)." Do you see that?

A. Yes, I do.

Q. And then the first column it says, not less than [redacted] percent and not more than [redacted] weight per weight on the volatiles freebases. Do you see that?

A. I do.

Q. So right above "Assay," it says "Total Related Substances." Do you see that?

A. I do.

Q. So here this is showing two separate ways that you can assess the purity of this substance, correct?

MR. POLLACK: Objection to form.

A. I'm afraid I don't understand your question.

Q. So earlier we discussed how "Total Related Substances" was a measure of purity, correct?

MR. POLLACK: Objection to form.

A. I'm afraid I still don't understand your question.

Q. Earlier when we discussed Dr. Walsh's declaration, we discussed how the measurement associated with "Total Related Substances" was a way to determine the purity of the substance, correct?

MR. POLLACK: Objection to form.

A. I think that we had used "Total Related Substances" to generate a -- an upper limit for what the purity of a substance could be.

Q. So it's one way to determine the purity, correct?

MR. POLLACK: Objection to form.

A. I think that I just said that it could be used to determine the upper limit of purity.

Q. And you see that "Assay" below

that is a different measurement, correct?

A. Different in what way? I'm afraid I don't understand the question.

Q. So according to this chart, "Assay" is listed separately from "Total Related Substances," correct?

A. That is correct.

Q. And both "Assay" and "Total Related Substances" are different ways that you can estimate the purity of a substance, correct?

A. They could be used to determine the purity of a substance, yes.

Q. And here it's showing that they're not necessarily the same number, correct?

A. I'm afraid I don't understand your question.

Q. Well, because they are listed separately, they're not one and the same thing, correct?

A. I'm afraid I still don't understand your question. There are different numbers given here for "Total

Related Substances" and "Assay" by HPLC.

Q. They are different analyses, right?

A. I'm afraid I don't understand what you mean by that.

Q. You don't understand whether or not the measurement for "Total Related Substances" is different than the measurement for the assay?

A. I understand the "Total Related Substances" as expressed here and the assay are different.

Q. Okay.

THE WITNESS: Excuse me. Excuse me. They're different but clearly related because the [redacted] percent HPLC assay certainly looks like it's correlated to the not more than [redacted] percent of "Total Related Substances," so I would think that these values are in fact related somehow.

Q. But in the last column, it says, not less than [redacted] percent for assay

but not more than [redacted] percent for total related substances, correct?

A. Yes, it does.

Q. So they are different analyses, right?

A. Based on that they would appear to be, yes.

Q. So we were talking about a reference standards and how sometimes they are used to compare samples against to determine a purity relative to the reference standard.

Do you understand that?

A. I think I do.

Q. So here when it talks about assay on a weight-per-weight basis, if assay means the amount of substance in a sample compared to the amount in a reference standard, that would be one way to determine the purity in relation to a reference standard, correct?

A. I could imagine the use of a reference standard to do that, yes.

Q. So if that were the case, if

the sample were made by an improved process that had a higher purity than the reference standard, you could get over 100 percent and it not be experimental error, correct?

MR. POLLACK: Objection to form.

A. That's not the way I understand the use of a reference standard.

Q. Well, if a reference standard, for example, has 99 percent of a substance and the sample you're comparing it against is 100 percent, then that would be 1 percent more than the reference standard, correct?

A. That is correct.

Q. And both of these --

A. But that would not give a calculated value of, say, of more than 100 percent purity. I don't think there is any logical calculation that would allow you to determine greater than 100 percent purity, at least none that

I've ever seen, before looking at these data.

Q. So in the "Assay" column --

MR. DELAFIELD: Strike that.

Q. In the "Assay" row on this page, you see that the proposed change is actually raising the upper limit from [redacted] percent to [redacted] percent. Do you see that?

A. I do see that.

Q. So then is it your belief that they're proposing more experimental error?

A. Well, if I see a number of [redacted] percent purity, that suggests to me that that value is going to be off by [redacted] percent.

Q. So you're not aware of a purity analysis where a sample is compared to a less pure reference standard ending up with over 100 percent?

A. In my experience of less than 100 percent pure reference standard should never give a value of more than

100 percent purity in the assayed material. There should be a correction for the lack of purity in the reference standard.

Q. So why --

A. Because it -- I'm sorry -- because it wouldn't make sense to one of skill in the art or myself to have a sample or have a measurement that would indicate that a sample was more than 100 percent pure.

Q. So you understand that this letter was submitted to the FDA to change the specification for treprostinil, correct?

MR. POLLACK: Objection to form.

A. I'm not sure exactly what the purpose was of this letter.

Q. Well, you understand that it was submitted to the FDA at least?

A. I do understand that it was submitted to the FDA, yes.

Q. And looking back at page 6,

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you also understand that the proposed change was to the assay, raising the assay from [redacted] to [redacted] to [redacted] to [redacted] percent. Do you see that?

A. I see -- I see a currently approved specification and then a proposed new specification.

Q. So you don't understand why it would be changed from [redacted] to [redacted] percent; is that correct?

MR. POLLACK: Objection to form.

A. I don't know, no.

Q. And it's your understanding that the experimental error in the first column is at least [redacted] percent because it says, not more than [redacted] percent, and the experimental error in the second column must be [redacted] percent because it says [redacted] percent. Is that fair to say?

MR. POLLACK: Objection to form.

A. I think what the data here indicate to me is that in the first

column, I would read this as saying that the experimental error could certainly be as high as [redacted] percent.

And that in the second column, the experimental error could be as high as [redacted] percent. Because I don't think it's possible to have a sample that is [redacted] percent pure.

Q. And you don't think it's possible that these numbers represent an analysis of a sample compared to a reference standard that is less pure such that's you would end up with over 100 percent? You don't think that's possible?

A. Well, again, I don't think that comparison of an unknown sample to a less than 100 percent pure reference standard should give a value of more than 100 percent purity in the unknown. I think one should be able to understand what the purity is of reference standard and to use that information to develop a more real picture of what the purity of

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the sample is, so that one doesn't get essentially auspicious result that is a purity of greater than 100 percent.

Q. But again, given all of these numbers, you don't know specifically what the experimental error associated with any measurement for treprostinil, correct?

MR. POLLACK: Objection to form.

A. I think the thing that I am able to conclude from the data that is on page 6 of this, of this letter is that the error in the HPLC assay could be as high as [redacted] percent in the first column and by my analysis could be as high as [redacted] percent in the second column.

THE WITNESS: Excuse me.

Q. Do you know why the assay is reported as not less than a number and not more than a number, as opposed to just not more than that, as the chromatographic purity was listed --

MR. DELAFIELD: Strike that.

Q. Do you know why the chromatographic purity in the second row is reported differently than the assay purity?

A. No, I don't.

Q. Sir, if you could look back at your declaration, at paragraph 68, if you recall, you reference in the first sentence, Moriarty's purity of 99.4 percent. Do you see that?

A. I'm sorry, could you repeat that?

Q. In paragraph 68 you reference Moriarty's purity as 99.4 percent, correct?

A. I actually describe Walsh's measurement of Moriarty's purity.

Q. Yes.

And that purity number came from subtracting from 100, the "Total Related Substances" in Walsh's declaration, correct?

A. That is correct.

Q. And then if you look at

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paragraph 70 --

A. Well, excuse me, that appears to be correct. That's the number that I think comes from the patent owner.

Q. Yes, the ".6 Total Related Substances."

A. Right.

No, the description of Moriarty's purity is 99.4 percent. If I'm not mistaken that number comes from the patent owner.

Q. I'm sorry, do you mean, do you the mean Walsh's declaration?

A. I think that in the documents of the -- for the petition or the response of the patent owner is where the 99.4 percent number comes from.

Q. I think I can help you there. If you look at paragraph 65?

A. Of?

Q. Your last sentence?

A. I'm sorry, 65 of what?

Q. Of your declaration?

A. Okay.

Q. The last line says, "While the prior art Moriarty reference achieved only 99.4 percent," and then you cite page 347.

A. Okay.

Q. And that is the file history which is Exhibit 4.

A. Okay. Oh, I see.

Q. Which reports the .6 total related --

A. Yes.

Q. So your number of 99.4 percent purity comes from subtracting the "Total Related Substances" from 100, correct?

A. Correct.

Q. So going back to --

A. As -- as a -- in this case, as an upper limit to the purity of that sample.

Q. Okay. So then if you could turn to paragraph 70 of your declaration, and in the second sentence you say, "In the present case we can estimate the precision of the equipment the inventors

actually used since the inventors found that Example 4's Batch 1 and HPLC assay of 100.4 percent which is obviously greater than 100 percent value theoretically achievable." Do you see that?

A. Correct, I do.

Q. And you are using here the assay number as a measure of purity, correct?

A. Yes.

Q. But "Assay" and "Total Related Substances" are different analyses, correct?

A. They can be different.

Q. Well, they're reported as different in the UTC's letter to the FDA, right?

A. Well, the -- the letter to the F -- I'm sorry, I'm confused. I was on the Walsh declaration.

In the letter to the FDA the chromatographic purity by measurement of total related substances appears to be

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different from the HPLC assay.

Q. Yes.

So because they are different measurements, you can't say specifically what the experimental error would be, correct?

MR. POLLACK: Objection to form.

A. Well, I think I can say what the experimental error would be, because, as I state in paragraph 70 of my report, given the fact that the assay comes up over 100 percent, comes up to 100.4 in batch 1 of example 4, from that, I can conclude that the error in these measurements or determinations must be at least .4 percent.

And in fact the, in the patent, in the '393, I think there is actually no measurement of impurities, but the purity was determined by this HPLC assay or this so called AUC, the area under the curve.

Q. Right, because the assay in

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"Total Related Substances" are different analyses, you can't say what the experimental error is with regard to the "Total Related Substances," correct?

MR. POLLACK: Objection to form.

A. Well, there is no description of the "Total Related Substances" in the '393 -- I'm afraid I don't understand your question.

Q. Well, the basis for you saying that it has a .4 percent experimental error is based on an assay analysis, not total related substance analysis, right?

A. The basis for my determination of experimental error is, among other things, the HPLC assay of 100.4 percent, the difference between the Walsh determination of the Moriarty purity and the Moriarty purity described by Moriarty, both being HPLC measurements and my understanding of HPLC. And the issues that I discussed in paragraph 70 where the relative standard deviation for

an HPLC instrument in the literature is being described about being about 1 percent.

Q. "Assay" and "Total Related Substances" two different analyses, right?

A. Yes.

Q. And they may have different experimental errors associated with them, correct?

A. Well, I think the point of my analysis is that HPLC carries experimental error, and that there would be experimental error in any HPLC determination, whether we're looking at an impurity profile or whether we are looking at the HPLC assay as described in the '393.

Q. But you don't know exactly what that number would be?

MR. POLLACK: Objection to form.

A. I don't know exactly what the experimental error will be, and but as I

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mentioned, based on the data that I've seen here, in my understanding of the HPLC, it looks like the error could be as high as 1 or 2 percent.

(Continued in nonconfidential portion of transcript.)

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THE WITNESS: Excuse me.

(Winkler Exhibit 6, Document entitled, "Getting Started in HPLC," [SteadyMed-Exhibit 1017], marked for identification, this date.)

THE WITNESS: Thank you.

Q. I've handed you what's been marked as Exhibit 6, which is a document entitled, "Getting Started in HPLC."

Do you recognize this document?

A. Yes, I do.

Q. And is this a document you relied upon in your declaration?

A. Yes, I did.

Q. Do you know who the author is of this document?

A. No, I do not.

Q. Did you obtain this from a website?

A. I did.

Q. Do you know if the website is maintained --

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MR. DELAFIELD: Strike that.

Q. Do you know who maintains the website?

A. I do not know the answer to that question.

Q. Now, you rely on this document for the argument you make that HPLC has 1 percent error; is that right?

A. I think what I -- excuse me -- what I stated here was that HPLC methods are expected to have CV values, and the CV is did he staled at coefficient of variance, which is an equivalent of the RSD of the relative standard deviation, and that that number is on the order of 1 percent for HPLC.

Q. So first, if you look at paragraph 70 in your declaration, you state that relative standard deviation is about 1 percent and you cite this exhibit?

A. Correct.

Q. And then you state that, in the last sentence, "This deviation

between the experimental and theoretical shows that the instrument can have variations of at least 0.4 percent, which is greater than the difference in purity that the inventors offered to support their contention regarding greater purity over the prior art," correct?

A. Correct.

Q. But you don't know what instruments were used to determine the purity in Moriarty, the Walsh declaration or the '393, correct?

MR. POLLACK: Objection to form.

A. The Moriarty reference may mention the HPLC equipment that was used but I don't remember.

Q. And this reference that you cite for the 1 percent, relative standard deviation, doesn't say that there would be a 1 percent error in all HPLC measurements, correct?

A. I think what the reference states is that for most purposes HPLC

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methods are expected to have CV values or coefficient of variation values on the order of 1 percent.

Q. But you don't know what it would be for any of the testing done for treprostinil, correct?

MR. POLLACK: Objection to form.

A. I'm afraid I don't understand your question.

Q. You don't know what the CV value would be for any of the HPLC tests performed on treprostinil that we discussed today?

MR. POLLACK: Objection to form.

A. I think I only know that the HPLC methods are expected to have CV values on the order of 1 percent.

Q. Is it fair to say that HPLC equipment may have different experimental errors associated with it, depending on the time frame that that the HPLC was used?

Winkler

MR. POLLACK: Objection to form.

A. Could you repeat that, please?

MR. DELAFIELD: Let me rephrase it.

Q. So if took an HPLC In the 1980s and HPLC today, they may very well have different experimental errors associated with it, correct?

A. I think it's possible that they could.

Q. And if you look at Exhibit 6, on page 3, at the bottom left it says, last revised April 6, 2001.

Do you see that?

A. Yes, I do.

Q. And the '393 patent wasn't filed until 2007, correct?

A. I'm sorry, could you repeat that, please?

Q. The '393 patent wasn't filed until -- let me get the -- I believe 2007.

Yes, the first application for

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the '393 patent wasn't filed until April 2007, correct?

A. The priority date I think is 2007.

Q. So HPLC determinations could have changed since 2001, correct?

MR. POLLACK: Objection to form.

A. I wouldn't have expected a large difference in HPLC performance over the six-year period, but I can't be certain.

Q. Now, looking back at your declaration, at paragraph 66, you say "First, the data in the Walsh declaration was derived from limited sample set - indeed, only two specific batches of treprostinil."

Do you see that?

A. Yes, I do.

Q. And you say, "There could be significant batch-to-batch variations in the impurity profile of each batch of treprostinil, which does not provide

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sufficient evidence to support the conclusion that purification method achieves 99.5 percent purity or above for the claimed treprostinil."

Do you see that?

A. Yes, I do.

Q. So if more batches demonstrated the same results then that would be additional evidence that it does achieve 99.5 percent purity, correct?

A. More batches would be -- would further support this idea, yes, that's true. Assuming that the numbers were consistent.

Q. Have you performed any of the synthetic steps identified in the '393 patent?

A. No, I have not.

Excuse me. I certainly prepared the salts of carboxylic acids and regenerated the acid, but I have not performed the precise steps that are described in the patent.

Q. So you have not performed

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those steps with respect to the treprostinil or treprostinil diethanolamine?

A. I have not prepared treprostinil or treprostinil diethanolamine.

Q. Has anyone under your direction prepared any of the steps in the '393 patent?

A. As I mentioned before, certainly people in my laboratory had prepared the salts of carboxylic acids and we generated the carboxylic acid, but we have not prepared in my laboratory treprostinil or treprostinil diethanolamine.

Q. And if you look at the next paragraph in your declaration, it says, "Variations in the processes of making the claimed product could also impact and vary the degree of purity of the product" --

A. I'm sorry, could you tell me where we are?

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Q. I'm sorry. Paragraph 67 of your declaration.

A. Yes, go ahead, please.

Q. So you state, "Second, variations in the processes of making the claimed product could also impact and vary the degree of purity of the product. Do you see that?

A. Yes, I do.

Q. So if variations in the process could have impact the degree of purity, then --

MR. DELAFIELD: Strike that.

Q. Do you know what the variations in the processes were used in the prior art versus the '393 process?

A. I'm afraid I don't understand the question.

Q. I'm just trying to understand the purpose of your paragraph 67 to say that variations in the process could impact the purity.

Basically if you change the process, you would not be able to tell

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what the experimental error is by comparing two different methods that have different solvents, processes, et cetera, right?

A. I'm sorry, I don't understand your question at all.

Q. In order to determine the experimental error between two different samples, don't they need to have been performed or made by the same process?

MR. POLLACK: Objection to form.

A. I guess I have no idea what you're talking about. When you ask about the experimental error in the substance, prepared by different processes.

I don't understand what you're saying.

Q. Let's look at prior art, and maybe that will help clear it up.

MR. POLLACK: If you are going to another exhibit, would this be a good time for a break?

MR. DELAFIELD: Sure.

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THE VIDEOGRAPHER: The time is 12:09 p.m. and we're going off the record.

(A brief recess was taken.)

(Winkler Exhibit 7, Excerpt from book entitled, "Organic Chemistry, Second Edition," [SteadyMed-Exhibit 1008], marked for identification, this date.)

THE VIDEOGRAPHER: The time is 12:27 p.m. and we're back on the record.

Q. Hi, Dr. Winkler, you've been handed what's been marked as Exhibit 7, which is an excerpt from a book entitled "Organic Chemistry," whose author is Ege. I'm not sure how to pronounce that. It might be Ege.

Do you recognize this document?

A. I do.

Q. And is this one of the documents that you relied upon in your declaration?

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A. Yes, it is.

Q. So if you could turn to page 8 in the document, I believe you cite this page and the second full paragraph says, "Carboxylate acids that have low solubility in water, such as benzoic acid, are converted to the water-soluble salts by reaction with aqueous base."

Do you see that.

Oh, page 8 of the Ege reference?

A. Oh, I'm sorry. Yes. I'm on page 8.

Q. And in the second sentence of the second paragraph says, "Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties of the carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds."

Do you see that?

A. Yes, I do.

Q. Is this what you were citing,

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from this reference, regarding your opinion on obviousness?

A. Can I take a look at my report?

Q. Sure, sure.

A. Yes, I'm sorry. Could you ask the question again, please?

Q. In your declaration you refer to page 8 of this document, are you citing the second paragraph that I just read from your declaration?

A. I think what I'm -- what I'm citing specifically is that the protonation of the carboxylate anion by a strong acid regenerates the carboxylic acid.

Q. And do you agree with the last sentence in that paragraph that states, "These properties of carboxylic acid are useful in separating them from reaction mixtures containing neutral and basic compounds"?

A. I think that these properties can be used to separate them from a

neutral and basic compounds, but the cite to Ege in my declaration was to the point of that protonation of a carboxylate anion is well-known with strong acid to regenerate the pure carboxylic acid.

Q. So is it your understanding that that process would remove impurities?

A. I think that process can be used to remove impurities, but the cite to Ege is specifically for the protonation of the carboxylate anion to deliver the free acid.

Q. By reacting a carboxylate anion with a strong acid, is it your opinion that that would remove impurities?

A. Again, the protonation of the carboxylate anion specifically with strong acid would simply regenerate the carboxylic acid, and that was the point that I was taking for that data.

Q. Well, I'm not referring to Ege now. I'm just saying in general, is it

your opinion that reaction of a strong acid with a carboxylate anion would remove impurities?

A. It's my opinion -- I'm sorry, could you repeat the question, please?

Q. Is it your opinion that regenerating the carboxylic acid by reacting a strong acid with the carboxylate anion would remove impurities?

A. No, it is not my opinion that protonation of a carboxylate by itself would eliminate impurities.

Q. So you disagree with this last sentence that states, "These properties of carboxylate acids are useful in separating them from reaction mixtures containing neutral and basic compounds"?

MR. POLLACK: Objection to form.

A. I do not disagree with that statement.

Q. So separating neutral and basic compounds from a reaction mixture,

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is that not considered removing impurities?

MR. POLLACK: Objection to form.

A. I think that in general terms separating compounds, separating impurities is a method of purification.

I'm afraid I don't completely understand your question.

Q. So maybe to clarify, if you could look at paragraph 88 in your declaration, you say, "Accordingly, a person of ordinary skill in the art would want to perform the treprostinil diethanolamine salt purify it and then convert it back to its free form in order to obtain excellent crystallinity and increased purity." Do you see that?

A. Yes, I do.

Q. So converting it back to its free form involves the reaction with a strong acid, correct?

A. That is correct.

Q. And you would do that in order

Winkler

to obtain excellent crystallinity and increased purity, right?

MR. POLLACK: Objection to form.

A. I think what I say in paragraph 88, in fact I know what I say, is that accordingly, a person of ordinary skill in the art would want to form the salt, purify it, and then convert it back to its free form, in order to obtain excellent crystallinity and increased purity.

Q. So is it your opinion that converting the salt back to the free form carboxylic acid increases its purity?

A. My opinion is that the protonation after the carboxylate salt in itself does not increase the purity.

Q. But it is your opinion that it would be obvious to do that still, correct?

A. I think what I state in paragraph 88 is that a person of ordinary skill would form a salt, purify it, and

Winkler

then convert it back to its free form to obtain excellent crystallinity and purity.

Q. So it's your opinion that the reaction with the strong acid does nothing in terms of purity of the substance; is that correct?

A. I think that the protonation of the carboxylate salt would not be expected to increase the purity of the final product, per se.

Q. So it would be unexpected if the purity did increase as a result of that step?

MR. POLLACK: Objection to form.

A. Well, I think what I say here is that one would form a salt, purify it, and then convert it back to its free acid, to obtain excellent crystallinity and increased purity.

Q. But you're referencing three steps to form the salt, purify it and convert it back, and then saying, you do

Winkler

that in order to obtain excellent crystallinity and increased purity.

So which of those steps are performed to obtain excellent crystallinity and increased purity?

MR. POLLACK: Objection to form.

A. Could you repeat the question, please?

Q. Well, in your sentence you reference three steps, correct? The first sentence of paragraph 88?

A. I reference the formation of the salt, its purification, and then its conversion back to the free form, yes.

Q. Yes.

And in that same sentence, you say, you do those three steps to obtain excellent crystallinity and increased purity, right?

A. That is correct.

Q. So which of those three steps are performed to obtain excellent crystallinity and increased purity?

Winkler

MR. POLLACK: Objection to form.

A. Well, the total process delivers a material with excellent crystallinity and increased purity.

Q. So you can't say which of those steps contributes to crystallinity or purity?

MR. POLLACK: Objection to form.

A. I think that it would be a combination of the steps that would lead to the excellent crystallinity and increased purity of the final carboxylic acid.

Q. But you can't say for sure that reacting a salt with a strong acid would increase its purity; is that correct?

MR. POLLACK: Objection, form.

A. Sitting here, I'm not certain that the protonation step, the final protonation step in itself would increase purity, but the protonation step would

Winkler

deliver the crystalline, the final crystalline material.

Q. So with that three-step process of forming a salt, purifying it and converting it back to the free acid, what types of impurities would that remove?

A. This process of forming which is, as I mentioned before is standard practice in organic chemistry, or organic chemistry 101 as I referred to in paragraph 3, of forming the carboxylate salt using an amine, and then purifying that salt, and regenerating the acid could eliminate any of a number of impurities.

Q. But you don't have an opinion as to the type of impurity it would eliminate?

A. I think there are examples in the literature of using exactly this kind of purification process to remove all different kinds of impurities.

Q. Does the pH of the impurity

Winkler

matter in terms of this process and whether or not it would be removed?

A. It might or might not.

Q. So if you can look back at the Ege reference on page 8, the last sentence of the second paragraph states that "These properties of carboxylic acids are useful in separating them from the reduction mixtures containing neutral and basic compounds." And you said you agreed with that?

A. "These properties of carboxylic acids are useful in separating them from reaction mixtures containing neutral and basic compounds," I agree with that statement, but I don't agree with the limitation of the statement; that is, I think that the formation of an amine salt of a carboxylic acid, purification of said salt, and then the regeneration of the carboxylic acid could be used to separate a carboxylic acid from any of a number of compounds, not limited to simply and neutral and basic

Winkler

compounds.

Q. So do you have any opinion as to the type of impurities that are removed in this process?

MR. POLLACK: Objection to form.

A. Sitting here I have no opinion about the types of impurities that are removed in the '393 process. I simply know as a function of my experience in organic chemistry that one can remove neutral compounds, one can remove basic compounds, one can in fact remove other acidic compounds using the formation of the amine salts, its purification, and then the regeneration of the carboxylic acid from the salt.

THE WITNESS: Excuse me.

Q. So if you had a mixture of two carboxylic acids --

A. Yes.

Q. -- and you formed a salt by reacting them with a base so that there is two carboxylic salts as a result, and

Winkler

then reacted that same mixture with a strong acid, both would be converted back to the free form carboxylic acid, correct?

A. In the hypothetical that we took a mixture of two amine carboxylate salts and treated them with acid, I would expect both of them to revert back to the parent, or the starting carboxylic acid, that's correct.

Q. Okay.

A. But the fact is that there is an extra step here that we're leaving out and that's the purification of the salt. At the step at which the salt is being purified, there is an opportunity to separate undesired compliments of the mixture that may or may not be available at the stage of the free acid. And so that's why the formation of a carboxylate salt, specifically an amine carboxylate salt, and its purification followed by the regeneration of the parent carboxylic acid could affect purification of the

Winkler

carboxylic acid from neutral components, basic compounds, as well as other acidic components.

And in fact there are examples of such in the literature.

THE WITNESS: Excuse me.

Q. So if you could turn back to Exhibit 4 which is the file history of the '393 patent.

MR. POLLACK: Are we done with this one?

MR. DELAFIELD: Yeah, we're done.

MR. POLLACK: You can clean up if you need to.

MR. DELAFIELD: Yeah.

Q. And if you turn to page 348 of the file history excerpt, in paragraph 7?

A. Yes.

Q. So paragraph 7 states that there are eight impurities analyzed for these samples. Do you see that?

A. Yes, I do.

Q. And it states 1AU90, 2AU90 and

Winkler

3AU90 each of which is a stereoisomer treprostinil. Do you see that?

A. Yes.

Q. So as a stereoisomer of treprostinil, each of those impurities are the also carboxylic acid, correct?

A. That is correct.

Q. And then --

MR. DELAFIELD: Well, strike that.

Q. So in the purification process you just described, there is no reason that only certain stereoisomers would be removed and others not removed, right?

A. No, that's not true.

Q. So are you able to predict which stereoisomer would not be removed?

A. I'm not sure that I could necessarily predict offhand which one would be removed, but it would be a standard practice for one of skill in the art to prepare the ammonium salts of the carboxylic acids, purify those salts, and then see what the composition of or what

Winkler

the -- what the impurity levels of these different materials would be.

Q. But the fact that this purification process does not remove all stereoisomers is not predictable, correct?

A. Well, I think the point is that it would be straightforward organic chemistry, or organic chemistry 101, to prepare salts, crystallize them, and then see which would be the most effective at removing impurities from the starting treprostinil.

There are certainly multiple examples in the literature of people preparing the amine salts of carboxylic acids that contain mixtures of stereoisomers and crystallizing them to obtain pure materials.

In other words, to separate the undesired stereoisomers.

Q. But there is nothing in the process you just described that would explain only why certain stereoisomers

Winkler

are removed and while others are not, correct?

A. Well, again, as I stated, I think this would be a straightforward empirical exercise to determine which salts would be optimal for the removal of which stereoisomers. So this wouldn't be a particularly difficult thing to do.

Q. But sitting here today, you can't explain why this process only removed certain stereoisomers and not others, correct?

MR. POLLACK: Object to form.

A. I think, as I stated, one would simply look at the crystallization of the amine salt or of a series of different amine salts and then look at the purity profile of the resulting crystalline ammonium salt before regenerating the carboxylic acid to determine which would be the most effective.

But it's certainly a standard practice in organic chemistry to do so.

Winkler

Q. So in paragraph 7 it mentions there's three stereoisomer impurities, correct?

A. Yes, it does.

Q. And also two dimers, correct?

A. Yes, it does.

Q. So in the purification steps of reacting the sample with the base to form salts, and then reacting with a strong acid to convert back to the free acid, all of the --

MR. DELAFIELD: Strike that.

Q. Treprostinil and its stereoisomers and dimers could all be theoretically transferred back to the free acids, correct?

A. That is correct, but as I've already stated, at the step of the ammonium salt formation, of the ammonium carboxylate formation, I would expect that that step of purification would be possible to remove undesired, undesired isomers or dimers of the desired carboxylic acid.

Winkler

THE WITNESS: Excuse me.

Q. It's also possible that an impurity that is a stereoisomer to treprostinil could increase as a result of those steps, correct?

A. It's not obvious to me how an impurity would increase during the course of this attempted purification. Because I don't see how there would be interconversion of the stereoisomers possible under the conditions of the salt formation, and then the regeneration of the acid.

Q. Well, what would make the different stereoisomers react differently depending on what salt form is used?

A. Well, it turns out that the literature, the chemical literature is replete with examples of the separation of diastereomer salts and even a separation of enantiomeric salts using this process of forming the ammonium carboxylate salt purifying that salt and then regenerating the acid.

Winkler

Q. Sitting here today, there is nothing in the prior art that you know of that would allow you to predict which specific stereoisomers would be removed as a result of the process in the '393 patent, correct?

A. I think my understanding of the scientific literature, of the chemical literature is such that there are sufficient examples of the purification of the carboxylic acids separating them from diastereomers, from enantiomers and from other undesired impurities that I would have a high level of confidence that I could form the ammonium carboxylate, purify that, and then regenerate the acid to obtain a product, as I state here, with -- in paragraph 88 with excellent crystallinity and increased purity.

Q. But none of the prior art you cited discloses separation of stereoisomers in treprostinil, correct?

A. Well, to reach the opinion

that I -- that I state in paragraph 88, it's not necessarily for me to see an example of the stereoisomers of treprostinil specifically, but simply to show that as a general rule, one of skill would know and be familiar with the formation of the ammonium salt of the carboxylic acid, its purification, and then the regeneration of the free acid to generate a material that, as I state here, could have excellent crystallinity and increased purity.

MR. DELAFIELD: Let's move on to a different topic.

Q. So generally speaking, the substances involved in a chemical reaction are described as reactants in products, correct?

A. You know, we were -- I just had one addition I wanted to make to what I had said previously.

In the FDA letter, they -- before I put this away, they actually describe or admit a [redacted] percent

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variability, United Therapeutics admits a [redacted] percent variability in the assay on page 3 of Exhibit -- I can't read -- of Exhibit 5.

They go on to describe essentially what I talked about, that the purity can't be greater 100 percent, and therefore, they have a [redacted]percent variability in their assay.

I'm sorry, where are we now?

Q. Well, since you brought that up, so what page were you looking at?

A. So I'm on page 3, Winkler Exhibit 5.

Q. Uh-huh.

A. And in the last paragraph it says, "During the initial analytical method validation, the results indicated there is about a [redacted] percent variability. Our spec of [redacted] to [redacted] was centered at [redacted] percent purity for the API." So again with a [redacted]percent variability, when the process for the manufacture was instituted in Silver Spring, the purity

Winkler

went up from a statistical standpoint, a variability of [redacted] percent may have result in -- I don't remember what "OOS" is -- on the high side when the upper limit of the spec is [redacted] percent.

So they're even now going, at least in their treatment of this, going over [redacted] percent, API cannot have a purity of greater than 100, so if the API 100 percent pure, there must be a [redacted] percent variability in the assay.

So that would suggest to me that these HPLC assays that they're essentially stating that they have a variability of [redacted] percent.

I just wanted to clear that up based upon what we talked about before.

Q. So if you look on page 4 of that same exhibit --

A. Yes.

Q. -- the first full sentence says, "UT proposes to shift the specification for the HPLC assay of treprostinil from [redacted] to [redacted] centered at

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[redacted] to [redacted] to [redacted] centered at [redacted] due to improved purity of the API produced in the Silver Spring, and an analytical variation of [redacted] percent in the HPLC assay method."

A. Yes.

Q. So the fact that it changed from a [redacted] to [redacted] percent was not because of an increase in the experimental error, it was due to an increase in the purity, correct?

A. Well, I think they're claiming increased purity, but I think the important thing here is that there is a variation of [redacted] percent that they're admitting to in the assay, which means that any number that they're obtaining here is going to necessarily, at least my interpretation, and I think one of skill would interpret this to mean that the number that they obtained is going to be plus or minus [redacted] percent.

They certainly are suggesting greater purity in Silver Spring, but

Winkler

they're still acknowledging the [redacted]percent variation, or [redacted]percent variability in the assay.

Q. Now, this is referring to the specification limits, correct?

A. Well, I think it's referring to the HPLC assay numbers which we see from the spec -- I don't know if they provide -- they don't provide any raw data here, but they certainly say that the spec can be as high as [redacted] percent.

And so, therefore, they explain that with a [redacted]percent variability in the assay. At least that's the way I understand this.

Q. So you understand that the specification is a range of allowable samples; is that a fair statement?

A. My understanding is that the specification that they're doing here is going to be either a range or a limit. In the case of the HPLC assay it certainly is a range.

Q. And if you look back at page

6, that we were discussing before, in assay, it changes the specification from not less than [redacted] percent to not more than [redacted] percent to not less than [redacted] percent, to not more than [redacted]. Do you see that?

A. Yes, I do.

Q. So because this is a specification, this is simply indicating what samples are, would pass specification, correct?

A. Yes. I think -- I'm reading this in the context of the sentence on page 3 that says that the results indicated [redacted] percent variability in the assay. That's really the most important sentence in the way I think, in explaining the variability that can be observed in these measurements; and therefore, if you will, the lack of precision in the assay. At least that's my interpretation on this statement. And I think how one of skill would interpret it.

Q. So for any specification

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submitted to the FDA, there must some range associated with the each value, correct?

A. Well, again, I can't speak to FDA submissions or specifications in general. I can only look at the one that is in front of me here. From chromatographic impurity of the impurities, it doesn't really supply a range, per se, but simply an upper limit. For the HPLC assay of the purity of the API, it does apply a range, but the range goes over 100 percent.

Q. So in your declaration, I believe you mention that there could be batch-to-batch variation, correct, in terms of the making treprostinil?

MR. POLLACK: Where are you pointing?

MR. DELAFIELD: Well, strike that.

Q. For the FDA specification, is it possible that these ranges are just an indication of what is acceptable and not

Winkler

what is experimental error?

A. Well, again I wasn't asked to opine on FDA specifications, specifically, if you will, but when I see that an assay range is over 100 percent, and I know that it's not physically possible to be over 100 percent, then that's suggests to me that the range that is over 100 percent is defining the error in the measurements that are being. Or at least the possible, as was stated by United Therapeutics in the letter on page 3, the [redacted] percent variability in the assay. And I think that's really the important thing, or that's the important thing that I note here.

Q. Okay. Going back to my question earlier, when you have a chemical reaction, you have both reactants in the products, correct?

A. Chemical reaction classically is consisting of reactants in products, correct.

Q. And usually the species

written on the left-hand side of a reaction arrow are called reactants, right?

A. The species on the left are typically the reactants, that's correct.

Q. And the species on the right are typically called products, correct?

A. And the species on the right of the arrow are typically called product, that's correct.

I guess, I'm sorry, the only thing that I would add to that is that the products could contain impurities. In other words, there could be desired products as well as undesired products in a chemical reaction of starting material product formation.

And excuse me, there is one other thing I would add, which would be that there could be reagents of some kind, that would be added to the reaction as well.

Q. Okay. But product typically refers to what is produced as a result of

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the chemical reaction, right?

A. The product is typically the result of a chemical reaction, right, and again the crude product could be contain impurities of some kind as well.

(Winkler Exhibit 8, Excerpt from textbook entitled "Chemistry," [UT Exhibit 2011], marked for identification, this date.)

Q. You've been handed what's been marked as Exhibit 8, I believe.

A. Yes.

Q. Which is an excerpt from a "Chemistry" textbook authored by a Steven Zumdahl?

A. Yes.

Q. Do you recognize this document?

A. I'm trying to remember whether I consulted this document in my --

Q. I don't believe you cited it.

A. Okay.

Q. But --

A. I'm familiar with Zumdahl,

yes.

Q. Sir, if you look on page 4, in the left column, it says --

A. I'm sorry. Could you just give me a minute to look at it?

Q. Sure, sure.

A. Thank you. Okay.

Q. So on the left column it lists the term "Product," do you see that?

A. Yes.

Q. And it says, "a substance resulting from a chemical reaction"?

A. Yes.

Q. Do you agree with that?

A. Well, as I mentioned, a product typically is a substance resulting from a chemical reaction. It's typically shown on the right of the arrow in the equation, but I think when we think about product or products of a reaction that could certainly include multiple products or impurities within the reaction would be part of the product of a reaction.

Winkler

Q. So purities would be part of the product of the reaction; is that correct?

A. I would certainly think of anything that is formed in a chemical reaction from a given starting material, to be part of the product of the reaction. It might or might not be the desired product. It might not or might not be the final product. It might or might not be the purified product, but it I would call all of that "product."

Q. And so if two products had different impurities, then they would be different products, correct?

A. Well, if two products contained different purities, the products, the desired material, if you will, is the same material, but the mixtures, the two mixtures would be different presumably prior to their purification.

Q. But because the product encompasses impurities, if impurities are

different, then the products are different, correct?

A. Well, I think that depends whether we're talking about the crude product reaction before purification or after purification.

Q. So if after purification the impurities are different, then the products would be different, correct?

A. I don't think I would call the products different. I think I would say that the products are the same. The impurities within the products could be different in different chemical reactions.

Q. But if impurities are part of the product, if impurities are different, then wouldn't that necessarily make the product different?

A. Well, I think the product is -- is the product. The product is the molecule. If, for example, it were treprostinil, it's treprostinil. That would be the product regardless of

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whether there were impurities there or not.

And so I'm not sure I completely understand your question.

Q. Well, I think maybe I'm misunderstanding your answer.

You said the product is the molecule regardless of whether there are impurities there or not, but early I believe you testified the impurities are part of the product.

MR. POLLACK: Objection to form.

Q. So which is it?

A. I think the impurities are part of what I call the crude product. In other words, the product mixture, if you will. And then on purification one could isolate a pure or relatively pure product.

Relatively pure, if you will, final product.

So I'm differentiating between the crude mixture, which would be

Winkler

containing the product, which would be the initially formed reaction, and the final purified material.

Q. So if the crude product --

MR. DELAFIELD: Strike that.

Q. If one crude product had different impurities from another crude product, would they be different products?

A. They would be different mixtures but they would contain the same desired product.

Q. But the products would be different?

A. The mixtures could be different. But I don't know that they would be different.

THE WITNESS: Excuse me.

Q. So as this definition states, "Product" does refer to the result of a chemical reaction, correct?

A. The Zumdahl definition here states that, or at least the definition in this glossary states that a product is

a substance resulting from a chemical reaction.

Q. And you agree with that definition?

A. Well, again, I agree with it in a sense, but I think it's a little simplistic, because there could be multiple, multiple products formed in a reaction. There can be desired and undesired products. There can be impurities. And all of this could be part of the reaction mixture that results from any chemical reaction.

Q. So not all chemical compositions are products, correct?

A. Not all chemical compositions are products? I'm thinking about that.

I'd have to think about that.

Q. Well, put it another way: Product refers to the result of a chemical reaction and not just the fact that it is a chemical, correct?

A. Well, I think I speak to this in my report.

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Q. Is that part of your report that you wanted to discuss?

A. I thought I -- I must be thinking of something else. I'm sorry.

Q. So going back to product --

A. Yes.

Q. -- the word "product" has a subset definition to chemical composition, right?

A. Well, the definition of product in Zumdahl here is clearly a substance resulting from a chemical reaction.

Q. And you agree with that?

A. I think that this is certainly a definition, but I think that it's rather broad in not making clear whether it's the crude product or the final product, the desired product or the undesired product.

Q. So if the definition of "product" was just a chemical composition, would you agree with that?

A. If the definition of a product

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is simply a chemical composition, I think that certainly is one definition of a "product," but there are other definitions obviously, including the one shown here in Zumdahl.

Q. But a chemical composition can be a lot more than just a product, correct?

MR. POLLACK: I am going to object to form, lack of foundation.

A. I guess hypothetically a chemical -- I'm sorry, I forgot the definition you just gave.

Could you repeat it, please?

Q. Well, let me rephrase.

A reactant can be a chemical composition, correct?

A. A reactant can be a chemical composition, yes.

Q. So chemical composition and product are not synonymous, correct?

A. I think that it's true that a chemical composition is not necessarily the same as a product, but could be the

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same as a product.

Q. Is it fair to say that the term "chemical composition" doesn't indicate whether it's a product or not?

A. I think I've just stated a chemical composition could be a product or might not be a product.

Q. But just the term "chemical composition," from that term alone, you can't determine whether it's referring to a product or a reactant or a reagent, correct?

A. I think a chemical composition could include a product or a starting compound or a reagent, among other things.

MR. DELAFIELD: I think we're almost out of time if we want to break for lunch.

THE WITNESS: I'm in no hurry to break. I could keep going.

MR. DELAFIELD: We've got five minutes left on the tape.

MR. POLLACK: You want to go

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the five minutes and then we will break.

MR. DELAFIELD: Sure.

Actually I need to get other exhibits, so I think now is a good time to break.

MR. POLLACK: All right. Why don't we break.

THE VIDEOGRAPHER: The time is 1:24 p.m. and we're going off the record.

(Lunch recess taken at 1:24 p.m.)

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A F T E R N O O N S E S S I O N

(Time noted: 2:24 p.m.)

THE VIDEOGRAPHER: This begins media unit number 3. The time is 2:24 p.m. and we're back on record.

MR. DELAFIELD: Welcome back, Dr. Winkler, I hope you had a good lunch.

THE WITNESS: Thank you.

(Winkler Exhibit 9, Excerpt from textbook entitled, "Chemistry, The Central Science," [UT Exhibit 2012], marked for identification, this date.)

J E F F R E Y D. W I N K L E R, resumed.

EXAMINATION (Cont'd.)

BY MR. DELAFIELD:

Q. So I've handed you what has been marked as Exhibit 9, which is an excerpt from another chemistry textbook entitled "Chemistry, The Central Science," by Theodore L. Brown and others.

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Have you seen this document?

A. I don't think so.

Q. Okay. Are you familiar with this book?

A. No, I am not.

Q. Are you familiar with Brown and LeMay in terms of being chemistry authors of textbooks?

A. No, I am not.

Q. Well, if you turn to page 4, which is the back, very last page, at the upper left is another definition of "Product." Do you see that?

A. Yes, I do.

Q. And it says, "product is a substance produced in a chemical reaction; it appears to the right of the arrow in a chemical equation." Do you see that?

A. Yes, I do.

Q. And do you agree with that?

A. I certainly agree that that's one definition of a product.

Q. And that's similar to the

definition from the other textbook we looked at right before lunch, right?

A. I would have to go back and look at that.

Q. Oh, that's okay. We can move on.

(Winkler Exhibit 10, Excerpt from textbook entitled, "Conceptual Chemistry, Understanding Our World of Atoms and Molecules," [UT Exhibit 2014], marked for identification, this date.)

Q. I've handed you what's been marked as Exhibit 10 which is another excerpt from another chemistry textbook entitled "Conceptual Chemistry, Understanding Our World of Atoms and Molecules," authored the by John Suchocki. Do you recognize this document?

A. No, I do not.

Q. If you look at page 3, about the middle of the left column, you see a definition for "Product"?

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A. I do.

Q. And it says, "a new material formed in a chemical reaction appearing after the arrow in a chemical equation." Do you agree with that definition?

A. I think that certainly represents a definition of product.

Q. Is it an accurate definition?

A. I don't know what you mean by "accurate."

Q. Is that how you use the term "product"?

A. I think that this would be a possible definition of product, but would not be the only possible definition of product.

Q. But is this how you would use the word "product"?

MR. POLLACK: Objection to form.

A. I don't think I would use this precise definition, no.

Q. Why not?

A. I don't think I would, I don't

think I would use these exact words for my definition of product.

Q. Why not?

A. Because I don't -- I just don't think I would state it this way.

Q. But you don't have a reason to disagree with this definition?

A. Well, again, I think it's limiting, and I think a product could be more than what's described in this definition.

I'm not disagreeing with this as a definition of product, but I wouldn't think that this would be the only or exclusive definition.

Q. Are products ever not formed in a chemical reaction?

A. I think one could say that, yes.

Q. So you've used the term product to mean something other than a substance formed in a chemical reaction?

A. I think one could use product in a different, in a way different from

that, yes.

Q. Can you think of an example?

A. I think one could think about a manufacturing product, for example, and that might not be the product of a chemical reaction, per se.

Q. Are you referring to the word "product" not referring to the chemistry definition of the word?

A. I'm afraid I don't understand your question.

Q. You said that one example would be a manufacturing product. Did you mean in a nonchemistry manner?

MR. POLLACK: Objection to form.

A. Not in a formal chemistry manner, but simply describing the word "product," a product could have numerous definitions and numerous meanings in different contexts.

Q. If you were teaching one of your chemistry students what a product is, would this be an acceptable

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definition?

A. I don't know that the material formed in the chemical reaction, for example, would have to be a new material to qualify as a product by my definition.

Q. Would a substance formed in a chemical reaction be an acceptable definition if you're teaching the concept of a chemical product to a student?

A. Well, I think I've discussed this question before. "Product" is a very broad descriptor and so the question is, is this initially formed product, is it the final product, is it the crude product? Those are all products, in fact. So the definition is rather a broad one.

The definition that I would use would be rather a broad one, in the absence of limiting descriptors.

Q. Well, if there were no other adjectives and you were just defining the word product, would this --

MR. DELAFIELD: Scratch that.

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Q. Assuming there are no adjectives defining the word "product," would the definition of substance formed in a chemical reaction be an acceptable definition to tell a chemistry student?

A. Well, again, the product of a chemical reaction would be essentially all of the substances that result from the treatment of a particular reactant with a particular set of reagents.

(Winkler Exhibit 11, A paper entitled, "A Pauson-Khand Approach to the synthesis of Ingenol," marked for identification, this date.)

Q. I'm handing you what's been marked as Exhibit 11, which is a paper entitled, "A Pauson-Khand Approach to the synthesis of Ingenol."

MR. DELAFIELD: I'm sorry.

MR. POLLACK: Do you have another copy?

MR. DELAFIELD: Sorry about that.

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Q. Do you recognize this document?

A. Yes, I do.

Q. Are you one of the authors of this document?

A. I am.

Q. So if you look at the abstract, the first sentence you state, "Pauson-Khand cyclization of dioxanone photoadduct 21 leads to the formation of a single product in good yield."

Do you see that?

A. I do see that.

Q. So your use of the word "product" here is in reference to what comes after the arrow in that chemical reaction, correct?

A. That is correct.

Q. And that's the result of the chemical reaction, correct?

A. I'm sorry, I don't understand your question.

Q. When you used the word "product" here, you're referring to the

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result of the chemical reaction, correct?

A. Yes, I am.

Q. So that's how you have used the word "product" in at least this paper, correct?

A. Well, in this paper, what I state is that "cyclization of the dioxanone photoadduct 21 leads to the formation of a single product in good yield." And so that single product in fact is the compound that's shown as 22 in the box above.

Q. Okay. And if you turn to page 1491, in the document, and the first full sentence on the left column underneath scheme 6 says, "The structure of 18, the sulfone derived from 17, was confirmed by x-ray crystallographic analysis" --

MR. DELAFIELD: Actually, strike that.

Q. The next sentence says, "Heating sulfoxide photoadduct 17 to 160 degrees celsius in quinoline led to the formation of the desired methylene

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photoadduct 11, the formal product of [2+2] cycloaddition of 10 (Scheme 4) in good yield."

Do you see that?

A. Heating sulfoxide 17 led to the formation of -- two plus two -- yes, I do see that.

Q. So in that sentence when you use the word "product," you're referring to the substance that forms in the chemical reaction, right?

A. Well, what I'm referring to here is that 11, structure 11 is the formal product that would result from two plus two cycloaddition of ten, the structure which is shown in scheme 4.

Q. And so the product is what results from the cycloaddition, correct?

A. Well, it's not actually true, because 10 does not undergo -- I think in this paper, we explain that at ten does not undergo the cycloaddition, and so instead, I refer compound 11 as the formal product of cycloaddition, and that

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is that is the compound that would have been obtained had chem undergone cycloaddition.

And that's why I refer to it as a "formal product."

So in fact 11 in this particular case never results from compound ten.

So that would be an example of -- of the vagaries, I guess, of the use of the word "product," because in this particular case, from my own, my own research work, you can see that I've characterized the compound as a product, when in fact it does not result from the reaction of the starting compound, because it is in fact, in this case, a formal product.

Q. But here you use the word "product" in the hypothetical sense as the result of the cycloaddition of 10; is that fair to say?

MR. DELAFIELD: Strike that.

Q. You wouldn't say the formal

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reactant of two plus two cycloaddition of 10, correct?

A. Well, I think the formal reactant in the two plus two cycloaddition would be compound 10.

But in fact compound 10 does not undergo -- excuse me -- cycloaddition.

Q. But that would be different than the formal product of two plus two cycloaddition of 10, correct?

A. I'm sorry, could you repeat the question?

Q. You said that the formal reactants of two plus two cycloaddition of ten would be ten, right?

A. That is correct.

Q. That's different than the formal product of two plus two cycloaddition of ten, correct?

A. The formal product of ten, right, is different from ten, that's certainly correct. But it underscores the idea that in this particular context,

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the product does not result from the starting tip.

Q. But the word "product" is just used to indicate had cycloaddition of ten worked, that would have been the result; is that fair to say?

I mean that's why you're using the word "product" as opposed to "reactant" as opposed to "chemical"?

A. Well, I don't know that the cycloaddition of ten would give 11, because it never -- it never transpired. But I describe in this paper 11 as the formal product of cycloaddition of ten, because that was the product that I anticipated would be formed if ten had been able to undergo reaction, which it could not, and in fact, that is really much of what the paper is describing, that, that chemistry.

Q. So let's look back at the '393 patent, which is Exhibit 3. If you look at claim 1, this is on column 17.

A. Column 17, okay.

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Q. And claim 1 says, "A product comprising a compound of formula I or a pharmaceutically acceptable salts thereof, wherein said product is prepared by a process." Do you see that?

A. Yes.

Q. So when it says, "A product comprising a compound formula I or a pharmaceutically acceptable salt thereof," is it your opinion that product includes any amount of impurities?

A. I think I addressed this question in my report. I guess it's not here. I must have read it somewhere else.

But my understanding is that the term "comprising" when added into the claim indicates that this describes the product comprising of formula compound I means that it includes the compound of formula I but can include other materials as well.

Q. Okay. But you don't offer any opinions regarding how the claim should

be construed, correct?

A. No. My understanding simply of what comprising means in the context of the claim is that it would include the product of, the compound of formula I, and the possibility of other things being there, such as impurities. That is my opinion.

Q. And similarly you weren't asked to apply certain claim constructions to your opinion, correct?

MR. DELAFIELD: Strike that.

Q. Your declaration does not contain any specific claim constructions with respect to the claim terms, correct?

A. I don't think so, but I would have to check.

Q. Well, when you just checked your declaration, were you checking for claim constructions?

A. I simply looked for a discussion of "comprising," which I know I have seen some of the documents and thought was in my report, but -- I'm

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sorry, in my declaration, but was not.

But my understanding of the common usage of "comprising" here is that we -- include the compound the formula I and other things, or at least it would allow the possibility for other things, including impurities being met.

Q. So then the term product --

MR. DELAFIELD: Strike that.

(Winkler Exhibit 12, Patent application WO 2005/007081, [SteadyMed - Exhibit 1005], marked for identification, this date.)

Q. Sir, you've been handed what has been marked Exhibit 12, which is a patent application WO 2005/007081, which we refer to as the Phares application. Do you recognize this document?

A. Yes.

Q. And this is one of the documents that you rely upon in your declaration, correct?

A. Yes, it is.

Q. So if you keep that open and

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if you could also look at your declaration.

So in your declaration, if you look at paragraph 48 --

A. Yes.

Q. -- you state, "Phares inherently discloses the same synthesis of treprostinil as set forth in the independent claims, Claims 1 and 9, of the '393 patent. "

And then at the bottom of that paragraph you say, "Accordingly, Phares inherently anticipates both independent of Claims 1 & 9."

Do you see that?

A. Yes, I do.

Q. Sir, what do you mean by "inherently anticipates"?

A. So what I mean by "inherently anticipates" in paragraph 48 is that what Phares describes on pages 41 and 42 -- actually starting -- excuse me -- starting at the bottom of 39, Phares teaches that minus treprostinil, which is

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the enantiomer of treprostinil, can be synthesized as follows, and then shows a synthetic re, which is quite similar to that shown in the Moriarty teaching, but this one delivers compound 2, which is the enantiomer of treprostinil, that is treprostinil minus.

And the point is that at the top of, in the middle of 39, it teaches that enantiomers can be synthesized using reagents and synthons of an enantiomeric chirality, which is to say that my opinion is that the scheme on page 40 teaches inherently anticipates the synthesis of the plus treprostinil.

Q. So I'm not clear about what is inherent about the disclosure you just described.

So why is it your opinion this inherently anticipates as opposed to just anticipates?

A. Well, I guess it's a subjective question of whether it anticipates inherently or expressly, but

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the reason I use the word "inherently" here is that the actual synthetic route that is shown on page 40 is for the synthesis of the enantiomer of the desired product, of the API.

But certainly one of skill and myself could look at this and see that one could use this information that's included on page 40 here to prepare either the enantiomer, the minus compound, as shown or could also be used to prepare the plus compound.

It does not show the plus compound, the synthesis of the plus compound explicitly, although it certainly shows compounds derived from the plus compound in the teachings of the patent.

But I say that there is inherent anticipation because one of skill would understand that the synthesis of the plus enantiomer is, if you will, anticipated by the chemistry that is shown on page 40.

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Q. So the Phares reference doesn't explicitly disclose the synthesis of treprostinil, correct?

A. Well, that's not actually true. The Phares reference explicitly discloses the synthesis of minus treprostinil, and explains that one of skill -- I'm sorry. It explains that enantiomers could be synthesized using reagents and synthons of enantiomeric chirality.

So inherently or implicit in the teaching on page 40 is the synthesis of the plus or, if you will, desired enantiomer of treprostinil.

Q. As written, the exact synthesis of plus treprostinil is not explicitly disclosed in Phares, correct?

MR. POLLACK: Objection to form, and asked and answered.

A. I think I've already answered that, that is that one of skill would look at the route that is provided on page 40 and the information that is given

on page 39 about being able to prepare either an enantiomer and use this teaching as, to inherently, if you will, understand that one could prepare either plus or minus treprostinil using the route that is shown here.

Q. So is it your opinion that it is explicitly disclosed or not?

A. I think what my opinion is on paragraph 48 that is that Phares inherently anticipates the claim because it shows the synthesis of treprostinil, it happens to show the synthesis of the minus compound, but one of skill could use essentially the same route to make the plus compound.

Q. And you used the word "inherently," because the plus compound synthesis is not explicitly disclosed in Phares?

A. I use "inherently anticipates" because it is the synthesis of the minus compound that is explicitly shown on page 40.

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Q. Okay.

A. But again, implicit in the disclosure on page 40 would be the synthesis of the plus compound as well.

Q. So is it your opinion that a person of ordinary skill would need to experiment based on this disclosure to get to treprostinil because it's not explicitly disclosed?

MR. POLLACK: Objection to form.

A. Well, my opinion is that the Phares patent on page 40 teaches the synthesis of the minus treprostinil and teaches by the information on page 39, that the enantiomer of the compound could be synthesized, using, if you will, enantiomeric starting compounds.

THE WITNESS: Excuse me.

Q. There are several different synthetic routes to make treprostinil; is that correct?

A. I have seen more than one synthetic route for the preparation of

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treprostinil, that is correct.

Q. And Phares lists several of those in the document, correct?

MR. POLLACK: Objection to form.

A. I don't remember. I'd have to look.

Q. If you look at page 99?

A. I'm sorry, 99 on Bates or 99 on the document?

Q. On Bates.

A. Okay.

Q. You cite this in your declaration as disclosing treprostinil diethanolamine salt, and that's at paragraph 51, if you want to look at that. Is that accurate?

A. Yes.

Q. But the Phares reference does not disclose the full synthesis of how that's made, correct?

MR. POLLACK: Objection to form.

A. Well, actually I think that it

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does disclose how that compound is made. In fact, it teaches on page 22, page 24 Bates, it teaches that treprostinil diethanolamine UT-15C is prepared by dissolving treprostinil acid acid -- I assume that's mistake. It should be acid once -- "is dissolved in a one-to-one molar ratio mixture of ethanol: Water and the diethanolamine is added and dissolved. The solution is heated and acetone is added as an antisolvent during cooling."

So the synthesis of the compound of claim 1 with the structure shown in 49 is described on page 24, Bates, of the Phares reference.

Q. And on that same page though where it says the treprostinil acid is dissolved, it doesn't indicate the source of that treprostinil acid?

A. It does not give us a cite to where in the patent the treprostinil acid comes from, but as we've already discussed, beginning the fact that the

synthetic route is shown on -- synthetic route is shown on page 42 of the Bates, the presumption that I made, and I think that one of skill would make is that this would be this route or the corresponding route that would lead to the preparation of the pulse treprostinil that would be used to prepare the compound that's discussed on page 24 of the reference.

Q. But as you mentioned earlier, the actual synthesis for plus treprostinil is not explicitly disclosed in Phares, right?

A. I think it's not explicitly shown, but it would certainly be apparent to one of skill and to myself that if one knows how to make the enantiomer, given the teaching on page 39 of the document, page 41 Bates, enantiomers of these compounds can be synthesized using reagents and synthons of enantiomeric chirality of the above reagents, certainly implicit in that statement is that the enantiomers of these compound,

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in other words, the enantiomer of minus treprostinil can be prepared in the same manner.

Q. If you look at page 11 in the Phares document, Bates page 11 --

A. Yes.

Q. -- at the bottom of the second paragraph, it says, "compounds of the present invention can also be provided by modifying the compounds found, U.S. Patent numbers 4,306,075 and 5,153,222 in like manner.

Do you see that?

A. Yes, I do.

Q. Do you recall reviewing either of those patents?

A. I don't remember those numbers offhand.

Q. So if treprostinil was made by a different process, and then subjected to form the diethanolamine salt, it could have a different impurity profile, correct?

A. I think the sort, the

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different synthetic procedures could lead to different impurity profiles, but again, my conclusion from reading Phares is that the material that's described on page 24 was prepared according to the synthetic scheme that is shown on page 42 Bates.

I think that would be the logical conclusion that one of skill would draw, and certainly what I concluded from reading Phares.

Q. But there is nothing in this document that says that is how they prepared treprostinil, correct?

MR. POLLACK: Objection to form.

A. I think the point is that if they go about showing a synthesis of treprostinil and they show subsequent chemistry of treprostinil, the opinion of one of skill, and certainly my opinion is that they would have use the treprostinil, the synthesis of which is described in the patent to make these

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other compounds.

Q. But as you said, the synthesis of the plus treprostinil isn't explicitly disclosed. It's only mentioned as a possibility from enantiomer, correct?

A. Well, I think --

MR. POLLACK: Objection to form. You can answer. Asked and answered. You can answer.

A. I think it would be obvious to one of skill having outlined the synthesis, the explicit synthesis of the enantiomer and acknowledging that one can prepare either of the two enantiomers depending on the chirality of the starting material, it would be obvious to one of skill that one could prepare plus treprostinil from the information that is given in the patent.

Q. Is there a difference between inherent anticipation and obviousness?

MR. POLLACK: Objection to form, lack of foundation.

A. So I think I discussed this in

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my report. I discussed the legal concepts that were explained to me in paragraph 16.

Excuse me.

I state that counsel explained to me that the law recognizes anticipation in which a single prior art reference must disclose each and every element of a claim either expressly or inherently to anticipate the claim and render it invalid. And then I understand on paragraph 18 that obviousness, a patent claim is invalid for obviousness if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious to a person of ordinary skill in the art at the time of invention. For, I understand, I go on to say, I understand that for a single reference or a combination of references to render the claimed inventions obvious a person of ordinary skill in the art must have been

able to arrive at the claims by modifying or combining the applied references.

So that's the difference as I see it.

Q. So then is it your opinion that the treprostinil diethanolamine disclosed in Phares would have been obvious based on the description of how you could make the enantiomer?

A. I think what I said in my -- in my declaration is that Phares inherently anticipates the claim and then I go on to say that under obviousness that the combination of Moriarty with Phares or Kawakami would have made the '393 obvious.

Q. The Phares reference doesn't disclose any specific impurities in treprostinil or treprostinil diethanolamine, correct?

A. The Phares teaching does not disclose the impurities, but it does in fact disclose the purity in the sense that we have the melting point by DSC by

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differential, D-I-F-F-E-R-E-N-T-I-A-L, scanning, S-C-A-N-N-I-N-G, calorimetry C-A-L-O-R-I --

THE WITNESS: You're going to have to do that one yourself.

A. Calorimetry that the melting point of the form B material that was obtained suggests based on the other information that I've seen that the material was quite pure.

Q. But Phares doesn't disclose any specific impurities, correct?

A. Phares does -- I don't think -- I would to check through, but I don't think that Phares discloses impurities, per se.

Q. And in terms of --

A. But does show -- excuse me -- but does this show this sharp melting form B material that suggests something of quite high purity.

Q. In terms of the level of purity, it doesn't disclose a number for that purity other than melting point,

correct?

A. I don't remember, but I think that's true.

Q. Now, you mentioned the --

A. I would have to check.

Q. If you turn to page 91 of Phares, it says on the first full paragraph, "The thermal data for form B as shown in figure 21, the DSC thermogram shows a single endotherm at 107 degrees celsius that is consistent with a melting event."

Is that the melting point that you referred to?

A. Yes, it is.

Q. So if we turn to figure 1, that's on page 121?

A. Yes.

Q. And it shows the melting point of 107.06; is that correct?

A. That is correct.

Q. And the substance starts to melt when that peak starts to go down and basically stops melting once the peak

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goes back up; is that a fair characterization of what that peak means?

MR. POLLACK: Objection to form.

A. I think that's right but I would have to check.

Q. Are you not sure what this graph represents?

A. Well, I know that this graph represents the DSC thermogram of the sample.

Q. So if you look at that peak in the temperature range at the bottom, it looks as though the peak starts at a little above 100 and stops at the next mark, which I guess is 110 or 115, based on the scale.

Do you see that?

A. It's hard for me to eyeball exactly where it starts and where it stops.

Q. But would you agree that it starts to melt close to 100?

MR. POLLACK: Objection to

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form.

A. I think you can certainly see a change in the thermogram starting at about 100 degrees.

Q. And then the peak ends close to 110?

A. I think that's about right.

Q. So that indicates a melting range of approximately 10 degrees; is that right?

MR. POLLACK: Objection to form.

A. I don't think that's how -- how DSC is typically interpreted, because, for example, if you look at figure 18 and look at the -- the thermogram for the form A, what you see is a similar, relatively similar range in terms of the width at the top of the scan. But in fact you see a very different, sharp minimum at 103 degrees. And so I think that these thermograms are taken to be quite accurate descriptors of melting temperature and you can see in

fact, even though the wide part of the thermogram is roughly comparable, I would say, in figure 18 and figure 21, we see very sharp minima with very different numbers, and so that's why I'm quite comfortable relying on figure 21 as expressing a melting temperature of 107 degrees.

Q. So you referred to figure 18 as form A -- excuse me -- the treprostinil diethanolamine; is that right?

A. Correct.

Q. And there is little hash marks to the left and right of the peak at 87.81 degrees celsius and 112.09 degrees celsius. Do you see that?

A. Yes. Excuse me.

Q. And so doesn't that indicate the start and stop of the melting range for form A?

MR. POLLACK: Objection to form.

A. I'm not sure exactly what the

significance is of the 87.81 and the 112.09. I think the thing that I focused on was the rather dramatic difference between 103 sharp peak and the 107 sharp peak in figures 18 and 21 respectively.

Q. But you relied on this information as a proxy for the purity of the treprostinil diethanolamine, right?

A. I did in the context of the 103 number, and 107 number that I see clearly as at the bottom of the sharp peaks that we see for the thermograms for these two substances.

Q. So sitting here today, you can't say whether the beginning of the peak on the left side is when the sample started to melt?

A. I think what I -- what I really mean to say here is that I don't know sitting here -- I can't remember sitting here today whether -- what the significance is of the 87.81 and 112.09 in figure 18 relative to the information that is imparted by the sharpness of the

peak at 103, and it's the difference between the sharpness of the peak at 103 in figure 18, and the sharpness of the peak at 107 in figure 21 that I use to conclude that the purity of the form B that was obtained in Phares was such that a 107-degree melting point was obtained.

Q. But looking at figure 21, the peak at its base is approximately 10 degrees across, right?

A. Well, I don't know what you mean by -- by the "base."

Q. When the peak starts to form and then when the peak stops on the other side.

A. Yes, there appears to be a range there of what could be about 10 degrees.

Q. And sitting here today, you can't say whether or not that indicates when it starts and stops melting?

A. I don't remember.

Q. This is a graph of heat flow versus temperature, right?

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A. That's correct.

Q. And as the temperature rises, it starts to melt and then degrade, or decompose after a certain temperature; is that fair to say?

A. I don't know whether it's decomposing or simply melting.

Q. So at 107 degrees you don't know if that is a melting point or a decomposition point?

A. No. In other words, I said that this is the melting point at 107 degrees. I thought you said that it decomposed, and I'm not sure that it undergoes decomposition at its melting temperature.

Q. Isn't it true that the peak is caused by the fact that melting is endothermic which causes a reduction in heat flow as the temperature rises?

A. That is my understanding, yes.

Q. And so when the melting starts, the endothermic reaction starts which causes the speak to start to form,

right?

MR. POLLACK: Objection to form.

A. I'm afraid I don't understand your question.

Q. So I guess I just want to clarify, sitting here today, you don't know whether the base of that peak at the top of the graph indicates the melting range or not?

A. I don't remember. I would have to check.

Q. If the melting range were 10 degrees, that would not be a very pure sample, correct?

MR. POLLACK: Objection to form.

A. You see the point is that, I have to go back and check, but from what I remember, the range that's observed above there in DSC does not directly correlate to the degree range that one typically sees on recording a melting point on a hot plate apparatus. So the

width there that you see is not necessarily the same thing that I would expect to see if I were recording a melting point with a melting point apparatus.

Q. Do you know if a melting point apparatus is more accurate than a DSC?

A. My understanding is that a DSC is more accurate for obtaining the number of 107.06 that we see there, but again, I would have to go back and check to see what the significance is of the width of the peak that you're describing.

Q. So sitting here today, you can't say for certain how pure the sample is, correct?

A. Well, sitting here today, what I can say is that the DSC shows a melting temperature of 107 degrees, and when I compare that 107 degrees melting point of the form B material to the information in the '393, I conclude that this material is, at least it's pure, if not more pure than the material obtained in the '393.

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Q. But other than statement you don't know any other evidence to support that, correct?

A. I think the evidence that I have to support that is the melting temperature of 107 degrees that is clear from -- to me from figure 21 for the form B material.

Q. Is it fair to say that a more narrow melting point range is more pure than a broad melting point range?

A. When those melting points are obtained with a melting point machine, typically what one does is to correlate the tightness of the melting point range to the purity of the material.

But -- excuse me -- in the case of the DSC measurement, I don't know what the significance -- I don't remember what the significance is of the distance at the top of the peak.

Q. So you don't know the melting point range for this sample, correct?

A. No. All I know is that the

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DSC is showing a melting point of 107 degrees.

Which is higher than the melting points that were described in the '393 for the same material.

Q. And based on Phares, there is no specific level of purity disclosed for the material, correct?

A. Well, the primary measure of purity in Phares in my opinion is the 107-degree melting point for the form B material, which is, as I mentioned before, is higher than the melting point that is described in the '393 for the form B material.

From that I would conclude that the material here is at least as pure if not more pure than the material described in the '393 patent.

Q. Phares does not disclose a specific level of purity. You're only basing your analysis on the DSC, correct?

A. I don't remember any other description of purity in the -- in Phares

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other than this melting point. But given two samples of the same polymorphic material, if one is higher melting than the other, I would assume that that compound would be the purer.

I would note, I would really know that that compound would be the purer. I can't think of any examples where two samples of the same material and the same polymorph where the lower melting material would be more pure.

In my experience, the higher the melting material is the more pure.

Q. And two samples --

MR. DELAFIELD: Strike that.

Q. If one sample had a high melting point impurity and it -- as opposed to another sample that did not have high melting point impurity, is it possible to have a higher melting point even if the overall purity is lower?

A. So it turns out that the way melting point depression works is that even if the additive or the impurity has

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a higher melting point itself, what it tends to do is to disrupt or disorganize the crystal structure of the -- of the primary component, to such an extent that ironically, even though it has a higher melting point by itself it can reduce and typically does reduce the melting point of the primary material.

So typically impurities do lower melting point.

Q. But you're not aware of any evidence in this case that that is necessarily true for treprostinil or treprostinil diethanolamine, correct?

MR. POLLACK: Objection to form.

A. I'm sorry, I don't understand your question.

Q. You didn't cite any documents or prior art that showed that a higher melting point was a higher purity with regard to the treprostinil or treprostinil diethanolamine, correct?

MR. POLLACK: Objection to

form.

A. I think that's a general teaching for one of skill would understand that the higher the melting point -- it may even be cited in some of the references that I supplied in some of these general textbooks, but I'm not positive. But in certainly any organic chemistry textbook, it would explain that the higher melting point the purer the sample is.

Assuming, of course, the same polymorph.

(Winkler Exhibit 13, copy of a Journal of Organic Chemistry paper entitled, "The Intramolecular Asymmetric Pauson-Khand Cyclization as a Novel and General Stereoselective Route to Benzidene Prostacyclins: Synthesis of UT-15 (Treprostinil)," [SteadyMed-Exhibit 1004], marked for identification, this date.)

THE WITNESS: Thank you.

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Q. You've been handed what's has been marked Exhibit 13 which is a copy of a Journal of Organic Chemistry paper entitled, "The intramolecular Asymmetric Pauson-Khand Cyclization as a Novel and General Stereoselective Route to Benzidene Prostacyclins: Synthesis of UT-15 (Treprostinil)."

Do you recognize this document?

A. Yes, I do.

Q. And is this one of the documents that you relied upon in your declaration?

A. Yes, it is.

Q. So if you turn to page 13, which is the last page in the document, and at the bottom of the left column it begins the final step in the synthesis for UT-15 compound 7. Do you see that? On the very last page?

A. I'm sorry, could you repeat that, please?

Q. Yes. If you could turn to the

very last page, 13?

A. Yes.

Q. And at the bottom of the left column, starts, the final step for the synthesis of UT-15?

A. Yes.

Q. Do you see that?

A. Yes, I do.

Q. And that continues on to the next column and at the bottom of the right column, right above "Acknowledgment," it says -- let's see. Purity 99.7 percent, I think you cite that number in your declaration?

A. Yes, I do.

Q. Now, do you know if that number refers to the total related impurities or the assay?

A. Well, the way it's described here, I think one of skill would assume, as I did, that this refers to the assay.

Q. Is there anything discussing the assay here?

A. No, there is not.

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Q. So it could be the total related impurities, correct?

MR. POLLACK: Objection to form.

A. Again, my assumption in reading this is that this is an assay for the treprostinil itself.

Q. But earlier today we saw several documents discussing the purity of the treprostinil in terms of the total related substances, correct?

A. Yes, we did.

Q. And so if one number is reporting total related substances and another is reporting assay, you can't really compare those numbers, right?

A. Well, I don't think that's true. I think what I can certainly compare here is that when I look at the 99.7, especially given the fact that there is no discussion of related impurities, I would presume here that this 99.7 refers to an HPLC assay of the treprostinil. I think that's what one of

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the skill would assume here.

Q. But there is also no discussion of assay, correct?

A. Excuse me.

MR. POLLACK: Objection to form. You can answer.

A. There is no discussion, I don't think that there is any discussion in the text of the assay conditions for the method that was used to establish purity.

So I assume it's just the area under of the curve for the treprostinil.

Q. So you assume it's that but you're not sure, right?

A. Well, like I said, in reading this, I would read this an HPLC assay.

(Winkler Exhibit 14, Japanese Patent Application 56-122328, [SteadyMed-Exhibit 1007], marked for identification, this date.)

Q. You've been handed what's been marked as Exhibit 14, which is a Japanese patent application 56-122328, which is

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referred to as the Kawakami reference.

Do you recognize this document?

A. Yes, I do.

Q. And is this a document that you relied on in your declaration?

A. Yes, it is.

Q. If you turn to page 4, at the top it says, "This reaction has an excellent yield but has a serious drawback of typically producing an unnecessary 7Z isomer as a byproduct," and then it cites a paper. Do you see that?

A. Yes, I do.

Q. And then it goes on to say, "In addition, the properties of the two are extremely similar for 7E, and 0.17 for 7Z; making separation and purification very difficult." Do you see that?

A. Yes, I do.

Q. Do you agree that it's typically difficult to separate and

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purify isomers?

A. I think that very much depends on the isomers.

Q. With respect to the prostacyclin and prostaglandin isomers?

A. I think it would be difficult for me make that kind of broad generalization. I think sometimes it's very difficult and sometimes it's quite straightforward.

Q. With respect to prostacyclin and prostaglandin --

A. Excuse me.

Q. -- isomers it's your opinion that it can be easy or it can be hard basically?

A. I think it depends on the isomers in question.

Q. It goes on to say that, "Also, the melting point of this compound is fairly low and crystallization is therefore severely impeded by the admixing of trace impurities." Do you see that?

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A. Yes, I do.

Q. So essentially it's saying that even trace impurities can greatly affect the outcome of the --

MR. DELAFIELD: Strike that.

Q. It's saying that even trace impurities can affect the overall purity in terms of crystallization, right?

A. I think what it's saying is that the crystallization is severely impeded by the admixing of trace impurities. That's how I read this.

THE WITNESS: Excuse me.

Q. So in some cases even trace impurities can be important to analyze and remove, correct?

A. I'm sorry, could you repeat that please?

Q. So in some cases even trace impurities need to be removed, correct?

A. I think all this teaches me is trace impurities can impede crystallization, in some cases, including the case that's shown here. That's the

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conclusion that I think a person of skill or a I, myself, would reach here.

THE WITNESS: Excuse me.

MR. DELAFIELD: Bless you.

Q. So this paper doesn't disclose treprostinil, correct?

A. Well, this paper describes a methanoprostacyclin derivative. So in other words, it's a compound that is replated to treprostinil.

Q. But none of the compounds have the three-ring structure that's formed treprostinil, correct?

A. None of the compounds in this paper or in this patent I think -- that's actually not true.

There are some compounds that are described in this patent that have as many as five rings.

But the compound that is shown in the -- in this scheme, compounds two and two prime, I think has shown each have two rings.

Some of the compounds under

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formula I, for example, on page 5 can have five rings, as I count them, depending on the nature of R-1.

Q. So let me rephrase.

None of the compounds disclosed in this paper has three fused rings; is that more accurate?

A. I think it is true that none of the compounds have three fused rings, although I should say add that the notion of taking carboxylic acid, preparing the amine salt as a purification technique and then regenerating the acid is not something that I would necessarily think would be dependent on the number of rings in the structure in terms of its efficacy, because I've seen this procedure used on any of a number of compounds with any of a number of different rings.

Q. But you agree that the paper doesn't disclose any structures of three fused rings, correct?

A. Excuse me. I agree that the

paper does not disclose any structures with three fused rings.

Q. Now, the separation procedure described is in reference to separating Z and E isomers, correct?

A. That is correct.

Q. And it's not in reference to separating stereoisomers, correct?

A. That is not correct.

Q. Why is that?

A. Because enantiomers are stereoisomers.

Q. Stereoisomers require a chiral center, correct?

A. No, that is not true.

Q. So this paper does not include separation of diastereomers, correct?

A. Actually compound 2 and compound 2 prime by the definition that I use in teaching stereochemistry, and that I've been using for, for over 30 years, two and two prime are in fact diastereomeric compounds.

Q. Do two and two prime have

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different chiral centers?

A. Two and two prime do not have different chiral centers or different stereo centers is the terminology that we typically use now.

Q. So to clarify, this paper doesn't describe the separation of compounds with different stereo centers, correct?

A. The paper does not describe the separation of compounds with different stereo centers, but it does describe the separation of diastereoisomers or stereoisomers, more general.

Q. And the only difference there is whether it's E or Z at the double bond at the top of the ring, correct?

A. That is correct.

MR. POLLACK: Would this be a good time for a break?

MR. DELAFIELD: Getting close. So just a little bit longer.

THE WITNESS: A break would be

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great.

MR. POLLACK: Do you want a break?

MR. DELAFIELD: Okay.

MR. POLLACK: We've been going over an hour and a half.

THE VIDEOGRAPHER: The time is 3:48 p.m. and we're off the record.

(A brief recess was taken.)

THE VIDEOGRAPHER: This begins media unit number 4. The time is 4:03 p.m., and we're back on the record.

Q. I just have a couple more questions. If you could turn back to your CV, Exhibit 1.

A. It's got to be here somewhere. Yep.

Q. On the first page, I was just curious what is the "Philadelphia Organic Chemists Club"?

A. The Philadelphia Organic Chemists Club is a consortium of organic chemists in the Delaware Valley area. So

it's been in existence for a long time and it -- like I said, it's basically a consortium of the pharmaceutical companies in the area and academics in the area.

Q. And what was the award for?

A. The award was in recognition of my research accomplishments.

MR. DELAFIELD: All right. I don't have any more questions.

MR. POLLACK: I have a very short redirect.

EXAMINATION BY

MR. POLLACK:

Q. If you could pull out Winkler deposition Exhibit 13, and that's the document also known as SteadyMed Exhibit 1004.

A. Yes.

Q. And if you could turn to page 13?

A. Yes.

Q. Are there any -- is there any information stated here regarding how the

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purity of 99.7 percent was determined?

A. Well, at the end of the experimental procedure for the preparation of UT-15, that is compound 7 in Moriarty, starting about three quarters of the way down, the paragraph on the right side of page 13, you can see that after the C13 MMR data, it describes a UV Landham Acts for the compound, as well as HPLC information.

And so it actually indicates the column that was used and the dimensions of the column. It indicates the flow rate that was used. And it also indicates the mobile phases, mobile phase A and mobile phase B, as well as the retention time of the treprostinil, treprostinil under these conditions.

Q. So what does that tell us about how the 99.7 percent was determined?

A. So that simply tells us what conditions they were using in the HPLC to determine this purity.

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Q. And what equipment, what piece of equipment was used to determine this purity?

A. Well, sir, this is indicating that the purity was determined by HPLC.

Q. Okay. And is that, what does that tell you about whether or not it's from assay or related impurities, what does that tell you about what method was used?

MR. DELAFIELD: Objection to form.

A. Well, I think this would indicate to me that, as I had indicated before, that the purity is being determined by the HPLC assay method.

MR. POLLACK: No further questions.

MR. DELAFIELD: I have one quick question, following up on that.

EXAMINATION BY

MR. DELAFIELD:

Q. If you recall we discussed two

ways purities were determined in the documents we looked at; the assay and the total related substances. Do you recall that?

A. Yes, I do.

Q. And you also recall that HPLC was used for both of those determinations, correct?

A. Yes, I do.

MR. DELAFIELD: Okay. I have no further questions.

THE VIDEOGRAPHER: The time is 4:07 p.m., June 14, 2016, and this completes today's video deposition of Jeffrey D. Winkler.

(Time noted: 4:07 p.m.)

STATE OF _____)

) :ss

COUNTY OF _____)

I, JEFFREY D. WINKLER, Ph.D., the witness herein, having read the foregoing testimony of the pages of this deposition, do hereby certify it to be a true and correct transcript, subject to the corrections, if any, shown on the attached page.

_____

JEFFREY D. WINKLER, Ph.D.

Sworn and subscribed to before me,

this _____ day of _____, 2016.

_____

Notary Public

C E R T I F I C A T E

STATE OF NEW YORK)

: ss.

COUNTY OF NEW YORK)

I, Jennifer Ocampo-Guzman, a Notary Public within and for the State of New York, do hereby certify:

That JEFFREY D. WINKLER, Ph.D., the witness whose deposition is hereinbefore set forth, was duly sworn and that such deposition is a true record of the testimony given by the witness.

I further certify that I am not related to any of the parties to this action by blood or marriage, and that I am in no way interested in the outcome of this matter.

IN WITNESS WHEREOF, I have hereunto set my hand this 15th day of June 2016.

J. Ocampo-Guzman

JENNIFER OCAMPO-GUZMAN, CRR, CLR

----------I N D E X ----------

---------- EXHIBITS ----------

EXHIBITS (Continued):

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Please read your deposition over carefully and make any necessary corrections. You should state the reason in the appropriate space on the errata sheet for any corrections that are made.

After doing so, please sign the errata sheet and date it.

You are signing same subject to the changes you have noted on the errata sheet, which will be attached to your deposition.

It is imperative that you return the original errata sheet to the deposing attorney within thirty (30) days of receipt of the deposition transcript by you. If you fail to do so, the deposition transcript may be deemed to be accurate and may be used in court.

E R R A T A

I wish to make the following changes, for the following reasons:

PAGE LINE

___ ___ CHANGE:_____

REASON:_____

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REASON:_____

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WITNESS' SIGNATURE DATE

ERRATA

I wish to make the following changes, for the following reasons:

Page, Line	Change	Reason
P10, line 15	delete "the"	Transcription error
P12, line 4	Add a semicolon after career	Transcription error
P13, line 21	"T-H-R-E-O" instead of "30, P-H-R-E-O."	Transcription error
P59, line 16	Replace "0.7%" with "0.07%"	Transcription error
P75, line 3	"Samples" should be "examples"	Transcription error
P88, line 3	"essentially auspicious" should be "essentially suspicious"	Transcription error
P98, line 13	"CV is did he staled at" should be "CV as is stated is"	Transcription error
P112, line 15	"perform" should be "form"	Transcription error
P120, line 18	"compliments" should be "components"	Transcription error
P120, line 25	"affect" should be "effect"	Transcription error
P126, line 24	should read "carboxylate salt, purifying that salt, and"	Transcription error
P127, line 12	there should be a comma after "acids"	Transcription error
P130, line 10	should read "if the API is" instead of "if the API"	Transcription error
P135, line 23	should read "reactants and products" instead of "reactants in products."	Transcription error
P159, line 3	"chem" should be "10"	Transcription error
P161, line 3	"starting tip" should read "starting materials"	Transcription error
P162, line 20	"of formula" should be "a formula"	Transcription error
P166, line 4	"synthetic re" should be "synthetic route"	Transcription error
P173, line 8	"pulse" should be "plus"	Transcription error
P188, line 24	"it's pure" should be "as pure"	Transcription error
P201, line 11	"replated" should be "related"	Transcription error
P203, line 12	"enantiomers" should be "diastereomers"	Transcription error
P207, line 10	"Landham Acts" should be "lambda max"	Transcription error

Jeffrey D. Winkler, Ph.D.

6-27-16
Date

Electronic Acknowledgement Receipt	
EFS ID:	26384888
Application Number:	14754932
International Application Number:	
Confirmation Number:	1865
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN2
First Named Inventor/Applicant Name:	Hitesh Batra
Customer Number:	22428
Filer:	Stephen Bradford Maebius/Karen Strawderman
Filer Authorized By:	Stephen Bradford Maebius
Attorney Docket Number:	080618-1550
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	NtfRltdProc.pdf	51558 1ef31532b7238db2377e386e6d77b183823 09a13	no	2

Warnings:

Information:					
2	Miscellaneous Incoming Letter	5-12-2016PublicInstitutionofIPR.pdf	1598383 2ba7afcfd5fe312bc9a184844d1168e5597b8576	no	53
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3	Miscellaneous Incoming Letter	7-13-2016PublicPatentOwnerResponsetoPetition.pdf	343563 2c0e7f3990fb732195bd9542442cf0eb65000ff9	no	57
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4	Miscellaneous Incoming Letter	Exhibit2020Public.pdf	350553 ef25158b9db44265e9fe4ac6e347b626f61d5b87	no	51
Warnings:					
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5	Miscellaneous Incoming Letter	Exhibit2021.pdf	1918078 89bcdd1c343dd9da762d4f932a4ed900dfc519e1	no	66
Warnings:					
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6	Miscellaneous Incoming Letter	Exhibit2022Public.pdf	302275 b610b11cd326f237ee1eaa823b974628cea3e8a8	no	38
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7	Miscellaneous Incoming Letter	Exhibit2023.pdf	362433 fed56009926bfcd5f04585549d4e344dae1fcdc6	no	115
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8	Miscellaneous Incoming Letter	Exhibit2024.pdf	300540 7635b7ef9c7bb7b5b138d683a6ca92fc4c88a563	no	36
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9	Miscellaneous Incoming Letter	Exhibit2025.pdf	14915056 658e62f4392c3ce7829f0852268458bfd1a4bb98	no	321
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10	Miscellaneous Incoming Letter	Exhibit2026.pdf	848305 131645095a3048c80080c1c6d9459402c0edd7a7	no	7
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11	Miscellaneous Incoming Letter	Exhibit2027.pdf	2676065 6f621789bd1066a569fbd7be2f02c5aea5d1de4b	no	23
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12	Miscellaneous Incoming Letter	Exhibit2028.pdf	826416 b39b1edf0626ce67ec9bb8e76fcb6fea7e455b0d	no	7
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13	Miscellaneous Incoming Letter	Exhibit2029.pdf	636267 de0d387dc5e8691a295db465819e7540dad50357	no	3
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14	Miscellaneous Incoming Letter	Exhibit2030.pdf	1444440 cbe4c905c2174ef7ee42ebc533e49aad79a8b3f7	no	8
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15	Miscellaneous Incoming Letter	Exhibit2031.pdf	2746950 2a2eca3df32e815ae106514a2c7eb650989c31a4	no	48
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Information:					

16	Miscellaneous Incoming Letter	Exhibit2032.pdf	6840224 db75f32e67720cecdbf4136cd291b961eaf6a8aa	no	54
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Information:					
17	Miscellaneous Incoming Letter	Exhibit2033.pdf	1117112 a6662d7651d43e990e796f68e2986204608afc1a	no	4
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Information:					
18	Miscellaneous Incoming Letter	Exhibit2034.pdf	7162174 3055f0a7fb890db0cff35764c15bb90be1f9115a	no	21
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Information:					
19	Miscellaneous Incoming Letter	Exhibit2035.pdf	1360069 451030d799e5076d21375ba21abe59c20323a924	no	33
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20	Miscellaneous Incoming Letter	Exhibit2036.pdf	5714806 db00914e2e8b4068ad7904567c0fd2fbddec7d5f	no	101
Warnings:					
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21	Miscellaneous Incoming Letter	Exhibit2037Public.pdf	1024843 fb1fa2245d36e4eac71106b30743412d60d8f490	no	242
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22	Miscellaneous Incoming Letter	Exhibit2038.pdf	4046157 2adecee4750ec89d7d8c44c5bd0e32903adc6639	no	15
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23	Miscellaneous Incoming Letter	Exhibit2039.pdf	12621894 f3a995802842e64c6fdab62efe7e795371b840c7	no	35
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Information:					
24	Miscellaneous Incoming Letter	Exhibit2040.pdf	980401 d7124e82672f1dd2346313737004aaa0ccfc44d8	no	55
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Information:					
25	Miscellaneous Incoming Letter	Exhibit2041.pdf	142352 163977e4fd8894c3ef6a4291ea2657b04a815228	no	2
Warnings:					
Information:					
26	Miscellaneous Incoming Letter	Exhibit2042.pdf	656999 094a62e5493e160f313e219b99c38e17ba3d71ef	no	12
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Information:					
27	Miscellaneous Incoming Letter	Exhibit2043.pdf	724511 0c6dc25a557e9211d90d61f41bb1c66e4e3990bb	no	79
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28	Miscellaneous Incoming Letter	Exhibit2044.pdf	294789 ac5ec9dccbd0a18afaa64eb87c0c9423d4d98082	no	57
Warnings:					
Information:					
29	Miscellaneous Incoming Letter	Exhibit2045.pdf	592600 3abc4a27155c793a78338a394c44a51df052f135	no	6
Warnings:					
Information:					

30	Miscellaneous Incoming Letter	Exhibit2046.pdf	11352305 b02393cda85b724bc52d252eed2d7aeced8e0ee1	no	141
Warnings:					
Information:					
31	Miscellaneous Incoming Letter	Exhibit2047.pdf	174986 7b527064c151bc2ebca7a2cd67667c625ca03d0f	no	10
Warnings:					
Information:					
32	Miscellaneous Incoming Letter	Exhibit2048.pdf	2472507 93f7ac702a57809979414 3720bee2e73ab0ad22d	no	11
Warnings:					
Information:					
33	Miscellaneous Incoming Letter	Exhibit2049.pdf	119520 8555471eea86f29c0521e63125c5fb0564980d9b	no	5
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34	Miscellaneous Incoming Letter	Exhibit2050.pdf	266854 8d641261fc163bf59fd4e15262b75c702f0e556f	no	40
Warnings:					
Information:					
35	Miscellaneous Incoming Letter	Exhibit2051Public.pdf	691756 d1365d532c194d2011ea5181259d24519b13f482	no	247
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Date Submitted: FEB 29 2016

(use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/754932
Filing Date	6/30/2015
First Named Inventor	Hitesh BATRA
Art Unit	1672
Examiner Name	Yevgeny Valenrod
Attorney Docket Number	080618-1550

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	C1	2001/0038855 A1	11/08/2001	Desjardin et al.	
	C2	2001/0056095 A1	12/27/2001	Mylari	
	C3	4,434,164 A	02/28/1984	Lombardino	
	C4	5,466,713 A	11/14/1995	Blitstein-Willinger et al.	
	C5	5,506,265 A	04/09/1996	Blitstein-Willinger	
	C6	6,706,283 B1	03/16/2004	Appel et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Documents	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	C7	WO 98/18452 A1	05/07/1998	Shire Laboratories, Inc.		

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	C8	BIGHLEY et al., "Salt Forms of Drugs and Absorption," Encyclopedia of Pharmaceutical Technology, Swarbrick et al., Eds., 1995, 13:453-499.	
	C9	SIMONNEAU et al., "Continuous Subcutaneous Infusion of Treprostinil, a Prostacyclin Analogue, in Patients with Pulmonary Arterial Hypertension," Am. J. Respir. Crit. Care Med., 2002, 165:800-804.	

Examiner Signature		Date Considered	

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ENCYCLOPEDIA OF PHARMACEUTICAL TECHNOLOGY

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PRESERVATION OF PHARMACEUTICAL PRODUCTS TO SALT FORMS OF DRUGS AND ABSORPTION

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Salt Forms of Drugs and Absorption

Introduction

Salt formation is frequently performed on weak acidic or basic drugs because it is a relatively simple chemical manipulation which may alter the physicochemical, formulation, biopharmaceutical, and therapeutic properties of a drug without modifying the basic chemical structure. Salt selection has been largely semi-empirical, based on consideration of cost of raw materials, yield, ease of preparation and purification, etc. Although attempts have been made to apply "decision analysis" and "potential problem analysis" to select salts and help predict salt performance [1], the choice of which salt to use remains a difficult decision.

The ideal characteristics of a salt are that it is chemically stable, not hygroscopic, presents no processing problems, dissolves quickly from solid dosage forms (unless it is formed with the intent to delay dissolution), and exhibits good bioavailability.

The literature contains a large amount of information on salts; however, much of the early research addresses the use of salt formation to prolong the release of the active component, thereby eliminating various undesirable drug properties[2–6]. This article supplements an extensive review published in 1977 [7], providing a literature overview of approximately 40–45 years. Its objectives are to present potentially useful salts, their effect on the properties of the parent drug, and a decision tree for choosing the most desirable salt form(s) for development.

Potentially Useful Salts

Salt formation is one of the simplest chemical reactions, involving either a proton transfer or a neutralization reaction between an acid and a base. The relative strength of the acid or base, or the acidity and basicity constants of the species involved, significantly influences the occurrence of the reaction and provides a measure of the stability of the resulting salt. Theoretically, every compound possessing acidic and/or basic properties can participate in salt formation.

Salt forms that have been clinically evaluated in humans or were commercially marketed through 1993 are shown in Tables 1 and 2, compiled from the drug monographs listed in *Martindale, The Extra Pharmacopoeia*, 30th ed. [8]. Table 1 gives all anionic salt forms, Table 2 all cationic forms. The relative frequency (as a percentage) of use for each salt type was calculated based on the total number of anionic or cationic salts used through 1993.

The monoprotic hydrochlorides are by far the most frequent choice of an anionic salt-forming radical, probably for physiological reasons and simple availability. For similar reasons, sodium is the most predominate cation. These findings are identical to those reported in a similar survey [7] from 1977, even though they are based on twice the number of salts as the earlier study. Other comparisons between this and the previous review show an increase of approximately 40% in the types of anionic salts and approxi-

TABLE 1 Anionic Pharmaceutical Salt Forms Currently in Use

Anion	Percent[a]	Anion	Percent[a]	Anion	Percent[a]
Aceglumate	0.07	Formate	0.07	Nicotinate	0.13
Acephyllinate (7-Theophyllineacetate)	0.26	Fosfatex (Metaphosphate)	0.07	Nitrate	1.18
Acetamidobenzoate	0.07	Fumarate	0.92	Oleate	0.13
Acetate	2.09	Gluceptate (Glucoheptonate)	0.13	Orotate	0.26
Acetylasparaginate	0.07	Gluconate	0.52	Oxalate	0.26
Acetylaspartate	0.07	Glucuronate	0.13	Oxoglurate	0.13
Adipate	0.13	Glutamate	0.07	Pamoate (Embonate)	1.37
Aminosalicylate	0.13	Glycerophosphate	0.52	Pantothenate	0.07
Anhydromethylenecitrate	0.07	Glycinate	0.13	Pectinate	0.07
Ascorbate	0.13	Glycollylarsinilate (*p*-Glycollamidophenylarsonate)	0.07	Phenylethylbarbiturate	0.13
Aspartate	0.33	Glycyrrhizate	0.07	Phosphate	2.48
Benzoate	0.20	Hippurate	0.07	Picrate	0.07
Besylate (Benzenesulfonate)	0.26	Hemisulfate	0.13	Policrilix (Methacrylic acid polymer)	0.07
Bicarbonate	0.07	Hexylresorcinate	0.07	Polistirex[b]	0.85
Bisulfate	0.13	Hybenzate *o*-(4-Hydroxybenzyl)benzoate	0.20	Polygalacturonate	0.07
Biartrate	0.52	Hydrobromide	1.37	Propionate	0.20
Borate	0.26	Hydrochloride	43.99	Pyridoxylphosphate	0.13
Bromide	3.79	Hydroiodide	0.07	Saccharinate	0.20
Butylbromide	0.07	Hydroxybenzenesulfonate	0.07	Salicylate	0.78
Camphorate	0.01	Hydroxybenzoate	0.07	Stearate	0.20
Camsylate (Camphorsulfonate)	0.59	Hydroxynaphthoate	0.07	Stearylsulfate	0.07

Butylbromide	0.07	Hydroxybenzenesulfonate	0.07	Stearate	0.20
Camphorate	0.01	Hydroxybenzoate	0.07	Stearylsulfate	0.07
Camsylate (Camphorsulfonate)	0.59,	Hydroxynaphthoate	0.07		
Carbonate	0.46	Iodide	1.11	Subacetate	0.07
Chloride	3.53	Isethionate (2-Hydroxyethanesulfonate)	0.52	Succinate	0.52
Chlorophenoxyacetate	0.07	Lactate	0.98	Sulfate	5.82
Citrate	2.81	Lactobionate	0.07	Sulfosalicylate	0.07
Closylate (4-Chlorobenzenesulfonate)	0.07	Lysine	0.65	Tannate	0.85
Cromesilate (6,7-Dihydroxycoumarin-4-methanesulfonate)	0.07	Malate	0.26	Tartrate	2.68
Cyclamate	0.13	Maleate	3.14	Teprosilate[c]	0.07
Dehydrocholate	0.07	Mandalate	0.13	Terephthalate	0.07
Dihydrochloride	1.37	Mesylate	3.20	Teoclate (8-Chlorotheophyllinate)	0.33
Dimalonate	0.07	Methylbromide	0.39	Thiocyanate	0.20
Edetate	0.07	Methyliodide	0.20	Tidiacicate (Thiazolidine-2,4-dicarboxylate)	0.07
Edisylate (1,2-Ethanedisulfonate)	0.20	Methylnitrate	0.13	Timonacicate (Thiazolidine-4-carboxylate)	0.07
Esolate (Lauryl sulfate)	0.13	Methylsulfate	0.98	Tosylate (Toluene-4-sulfonate)	0.39
Esylate (Ethanesulfonate)	0.13	Monophosadenine (Adenylic acid)	0.07	Triethiodide	0.07
Ethylbromide	0.07	Mucate	0.07	Undecanoate	0.13
Ethylsulfate	0.07	Napadisylate	0.13	Xinafoate	0.07
Fendizoate (Hydroxyphenylbenzoylbenzoate)	0.07	Napsylate (1,5-Naphthalenedisulfonate)	0.20	(1-Hydroxyl-naphthoate)	

[a]Percent is based on total number of anionic salts in use through 1993.
[b]Sulfonated diethenylbenzene-ethenylbenzene copolymer complex.
[c]1,2,3,6-Tetrahydro-1,3-dimethyl-2,6-dioxopurine-7-propanesulfate.

TABLE 2 Cationic Pharmaceutical Salt Forms Currently in Use

Organic Cation	Percent[a]	Metallic Cation	Percent[a]
Ammonium	1.95	Aluminum	1.35
Benethamine (*N*-Benzylphenethylamine)	0.15	Bismuth	0.30
Benzathine (*N,N'*-Dibenzylethylenediamine)	0.45	Calcium	12.18
Betaine ((Carboxymethyl)trimethylammonium hydroxide)	0.15	Lithium	0.90
Carnitine	0.15	Magnesium	4.51
Clemizole[b]	0.15	Neodymium	0.15
Chlorcyclizine 1-(4-Chorobenzyhydryl)-4-methylpiperazine)	0.15	Potassium	9.77
Choline	0.60	Rubidium	0.15
Dibenzylamine	0.15	Sodium	57.74
Diethanolamine	0.45	Strontium	0.30
Diethylamine	0.60	Zinc	1.05
Diethylammonium	0.15		
Eglumine (*N*-Ethylglucamine)	0.15		
Erbumine (*t*-Butylamine)	0.15		
Ethylenediamine	0.15		
Heptaminol (6-Amino-2-methylheptan-2-ol)	0.15		
Hydrabamine (*N,N'*-Di(dihydroabietyl)ethylenediamine	0.15		
Hydroxyethylpyrrolidone	0.15		
Imidazole	0.30		
Meglumine (*N*-Methylglucamine)	2.41		
Olamine	0.45		
Piperazine	0.90		
4-Phenylcyclohexylamine	0.51		
Procaine	0.15		
Pyridoxine	0.15		
Triethanolamine	0.15		
Tromethamine (Tris(hydroxymethyl)aminomethane)	0.90		

[a]Percent based on total number of cationic salts in use through 1993.
[b]1-*p*-Chlorobenzyl-2-pyrrolidin-1'-ylmethylbenzimidazole

mately 80% in the types of cationic salts in use. This may be indicative of a trend to modify or optimize the properties of a substance through salt formation as opposed to more complex molecular modifications. In addition, the interest in polymer–drug salts for controlling drug release is indicated by the appearance of polistirex and policrilix salts.

It is well documented that due to differences in physical, chemical, and thermodynamic properties imparted by the salt-forming species, various salts of the same com-

pound often behave differently. Knowledge that a particular salt form imparts enhanced water solubility, reduced toxicity, or slow dissolution rate to a drug molecule greatly benefits chemists and formulators. Although some generalizations such as the statement by Miller and Heller on water solubility associated with carboxylic acid salts [9] can be followed, drug use and history frequently dictate the salt form selected. For example, most contrast agents requiring water solubility are meglumine salts, whereas many of the newer therapeutic peptides (i.e., buserelin, nafarelin, octreotide) are acetates. Many of the antibiotics administered intravenously are sodium salts. This indicates that the drug class, history of use and local tolerance, and possibly regulatory acceptability influence the selection of the salt form.

Both pamoic acid and alginic acid have been shown to prolong action by forming slightly soluble salts with certain basic drugs. The incorporation of pamoate salts in sustained-release preparations has been reviewed by Saias et al. [10]; numerous examples can be found in the literature [11–16]. Alginic acid salts of streptomycin [17] and pilocarpine [18] have been prepared and shown to provide sustained action.

A unique way of prolonging action through salt formation was demonstrated by Malek and co-workers [19]. Utilizing the knowledge that macromolecules have an affinity for the lymphatic system, salts of four antibiotics were prepared with high-molecular-weight polyacrylic acids, sulfonic or phosphorylated polysaccharides, and polyuronic derivatives. Parenteral administration of these macromolecular salts produced low antibiotic blood levels for long periods while lymph levels were high. Since lymphatic circulation is slow, the preferential distribution of the antibiotics to the lymphatic system prolonged the passage through the body.

The lauric acid salt of propranolol was studied as an alternative to polymeric formulations for sustaining the release of propranolol HCl. The findings indicated that the laurate salt increased the bioavailability. This was attributed to micellar solubilization or ion-pairing which could lead to lymphatic absorption or lower efficiency of extraction by the liver [20].

Toxicity is reduced by choosing the appropriate salt form; two different strategies have been utilitzed to accomplish this. One is based on organic radicals that occur naturally and are readily excreted or metabolized. Using this approach, salts formed with choline [21–23], amino acids [24,25], and vitamins [24, 26–32] have been prepared that exhibit lower toxicity and fewer side effects than the parent molecule or other salts. The second strategy is to select a salt component that pharmacologically overcomes an unfavorable property or properties of the principal agent. Salts incorporating *N*-cyclohexylsulfamic acid, better known as cyclamates, can make bitter-tasting drugs acceptable because of their characteristic sweet taste. Cyclamate salts of dextromethorphan and chlorpheniramine [33] raise the bitterness thresholds compared to commonly occurring salts. The preparation and characterization of other cyclamic acid salts have been reported [34–37].

Other examples include the preparation of the benzhydralamine salt of penicillin [38] and the 8-substituted theophylline salts of several antihistamines [39–42]. Benzhydralamine is an antihistamine. The preparation of the benzhydralamine salt of penicillin was an attempt to produce a repository form of penicillin with antiallergic properties. The synthesis of the xanthine salts of several antihistamines was an attempt to counteract the drowsiness caused by the antihistamines with the stimulant properties of the xanthines. A number of other 8-substituted theophyllines have been prepared [21, 43–49].

A quinidine salt with reduced toxicity has been prepared from polygalacturonic acid, a derivative of pectin [50,51]. This substance possesses special demulcent properties and inhibits mucosal irritation. It is used to reduce the shock to the gastrointestinal (GI) mucosa resulting from the liberation of irritating ions caused by the rapid dissociation of the conventional inorganic quinidine salts. Quinidine polygalacturonate is one-fourth as toxic orally as the sulfate.

The *N*-(2-hydroxyethyl)pyrrolidine salt of diclofenac (DHEP) was prepared as part of a study to obtain salts with balanced hydrophilic and hydrophobic properties [52]. Of the 24 salts synthesized, DHEP had the greatest solubility in both water and octanol. In addition, it exhibited surfactant properties and the ability to solubilize lipid materials above its critical micelle concentrations. These properties suggest that this salt is preferable to topical administration since it could promote its own absorption by interacting with the membrane components. Other compounds reported to be potentially useful as pharmaceutical salt forms are shown in Table 3.

Physicochemical Studies

Although different salts of the same drug elicit similar biological responses, the intensities of response may differ markedly [96,97]. A knowledge of the physicochemical properties of a salt and its influence on pharmacokinetics is necessary to understand the onset, duration, and intensity of action, relative toxicity, and possible routes of administration [2]. The influence of salt form on volatility and hygroscopicity has been investigated in preformulation studies [98].

Solubility

Solubility is an important factor in chemical stability, the formation of dosage forms, and the overall drug-absorption process.

Common-Ion Effect

Hydrochloride salts are the most common anionic salt-forming species [7]. However, they do not necessarily enhance the solubility of poorly soluble basic drugs in a chloride-containing medium because of the common-ion effect which suppresses the solubility product equilibrium [99–105]. In some instances, the solubility of various hydrochlorides was less than that of the corresponding free base at gastric pH. The practical effect of reducing solubility could ultimately be a reduction of the dissolution rate in gastric juice. The Setschenow salting-out constants for chloride are highest for these slightly soluble hydrochlorides [106]. However, the relationship between the aqueous solubility of sparingly soluble salts and the empirical Setschenow salting-out constant is valid only at low concentrations of added salt [107].

Prazosin is an example of a drug with a strong chloride-ion dependence. The hydrochloride salt in water has a solubility of 1.4 mg/mL at 30°C, whereas in 0.1M HCl it is 0.037 mg/mL [108].

A common-ion effect on the sodium salt of an organic acid has also been reported [109]. The solubility and dissolution rates decreased with varying sodium ion concen-

TABLE 3 Potentially Useful Salt Forms of Pharmaceutical Agents

Salt-Forming Agent	Compound Modified	Property Modified	Reference
p-Acetamidobenzoic acid	Various amines	Hygroscopicity	53
Acetylaminoacetic acid	Doxycycline	Solubility	54
N-Acetyl-L-asparagine	Erythromycin	Solubility, activity, stability	55
N-Acetylcystine	Doxycycline	Combined effect useful in pneumonia	56
Adamantoic acid	Alkylbiguanides	Prolonged action	57
Adipic acid	Piperazine	Stability, toxicity, organoleptic properties	58
N-Alkylsulfamates	Ampicillin	Absorption (oral)	59
	Lincomycin	Solubility	60
Anthraquinone-1,5-disulfonic acid	Cephalexin	Stability, absorption	61
Arabogalactan sulfate (arabino)	Various alkaloids	Prolonged action	62,63
Arginine	Cephalosporin	Toxicity	64
	Sulfobenzylpenicillin	Stability, hygroscopicity, toxicity	65
Aspartate	Erthromycin	Solubility	66
Betaine	Tetracycline	Gastric absorption	67
Bis(2-carboxychromon-5-yloxy)alkanes	7-Aminoalkyltheophyllines	Activity, prolonged prophylactic effect	68
Carnitine	Metformin	Toxicity	69
4-Chloro-*m*-toluenesulfonic acid	Propoxyphene	Organoleptic properties	70
Decanoate	Heptaminol	Proplonged action	71
Diacetyl sulfate	Thiamine	Stability, hygroscopicity	72
Dibenzylethylenediamine	Ampicillin	Prolonged action	73,74
Diethylamine	Cephalosporins	Reduced pain on injection	75
Diguiacyl phosphate	Tetracycline	Activity	76
Dioctyl succinate	Vincamine	Organoleptic properties	77
Embonic (pamoic) acid	Kanamycin	Toxicity	78
	2-Phenyl-3-methylmorpholine	Toxicity	79
Fructose-1,6-diphosphoric acid	Tetracycline	Solubility	80
	Erthromycin	Solubility	80
Glucose-1-phosphoric acid,	Tetracycline	Solubility	80
Glucose-6-phosphoric acid	Erythromycin	Solubility	80
L-Glutamine	Erythromycin	Solubility, activity, stability	55
Hydroxynaphthoate	Bephenium	Toxicity	81
2-(4-Imidazolyl)ethylamine	Prostaglandin	Prolonged action	82
Isobutanolamine	Theophylline	Stability	83
Lauryl sulfate	Vincamine	Organoleptic properties	84
Lysine	Sulfobenzylpenicillin	Toxicity, stability, hygroscopicity	65
	Cephalosporin		64
Methanesulfonic acid	Pralidoxime (2-PAM)	Solubility	85
N-Methylglucamine	Sulfobenzylpenicillin	Toxicity, stability, hygroscopicity	65

TABLE 3 *(Continued)*

Salt-Forming Agent	Compound Modified	Property Modified	Reference
	Cephalosporins	Reduced pain on injection	75
N-Methylpiperazine	Phenylbutazone	Toxicity, faster onset of action	86
Morpholine	Cephalosporins	Reduced pain on injection	75
2-Naphthalenesulfonic acid	Propoxyphene	Organoleptic properties	87
Octanoate	Heptaminol	Prolonged action	71
Probenicid	Pivampicillin	Organoleptic properties	88
Tannic acid	Various amines	Prolonged action	89,90
Theobromine acetic acid	Propoxyphene	Activity	91
3,4,5-Trimethoxybenzoate	Tetracycline	Organoleptic properties	92
	Heptaminol	Prolonged action	71
Tromethamine	Aspirin	Absorption (oral)	93
	Dinoprost (prostaglandin F)	Physical state	94
Xinafoate	Salmeterol	Local tolerance	95

trations. The reduction in solubility product in the presence of NaCl was attributed to a decrease in the degree of self-association of the drug in aqueous media.

Formulation

The choice of salt can have significant benefits for the formulation of a drug as, for example, with the cytotoxic drug, coralyne sulfoacetate. The solubility of coralyne chloride in water is 4.5 mg/mL, and that of the sulfoacetate is 6.5 mg/mL; however, solutions containing 25 mg/mL were required for iv infusion [110,111]. The solubility of the chloride salt was no higher in weakly alkaline aqueous media than in distilled water since it is a salt of a quaternary ammonium ion and the conjugate base of a strong acid. Adding sodium hydroxide greatly enhanced the solubility of the sulfoacetate. The reason is that the sulfoacetate anion is an acid which is ionized by the added base, resulting in an increase in the concentration of coralynium ion in solution.

The solubility of a salt can influence the use of formulation adjuvants. In the presence of methanesulfonic, acetic, and hydrochloric acids, 2,3,4,5-tetrahydro-8-(methylsulfonyl)-1-H-3-benzazepin-7-ol had water solubilities of approximately 440, 320, and 1 mg/mL. Addition of sodium chloride to a saturated solution of the mesylate (methanesulfonic) salt, reduced the solubility to approximately 60 mg/mL, even with a sodium chloride concentration as low as 0.05 M. This was probably due to the rapid conversion of the mesylate to the hydrochloride salt and may preclude the use of sodium chloride as an isoosmotic agent or the use of saline as diluent [103].

In addition to its effect on solubility, the choice of salt is important to the usefulness and efficacy of the formulation. For example, hydrochloride salts in aqueous solution may lower the pH, which can adversely affect their use in parenteral dosage forms because of the incidence of pain and subsequent venous inflammation [112]. It could also lead to incompatibilities with metal aerosol containers [108].

Sciarra et al.[113], using epinephrine as a model compound in an aerosol delivery system, points out that the solubility of the salt form in various propellants is important in products intended for local action in the lungs or for systemic therapy; furthermore, the salt form should be soluble in extracellular fluids.

Complex Salt Formation

Organic acid salt forms of basic drugs, such as amines, frequently have higher aqueous solubilities than their corresponding inorganic salts. Hydrochloric, nitric, sulfuric, and phosphoric salts of triamterine form insoluble complex salts [114]. Acetic acid produced solubilities higher than those observed with any of the inorganic acids. Although acetic acid complexes with triamterine, an insoluble complex was not found. This is important in the synthesis and selection of a salt form that exhibits enhanced bioavailability and desirable formulation characteristics.

Studies have been conducted on the complexation of some drugs with sodium polyphosphate [115]. Insoluble complex salts formed with amethocaine, amitriptyline, propranolol, and verapamil, but not with atropine, ephedrine, and procaine. The complex salt formed with verapamil produced a prolonged dissolution profile in acid compared to pure verapamil, but because of hygroscopicity it was difficult to process and store.

The solubility also of organic carboxylic acids is also affected by salt formation, in some cases adversely. For example, *N*-[4-(1,4-benzodioxan-6-yl)-2-thiazolyl] oxamic acid was less soluble in the presence of sodium, potassium, and calcium ions. However, these ions increased the distribution coefficients significantly between water and 1-octanol, even at low concentrations. The lower solubility was attributed to the formation of less soluble salts, whereas the increase in distribution coefficients was explained by ion-pairing and/or complexation [116].

Solubility Predictions

The solubility of a salt can be influenced by the structure of the organic moiety or by the hydrophilic properties of the anion or cation. A higher crystal lattice energy (crystallinity) is generally reflected by a higher melting point. An increase in melting point, usually by maximizing or encouraging crystal symmetry, reduces solubility. Gould [108] reports that the solubility of a drug frequently decreases by an order of magnitude with an increase of 100°C in its melting point. Where solubility and resultant pH are major issues, a low melting salt of a drug produced from a soluble, fairly weak acid or base, probably made in situ, is usually preferred.

The increase or decrease in melting point of a series of salts of basic compounds depends on the controlling effect of crystallinity from the conjugate anion. This is exemplified by an experimental drug candidate, UK47880, which has a basic pKa of 8 [108]. Salts prepared from planar, high melting aromatic sulfonic or hydroxycarboxylic acids yield high melting crystalline salts. However, flexible aliphatic acids such as citric and dodecylbenzene sulfonic yielded oils. Gould [108] discussed how crystal lattice forces of drugs with good hydrogen bonding potential could be built up by considering the symmetry and hydrogen bonding potential of the conjugate acid. He used epinephrine as an example, which gives high melting salts with small, strongly hydrogen-bonding acids like malonic and maleic. The larger bitartrate and presumably symmetrically unfavored fumarate give lower melting salts.

Various salts of α-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol [102], chlorhexidine [117], erythromycin [118], and the *N*-alkylsulfamates of lincomycin [60] show enhanced solubility which can be attributed to a lower melting point and the hydrophilic properties of the anion. Organic salts may increase aqueous solubility through decreased crystal lattice energy, lowered melting point, increased hydrogen bonding of the salt counterions with water, etc.

There are exceptions to the solubility–melting point and solubility–hydrophilicity relationship. For example, the THAM, tris(hydroxymethyl)aminomethane, salts of certain analgesic-anti-inflammatory agents showed no simple solubility–melting point relationship [119]. Anderson and Condradi, using organic amine salts of flurbiprofen to predict water solubility, found a strong dependence of the solubility product on melting point; however, there was no significant correlation between solubility product and counterion hydrophilicity [120]. The authors concluded that this is in conflict with the notion that higher salt solubilities can be achieved by selecting more hydrophilic counterions, since such arguments neglect the likelihood that interactions in the crystal become stronger as the salt-forming species are made increasingly polar.

Rubino [121] found that the logarithms of the molar solubilities of a number of sodium salts of drugs were inversely related to their melting points, but a good correlation was not evident. However, the logarithms of the molar solubilities were inversely related to both the melting points and stoichiometric amounts of water in the crystal hydrates, but unrelated to the polarity of the corresponding acid forms of the drugs. It was concluded therefore that the melting point and the degree of crystal hydration of the solid phase are most important in determining the solubilities of the sodium salts of some drugs.

The solubilities of the sodium salts of some weakly acid drugs have been determined in mixtures of propylene glycol and water. The solubility in the mixed solvent of compounds with low temperatures of desolvation had increased, whereas the solubility of compounds with high desolvation temperatures had decreased. These data indicate that crystal hydrate formation plays a significant role in determining if a cosolvent can be used to enhance the solubilities of certain sodium salts [122].

The hydrogen ion concentration can significantly affect salt solubility. Anderson [123] discussed the influence of pH on the solubility of therapeutically useful weak acids and bases and their salts. This was followed a few years later by an extensive study on the solubility interrelationships of the hydrochloride and free base of two amines [124]. Mathematical equations describing the total solubility at an arbitrary pH in terms of the independent solubilities of the hydrochloride and free base species and the dissociation constant of the salt were derived and fitted to experimental data with good results. This report made the point that, although the solubility of an amine hydrochloride generally sets the maximum obtainable concentration for a given amine, the solubility of the free base and the pKa determine the maximum pH at which formulation as a solution is possible. This assumes that the desired concentration exceeds the free base solubility. Shifting the pH–solubility profile to higher pH values for formulation purposes may require increasing the solubility of the free base with the help of an appropriate cosolvent. Because the dissociation characteristics of carboxylic acids and other organic species are similar to those of organic hydrochlorides, the pH–solubility profiles could be characterized theoretically by the same treatment.

Chowhan [125] studied the solubilities of three organic carboxylic acids (naproxen; 7-methylsulfinyl-2-xanthonecarboxylic acid; and 7-methythio-2-xanthonecarboxylic acid) and their sodium, potassium, calcium, and magnesium salts as a function of pH. The

data were fitted to mathematical relationships similar to those used by Kramer and Flynn [124]. The results on the solubility of naproxen and its salts were in excellent agreement with theory. The solubilities of the two xanthone carboxylic acids and their salts were higher at higher pH than the values calculated for complete dissociation in solution.

Surface Activity

The salts of some compounds are surface active [126–128]. If the saturation solubility enables the critical micelle concentration (CMC) to be reached, solubility is enhanced significantly via micellar solubilization. A study of the colloidal properties of some chlorhexidine salts showed that the counterion can affect the CMC which was usually associated with a change in micellar size [126]. For example, the diacetate displays a higher CMC than the digluconate [126].

The hydrochloride salt of 2-butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone is capable of forming micelles. Anions such as chloride, sulfate, acetate, tartrate, and citrate significantly affect the equilibrium solubility of the compound, which is partly related to the effect on the CMC by the anionic environment [127].

The nonopioid kappa agonist analgesic amine, DuP 747, as the hydrochloride salt, exerts surface activity in aqueous solutions; however, the critical micellar concentration is not reached at the saturation solubility [128]. On the other hand, the methanesulfonate salt formed a micellar solution and allowed for a solubility of 60 mg/mL as opposed to 3 mg/mL for the hydrochloride.

Zomepirac, an insoluble, carboxylic, non-narcotic analgesic, has a solubility in water of 0.02 mg/mL. In a developing zomepirac solution containing 100 mg/mL [129], THAM was found to be a satisfactory solubilizer at a concentration where equivalent concentrations of sodium or potassium hydroxide were not. The solubility was achieved by a micellar mechanism. It is interesting that potassium hydroxide was more effective in solubilizing zomepirac than sodium hydroxide. Walkling et al. attributed the difference in their performance as solubilizers to the difference in their charge densities [129]. Additional references on the relationship of salt form and solubility are listed in Table 4.

Dissolution Rate

In many cases, the dissolution rate can be a good indicator of bioavailability, especially of poorly soluble drugs. A salt form frequently exhibits a higher dissolution rate than the corresponding conjugate acid or base at the same pH, even though they may have the same equilibrium solubility. In a review article on the biopharmaceutical basis for drug design, Nelson [150,151], and later Benet [152], referred to the self-buffering action of the salt form in the diffusion layer. The dissolution rates are determined by the pH values of the diffusion layer and are independent of the pH_{bulk} of the media used. Therefore, the difference in diffusion-layer pH between a parent compound and its salt accounts for the difference in the dissolution rates in a particular medium.

Effect of Salt Form

Nelson, using theophylline salts, was the first to show the correlation between diffusion-layer pH and dissolution rate [150]. Salts with a high diffusion-layer pH had higher

TABLE 4 References on Salt Form and Solubility

Topic	Reference
Mineral acid salts of lidocaine	130
Nonionic surfactant effect on rate of release of drugs from suppositories	131
Influence of solubility of salicyclic acid on diffusion from ointment bases	132
Influence of solubility on rate of GI absorption of aspirin	133
Effect of dosage form on GI absorption rate of salicylates	134
Physical-chemical properties of polyene macrolide esters and their water-soluble salts	135
Isolation and reaction products of orotic acid and amines and their water solubility	136
Solubility and stability of erythromycin salts	137
Pharmaceutical preparations of orotic acid; water-soluble properties of orotic acid salts	138
Solubility of antibiotics in 24 solvents	139,140
Solubility of antibiotics in 26 solvents	141
Aqueous stability and solubility of CI-988, a novel dipeptoid cholecystokinin-B receptor antagonist	142
Quaternary ammonium salts of dantrolene and clodanolene	143
Acetylacroninium salts as soluble prodrugs of the antineoplastic agent acronine	144
In vitro release characteristics of a membrane-coated pellet formulation of metroprolol salts, influence of drug solubility and particle size	145
Physical properties and solubility of different salts of fenoprofen	146
pH-Solubility profile of papaverine hydrochloride and its relationship to the dissolution rate of sustained-release pellets	147
pH-Solubility profiles of organic bases and their hydrochloride salts	148
Synthesis and properties of benzathine and embonate salts of some beta-lactam antibiotics	149

in vitro dissolution rates than those with a lower diffusion-layer pH. These salts effectively act as their own buffer to alter the pH of the diffusion layer and increase the apparent solubility of the parent compound in that layer. The rank order of dissolution rates correlated well with blood levels for these salts. Similar findings have been reported for other compounds [151,153–157].

These studies lay to rest the misconception that absorption is related only to solubility in the appropriate medium. They show that solubility affects absorption only to the extent that it affects dissolution rate, since dissolution is the preceeding process.

Although salt formation generally increases the dissolution rate, formulations of some salts may actually slow dissolution and absorption due to the precipitation of insoluble particles or film on the surface of the tablet [158–163]. This reduces the effective surface area and prevents deaggregation of the particles.

The deaggregation behavior of a relatively insoluble benzoic acid derivative and its sodium salt was postulated to be a possible rate-limiting step in the absorption of the drug [164]. Although no direct comparisons of the two forms were made, inspection of the data shows that the deaggregation of the salt was considerably more rapid than that of the free acid in equivalent dosage forms. Therefore, if absorption is dependent on the dissolution rate, which in turn is dependent on the deaggregation rate, the salt should produce the highest and earliest blood levels. On the other hand, it is possible

that hygroscopic (and deliquescent) salts absorb atmospheric moisture, cause a sticky surface, and inhibit deaggregation.

Effect of Formulation

Tablet processing and formulation factors can reduce the dissolution rate of a salt more than that of the nonionized form in human gastric juice and 0.1N HCl [165]. Granulation and tableting lower the dissolution rate of phenobarbital sodium, but have the opposite effect on phenobarbital. This was attributed to the fact that sodium phenobarbital tablets do not disintegrate in acidic media, but swell and dissolve slowly from the surface. The phenobarbital tablets, however, disintegrate very rapidly in acidic media. In some instances, rapid dissolution may be a problem even with very soluble drugs.

Common-Ion Effect

The common-ion effect can significantly alter the dissolution rate of a drug. Hydrochloride salts frequently have a dissolution rate lower than the nonionized form in chloride-containing media [99,166,167]. This is due to the solubility product equilibria strongly affecting the dissolution of these salts. In these cases, an alternative salt form or a less soluble free base may improve dissolution and bioavailability [167]. Because of the common-ion effect, absorption following administration of salts of basic drugs, and especially the hydrochloride salt, is probably dependent on stomach emptying rather than in vivo dissolution. Additional references on the influence of salts and salt forms on dissolution rate are listed in Table 5.

Organoleptic Properties

With a drug administered as a tablet or capsule and swallowed as an intact unit, taste is less of a problem. Taste acceptability is a primary consideration in the formulation of a liquid, chewable tablet, or lozenge. The examples outlined below are approaches to make drugs organoleptically acceptable. The most recent applications of taste masking in oral pharmaceuticals are reviewed in Ref. 193.

Poorly Soluble Salts

An example of the preparation of poorly soluble salts to minimize undesirable organoleptic properties is the formulation of erythromycin estolate (lauryl sulfate) [194] in an oral suspension. The level of bitterness of various salts of erythromycin is related to the size of the alkyl group attached to the acid and to the stability of the salt which is a function of the strength of the acid used to prepare the salt [118].

A similar solution has been applied to bacitracin by using the relatively insoluble zinc salt [195], whose taste is easily masked by sucrose [196].

A different approach is to form water-insoluble salts with ion-exchange resins. The practical application has been described and tested by several investigators [198–201].

Common-Ion Effect

In another method a poorly water soluble salt is treated with a common ion to further reduce solubility. The taste of propoxyphene napsylate suspensions can be improved

TABLE 5 References on Salt Form and Dissolution Rate

Topic	Reference
Effect of dissolution rate on absorption, metabolism, and pharmacologic activity of drugs	168
Scientific principles in design of drug dosage formulations	169
Dissolution rate of mixtures of weak acids and tribasic sodium phosphate	170
Physiological availability and in vitro dissolution characteristics of some solid dosage formulations of aminosalicylic acid and its salts	171
Biopharmaceutics, rate of dissolution (chronological bibliography)	172
Biopharmaceutics, rate of dissolution in vitro and in vivo	173
Dissolution tests and interpretation of anomalies observed in the dissolution process of sulfaquinoxaline based on salt formation	174
Influence of the dissolution rate of lithium tablets on side effects	175
Dissolution kinetics of drugs in human gastric juice	176
Comparison of dissolution and absorption rates of different commercial aspirin tablets	177
In vitro dissolution rates of aminorex dosage forms and their correlation with in vitro availability	178
Polymer-drug salts as an approach to physicochemical design of dosage forms	179
Release of acidic drugs from anionic exchange resinate complexes	180
Sustained-release microcapsules containing ion exchange resin-dextromethorphan HBr complex	181
Ion exchange resins as matrices for controlled drug release	182
Influence of polymeric excipients on drug release from hydroxypropylmethylcellulose matrixes	183
Effect of surfactants on release of a highly water-soluble compound from an inert, heterogeneous matrix	184
Effect of diffusion-layer pH and solubility on the dissolution rate of pharmaceutical bases and their HCl salts	185
Preparation and in vitro evaluation of salts of an antihypertensive agent to obtain slow release	186
Some factors influencing in vitro release of phenytoin from formulations	187
Effect of diffusion-layer pH and solubility on the dissolution rate of pharmaceutical acids and their sodium salts	188
Dissolution behavior of diclofenac salts with hydrophilic bases	189
Dissolution studies on naproxen and its sodium and piperazine salts	190
Dissolution profiles of ibuprofen, fenbufen, and their sodium salts	191
Relationship between intrinsic dissolution rates and rates of water absorption	192

significantly by adding a common ion like sodium or calcium napsylate to depress solubility even more [197].

Soluble Salts

The taste of a drug may be improved by formation of a soluble salt. However, solubilization does not always improve the taste. For example, potassium salts frequently have

Reference

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an unpleasant taste and leave a metallic aftertaste. A study of inorganic potassium salts showed similar taste thresholds in water, but varying degrees of palatability at therapeutic potassium concentrations. Although flavored vehicles and sucrose improved palatability, all potassium salts exhibited poor taste [202]. Some success has been reported using cyclamate sodium and saccharin. *N*-Cyclohexylsulfamate salts of several drugs have improved taste and enhanced solubility properties [33,34]. Conventional quaternary ammonium compounds have a very bitter taste, but benzalkonium saccharinate and a series of saccharinates of other quaternary ammonium compounds are sweet [203]; water solubility of the saccharinate anlogs differs greatly.

Stability

The chemical and physical stability of a pharmaceutical can significantly affect the choice of dosage form, manufacturing technique, packaging, and therapeutic efficacy of the final preparation. In some cases the salt-forming radical itself may enhance stability of the parent compound or contribute to its instability.

Hygroscopicity

The stability of the drug in the dry state can be influenced by differences in hygroscopicity of the salts. Hydrochloride salts, as well as some sulfates, and especially dihydrochlorides or disulfates, are very polar in nature. The polar ionized groups exposed on the crystal surfaces result in a highly hydrophilic nature which favors wettability and can lead to hygroscopicity [108,204]. This may present processing difficulties or reduced stability if the drugs are easily hydrolyzed. To improve drug stability through salt formation, hygroscopicity has to be controlled and the strength of the conjugate acid used to form the salt considered, especially with compacted dosage forms [108]. This is very important where the available moisture is shared by the salt and excipient, and when most of the moisture comes from the excipient rather than the drug. Salts of mineral acids, which produce a low pH and a high solubility in the available moisture, produce a more hostile environment than a sulfonate or carboxylate salt. Obviously, a balance between hygroscopicity and wettability must be struck to avoid interference with the bioavailability of the compound.

Moisture associated with certain salts of weak bases can be very acidic and potentially cause stability problems related to hygroscopicity, aqueous solubility, and resulting pH. The mononitrate salts of thiamine and various vitamin B complex formulations are less hygroscopic and much less water soluble than the hydrochloride salts [205–209]. The stabilities of numerous thiamine salts were studied in aqueous solution and in dry powder preparations with various excipients [210,211]. In aqueous solution, the resulting pH was the chief factor controlling hydrolysis and oxidative decomposition. The stability of powder preparations was related to their aqueous solubility, with sparingly soluble salts being more stable and presumably less hygroscopic.

The THAM salt had superior hygroscopic properties compared to the sodium salt for naproxen, ketorolac, RS-7337, and RS-82917 [119]. With the exception of naproxen, THAM salts did not have lower aqueous solubility or intrinsic dissolution rate than the sodium salt. The less hygroscopic THAM salts offer handling advantages in formulation and storage.

A study of the effect of moisture on the stability of penicillin salts found the calcium salt to be less hygroscopic than the sodium salt, and, therefore, more stable in moist atmospheres [212]. Penicillin G potassium is much less hygroscopic than penicillin G sodium and is the preferred form for marketing in the dry state [213].

Salt stability also depends on the hydrophobic portion of the conjugate acid [214]. The aryl sulfonic acids protect xilobam which is easily hydrolyzed. The aryl groups present a hydrophobic barrier to minimize hygroscopicity and dissolution in the surface moisture. The napsylate salt is the most stable.

Disproportionation

Dihydrohalide salts of pharmaceutical compounds may undergo facile dissociation/disproportionation of HCl or HBr, leading to release of hydrohalide gas or reaction with excipients or process-related chemicals. In one study [166], a difference in strength of the basic centers in a dihydrochloride salt led to a loss of one hydrogen chloride molecule by release of hydrogen chloride gas. Dihydrochloride or dihydrobromide salts of pharmaceuticals have been reported to cause in the reduction of dimethyl sulfoxide to dimethyl sulfide under mild conditions [215]. In this study, no reactions were observed with the monohydrohalide salts.

A hydrochloride salt was reported to cause in rusting of tooling material used for tablet manufacturing [216]; hydrogen chloride liberated from the salt was the cause.

Thermal Stability

Lincomycin cyclamate exhibits enhanced thermal stability over the hydrochloride salt [35]. Differential thermal and thermogravimetric analyses showed that the hydrochloride undergoes thermal degradation, whereas the cyclamate anion is considerably more stable [217].

Similar differences in thermal stability are seen with penicillin G salts. Penicillin G procaine is stable in aqueous vehicles, but has less thermal stability than the sodium or potassium salts, and decomposes about 60°C. The sodium and potassium salts withstand heating up to 100°C for four days with little loss in potency [218]. These discrepancies may be due to differences in melting points among the salts.

Crystallinity

As Guillory and Higuchi [219] point out, the stability of organic compounds in the solid state is intimately related to the strength of the crystal lattice. Because the intermolecular forces in a crystal are small compared with the energy necessary to break chemical bonds, liquifaction occurs before degradation begins. Therefore, the melting point of a compound may be an important factor in determining stability. In general, an increase in melting point, usually by maximizing or encouraging crystal symmetry, improves stability, particularly if salt formation results in a crystalline solid [108,219].

Degradation of solid drugs usually occurs in the surface film phase and is accomplished by the formation of a liquid phase at temperatures below the normal melting point of the solid. Guillory and Higuchi [219] investigated the stability of esters and determined the relationship between degradation rate and melting point. Gould [108]

has postulated that this approach may have utility as a a method of assessing the bulk stability of nonhygroscopic salt forms.

Solubility

Low solubility can contribute significantly to the stability of a salt since it reduces the amount of drug in solution and hence its degradation. Low solubility is especially important in developing a stable aqueous suspension of a hydrolytically unstable water-soluble drug. This has been extensively studied with penicillin G and its salts because of the drug's therapeutic importance and its characteristic instability [220–223]. The 8-chlorotheophylline salt of penicillin was reported to be water soluble, yet stable in solution [224]. Inasmuch as it is acidic, it has been postulated that its stability is due to a buffer effect.

Polymorphic Forms

A study of the physical and chemical stability of the free acid and the salts of novobiocin showed that the amorphous calcium salt is the choice for the formulation of a liquid preparation [225]. The sodium salt is unstable in liquid form and the crystalline free acid is not absorbed from the GI tract. Amorphous novobiocin is metastable in solution and slowly converts to the unabsorbed crystalline form.

Hydrate Formation

If a compound readily forms a hydrate, production of an amorphous solid does not necessarily increase the dissolution rate and stability may be impaired. This was illustrated with ethacrynic acid and sodium ethacrynate [226]. The stability of both the amorphous ethacrynic acid and amorphous sodium ethacrynate was lower than that of crystalline samples or hydrated sodium ethacrynate. As expected, dissolution of the amorphous ball-milled ethacrynic acid was more rapid than that of the crystalline material. Dissolution of the amorphous sodium ethacrynate, however, was slower than that of its crystalline counterpart. The explanation offered was that the amorphous sample of sodium ethacrynate crystallized as the hydrate in the presence of the dissolution medium.

Stability of the water of hydration is important since the stability of salt hydrates is known to change with the counterion. Hirsch et al. [227] found that the dihydrate of both the sodium and the calcium salt of fenoprofen had suitable physical characteristics with respect to crystallinity and appreciable water solubility. However, attempts to formulate fenoprofen sodium dihydrate with either propoxyphene salts (hydrochloride or napsylate) or codeine salts (sulfate or phosphate) failed. The combinations were chemically and physically incompatible since eutectic melts and/or acid–base reactions resulted in all instances. The fenoprofen calcium dihydrate, on the other hand, was compatible. These differences are postulated to be due to the integrity of the bound water of hydration of the calcium salt compared to that of the sodium salt.

The effect of counterion charge on crystal structure and the hydration–dehydration behavior of the potassium, sodium, calcium, and magnesium salts of *p*-aminosalicylic acid was studied [228]. Within the series, the propensity to form hydrates increased with increasing ionic potential of the cations. This was evident from the increase in the

number of moles of water associated with the salts as the ionic radius decreased and the charge on the cation increased. The divalent salts had higher threshold temperatures of water loss due to stronger ion–dipole interactions because of short bond distances. The sodium salt had a lower threshold temperature of water loss due to weak ion–dipole interactions because of larger bond lengths and its more open structure. Hydrate stability is important since processing operations such as drying or compaction could potentially remove water of crystallization or cause a mixture of hydrated and dehydrated forms to exist within formulations. Hydrate stability can also affect formulation and drug combination compatibility as the fenoprofen salts demonstrated.

Changes in crystal form may affect the physical and/or chemical stability of tableted dosage forms. For instance, Yamaoka et al. [229] observed cracking of tablets of carbochromen HCl as an anhydrate changed to a hydrate under high-humidity conditions.

Oral Absorption

Drugs that are administered orally and are sensitive to acid environments benefit from forming salts which are poorly soluble in acidic solution. Generally, they must pass intact through the acidic environment of the stomach in order to exhibit therapeutic effect. Erythromycin estolate, because of its low solubility in gastric juice, is more stable than the free base, therefore it can be administered with food [194].

An interesting example of selecting a proper salt of a compound (RS-82856) displaying both weak acid and weak base characteristics was presented by Gu et al.[230]. This compound has a very high pKa (11.2) as an acid and a very low pKa (3.5) as a base, which gives a solubility minimum between pH 4 and 10. Their goal was to maximize drug absorption while achieving good physical and chemical stability. Because of the weak acid-base properties of the parent compound, only very strong bases and acids could be used to make physically stable salts. The phosphate salt was not physically stable. The hydrogen sulfate and chloride salts were less hygroscopic and more soluble in water than the sodium and potassium salts. All salts studied showed only slightly better intrinsic dissolution rates (approximately twofold) than the parent drug at pH 3.1 and 7.0; only the hydrogen sulfate and potassium salts had better dissolution rates at pH 1.2. The dissolution behavior was explained as being due to the extremely low buffering effect of the salts in the dissolution medium. The hydrogen sulfate salt was recommended for development and was supported by a twofold increase in absorption over the parent drug in a dog study. Gu et al. [230], suggested that since the buffering effect of the sodium and potassium salts of a weak acid-base would be expected to increase the pH of the medium and thus reduce solubility, anion salts of weak acid-base drugs are preferred to be developed. Additional references on the influence of salts on stability are included in Table 6.

Pharmaceutical Technology

The salt form of a drug may have a pronounced effect on the formulation of the parent compound, inasmuch as it influences the melting point, hygroscopicity, and the strength of the conjugate acid used to form the salt.

TABLE 6 References on Salt Form and Stability

Topic	Reference
Stability of chlorhexidine solutions	231
Stability of autoclaved chlorhexidine	232
Anhydrotetracycline and 4-epianhydrotetracycline in commercial tetracycline and aged tetracycline products	233
Physicochemical studies of the stability of penicillin salts	234
Light sensitivity of tetracyclines	235
Hygroscopic properties, thermostability, and solubility of oleandomycin salts	236
Stability of orotic acid and its amine salts in aqueous solution	237
Factors influencing the stability of aspirin tablets	238
Stability of aqueous solutions of sodium aminosalicylate	239
Hygroscopic properties of various preparations of erythromycin	240
Physicochemical studies on the decomposition of aminosalicylic acid and its salts	241
Stabilities of aqueous solutions of 2-diethylaminoethyl-3-methyl-2-phenylvalerate HCl and methobromide	242
Investigation of properties of penicillin G salts	243
Stability of ferrous iron tablets on storage	244
Stability of aspirin aluminum compounded with antacids	245
Dissociation and stability of insulin zinc and sodium insulin oligomers by bile salt micelles	246
Thermal stability of various epinephrine formulations	247
Forms of *p*-aminosalicylic acid and its salts in pharmaceutical practice	248

Melting Point

The melting point plays a crucial role in the comminution and tableting of drugs [108]. Because low-melting compounds tend to be plastic rather than brittle, they comminute poorly and frictional heating causes melting and deposition of the drug on the screens and pins of the mill causing "blinding." Therefore it may be difficult to obtain a free-flowing powder.

The melting point can have important implications for particle bonding during tablet compression. On compression, bonding occurs by point welding at the deformed or fragmented particle surfaces. At a certain temperature and pressure, a low-melting material would be expected to have better bonding. The pressure on the powder and the eutectics formed with other excipients can depress the melting point further. Gould [108] lists the melting points and heats of fusion of salts of an experimental drug candidate and suggests that the low melting point and heat of fusion for the mesylate salt would make it the most suitable candidate for bonding reasons of the salts studied for a direct compression tablet. Since the melting points of compounds are reduced under pressure, the solubility of salt forms would be expected to increase with increasing pressure. This can potentially cause the formation of solutions of the salts in the film of adsorbed moisture on the surface of the drug and excipient particles which may have an effect on drug bonding [249].

Hygroscopicity and Strength of Conjugate Acid

The effect of hygroscopicity and the strength of the conjugate acid is especially important for compacted dosage forms where most of the available moisture comes from the excipient rather than the drug. Salts of mineral acids have a lower pH and higher solubility in the available moisture and have a tendency to be less stable than the sulfonate or carboxylate[108].

Other Properties

The salt form can influence other physicochemical properties of a drug substance. The effect of the salt-forming radical on surface tension, ion-pair extraction, solubility, and zwitterion flux have been studied.

Surface Activity

The influence of the anion on the absorption of dextromethorphan and tetracycline in the rat stomach was studied [250,251]. A linear relationship existed between the rate of absorption from buffer solutions of anions being investigated and their surface tensions. Therefore, the absorption process was attributed to the surface activity of the various salts and not to their lipid solubilities. These results are similar to those reported on the surface activity of various phenothiazine salts [252].

Ion Pairing

Higuchi and co-workers extensively studied the physicochemical basis of the ion-pair extraction of pharmaceutical amines [253,254]. They found that the distribution ratios between an organic layer and water were highly dependent on the concentration and nature of anion present. Less hydrophilic anions yielded more readily extractable ion pairs. The entropy change associated with transfer of the different anions between phases is the mean controlling factor in the extraction process [255].

Zwitterions

Salts of zwitterions increase the solubility in both polar and nonpolar media as well as the flux in skin and membranes [256,257]. The rules for counterion selection followed the covalency of the salt formed and the solubilities of the salt in both the solvent and the membrane.

Biopharmaceutical Studies

The amount of drug absorbed from a dosage form and the onset, duration, and intensity of action depend on the physicochemical properties of the drug. These differences are due primarily to differences in amount and rate of absorption. Administration of a drug in salt form can be frequently used to alter the solubility and dissolution rate in order to increase bioavailability.

Influences on Bioavailability

Disintegration

The effect of formulation on the bioavailability of warfarin sodium was compared with that of warfarin [160,258]. Absorption from a lactose-base tablet was no better with warfarin sodium than with warfarin free acid. With starch in place of lactose, salt absorption was even worse, in spite of the fact that the in vitro water dissolution rate for warfarin sodium is 350 times that of warfarin free acid; yet, the latter exhibited rapid and complete absorption in vivo. The primary factor accounting for this apparent anomaly is that a strongly acidic medium is necessary for tablet disintegration. Following initial exposure to 0.1N HCl, in vitro dissolution of the warfarin tablet in pH 7.4 buffer was 14 times faster than that of the sodium salt, a result that explained the otherwise contradictory in vivo blood level. Therefore, absorption was ultimately dependent upon gastric emptying rate and gastric pH, as long as the formulation distintegrated properly in the stomach.

Common-Ion Effect

Hydrochloride salt formation does not necessarily enhance the bioavailability of basic drugs due to the common-ion effect. This is illustrated in studies comparing the absorption of the tetracyclines with that of their salts [156,251,259–262] or salts of lincomycin [263]. Administration of the free base of several tetracyclines resulted in higher plasma levels than after administration of the hydrochloride salt. Similar to reports for the tetracyclines, the hydrochloride salt of lincomycin did not produce as great an area under the curve as the hexadecylsulfamate salt. It is likely that the differences in bioavailability are due to differences in solubility at gastric pH because of the common-ion effect. It can be postulated that stomach emptying rather than in vivo dissolution is the rate-limiting factor in absorption. This may be a plausible explanation, since subcutaneous administration of the various lincomycin salts did not produce significantly different fractions absorbed, regardless of which salt was administered.

Ion Pairing

The absorption of drugs across membranes is usually attributed to the unionized form [264]. Many drugs are only weakly acidic or basic and a large fraction of the drug exists in the unionized form at some pH found in the GI tract. However, some drugs are sufficiently strong acids or bases so as to be largely ionized throughout the GI tract. An example of this is proxicromil [265], which, however, is reported to be reasonably well absorbed. The authors indicate that proxicromil possesses intrinsic lipophilicity and requires only charge neutralization to partition into lipoidal phases. It was suggested that ion-pair formation with naturally abundant cations such as sodium plays a significant role in the absorption of proxicromil.

The increase in lipophilicity and the transport of various cationic drugs across an artificial lipid membrane [266], rabbit skin [267], or human skin [268] has been attributed to ion-pair formation with the carboxylate anion of oleic, lauric, or myristic acids.

The absorption characteristics of an ionized dianionic drug was claimed to be altered upon ion association with a quaternary ammonium salt [269]. Using a human

buccal absorption test, approximately a tenfold increase in the uptake of cromoglycate ion in the presence of alkylbenzyldimethylammonium chlorides was reported compared with uptake of the drug alone.

Propranolol laurate was investigated for the possibility of using a fatty acid salt as an alternative to polymeric formulations in a sustained-release preparation [20]. Studies in dogs showed an increase in bioavailability over the immediate release or sustained-release formulations of propranolol HCl. Explanations offered for the improved bioavailability include association of the drug with a fatty acid, micellar solubilization, or ion-pair association.

Ion-pair absorption of drugs has been reviewed [270]. The authors conclude that ion-pair absorption may occur, but that the literature on the subject is controversial.

Bioavailability

The bioavailability in rats of magnesium and calcium salts of indomethacin was compared with that of indomethacin [271]. The mean plasma levels after a single oral dose of the salts were significantly higher and the area under the plasma curve after multiple oral dosing of the salts was significantly larger than after administration of indomethacin free acid. There was no significant change in plasma protein binding between the two groups of rats. The increased absorption was attributed to enhanced lipid solubility and increased solubility in bile and intestinal juice.

Aerosolized pentamidine isoethionate is retained in the lung and appears to prevent *Pneumocystis carinii* pneumonia in many AIDS patients; in an attempt to find salts that would reduce the airway irritation, the gluconate and lactate salts were also prepared [272]. More than 50% of pentamidine, aerosolized as the three different salts, is retained in the lung for at least two weeks after a single dose, and each salt produced a high lung-to-extrapulmonary drug ratio. None of the three pentamidine salts produced histological evidence of organ toxicity even when aerosolized daily for two weeks at very high doses.

In a comparison study of ampicillin sodium and potassium with ampicillin trihydrate [273], the absorption rate constants were higher for the salt forms, but the overall bioavailability was unaffected. This indicates that the dissolution of the ampicillin trihydrate was the rate-limiting step in its absorption.

Salt forms of a compound may influence the kinetics of drugs which exhibit nonlinear pharmacokinetics. For example, the area under the plasma concentration–time curve of unmetabolized drug from aminosalicylic acid administration was smaller than for salts of this compound [171,274–276]. This was attributed to concentration-dependent metabolism during absorption. At a high rate of absorption, the metabolic processes become saturated and more unmetabolized drug remains in the blood. At a low absorption rate, as for the free acid, a higher percentage of drug is metabolized.

The relative bioavailability of the vasodilator naftidrofuryl as the oxalate or citrate salt was studied [277]. The relative rate of absorption, but not the extent, was higher for the citrate salt than for the oxalate salt. The degree of intersubject variability was similar after administration of either compound.

Blood levels in rabbits were significantly higher during the 30 min to 2 h post-administration period after rectal administration of suppositories containing amino acid and choline salts of phenobarbital compared with those from a suppository of phenobarbital [278].

A significant amount of work has been done on the salts of theophylline [150,279–281], the penicillins [153,282–291], and ampicillin [292].

Throughout the years numerous studies have been, and continue to be, published on erythromycin and its salts and esters [293–300]. The pharmacokinetics and bioavailability of various erythromycin salts and dosage forms have been discussed [301], including the effects of clinical protocol and formulation differences on drug serum levels.

Steady-state bioavailability and day-to-day variability of plasma levels of metoprolol were evaluated in 18 subjects in a crossover study of a multiple unit (CR) and a single unit (OROS) delivery system [302]. The CR contained metoprolol succinate and the OROS an equal dose of metropolol fumarate. Although there were minor dissimilarities in the in vitro dissolution profiles, perhaps the result of different salts or delivery systems, both formulations were bioequivalent for C_{max} and area under the curve (AUC); however, the day-to-day variability of AUC was significantly lower for the CR formulation. The authors concluded that salt forms did not influence the variability, which was rather due to formulation-related differences. Additional references on the influence of salts on bioavailability are given in Table 7.

General Pharmacy

Pharmacological Effect

Calcium pectinate, the insoluble salt of pectin, can potentially be used as a colon-specific drug-delivery system compressed into tablets with insoluble drug [350]. Like pectin, calcium pectinate can be decomposed by specific pectinolytic enzymes in the colon, but retains its integrity in the small intestine.

The effects of chlorpromazine hydrochloride and quaternary chlorpromazine chloride on the central nervous system were examined [351]. The quaternized compound was less potent and more toxic to rodents than the parent tertiary compound.

A series of salts of 9-aminoacridine and its derivatives were screened for antifungal and antibacterial activity [352–354]. The antifungal action paralleled the length of the carbon chain of the anion. These results were attributed to the lipid solubility of the salts which would allow them to pass through the cell wall of the microorganism more readily, possibly as an ion pair.

The efficacy of bases or salts as topical anesthetics for relieving cutaneous itch, burning, and pain in unbroken skin was investigated [355]. Aqueous solutions of the salts did not alleviate itching or burning in any of the subjects; however, saturated solutions of their bases in a mixture of water, 40% alcohol, and 10% glycerol were claimed to be effective. Transport phenomena across the stratum corneum are often dependent on the polarity of the drug and vehicle and on binding of the drug to keratin. Additional references on pharmacological effects can be found in Table 8.

Other Studies

Numerous investigators [179,180,183,201,370–372] reported on the formation of polymer–drug salts or resinate–drug salts as an approach to the physicochemical design of dosage forms. These include organic carboxylic acids with anionic exchange resins

TABLE 7 References on Bioavailability

Topic	Reference
Comparison of the GI absorption of aluminum acetylsalicylate and acetylsalicylic acid	158
Unusual dissolution behavior due to film formation	159
Effect of salts on release of proflavine	303
Drug absorption of aspirin and derivatives from the rectum	304
Effects of various substances on the absorption of tetracycline in rats	305
Effects of dosage form upon the GI absorption rate of salicylates	134
Determination of in vivo and in vitro release of theophyllineaminoisobutanol in a prolonged-action system	306
Ion-exchange resin salts for oral therapy (carbinoxamine)	307
Latentiation of dihydrosteptomycin by pamoate formation	11
Solid-state ophthalmic dosage systems in effecting prolonged release of pilocarpine in the cul-de-sac	18
Absorption of erythromycin in various pharmaceutical forms	308
Comparative study of the absorption of drugs from old and new rectal preparations	309
Absorption of salts of streptomycin, neomycin, viomycin, and streptothrycin	19
Influence of salt on onset and duration of tolbutamide action	154
Blood levels produced by three theophylline-containing elixirs	310
Oral absorption characteristics of naproxen	311
Effect of food on absorption of a new form of erythromycin propionate	312
Anion effect on the absorption of tetracycline from the rat stomach	251
Blood levels following oral administration of different novobiocin preparations	313
Absorption of iopanoic acid and its sodium salt	314
Oral absorption of secobarbital (quinalbarbitone) and its sodium salt	315
Absorption rate of barbiturates in humans	316
Morphine and atropine mucate	317
Excretion of bephenium salts in urine of human volunteers	318
Antituberculosis activity of polymethylenebis(isothiuronium) salts	319
Prolonged antitussive action of a resin-bound noscapine preparation	320
Pharmacology of sulfapyridine and sulfathiazole	321
Evaluation of plasma concentrations of propoxyphene utilizing a hybrid-principal-component analysis of variance technique	322
Antrycide, a new trypanocidal drug	323
Pralidoximemethanesulfonate, plasma levels and pharmacokinetics after oral administration to humans	324
Intestinal absorption of pralidoxime and other aldoximes	325
Blood plasma levels and elimination of salts of pralidoxime (2-PAM) in humans after oral administration	326
Enhancement of GI absorption of a quaternary ammonium compound by trichloro-acetate	327
Relative bioavailability of phenoxymethylpenicillin preparations	328
Behavior of diclofenac salts in polar and poorly polar solvents	52
Sulfadiazine salts to reduce gingivitis	329
In vitro/in vivo evaluation of a liquid sustained-release dosage form of chlorpheniramine	330
Biliary excretion of erythomycin base and erythromycin estolate	331
Pharmacodynamics of fosfomycin after intravenous administration to humans	332
Pharmacodynamics of phosphonomycin after oral administration to humans	333
Comparative studies of distribution, excretion, and metabolism of ^{3}H-hydroxyzine and its ^{14}C-methiodide in rats	334

TABLE 7 *(continued)*

Reference	Topic	Reference
158	Pharmacokinetics of ampicillin trihydrate, ampicillin sodium, and dicloxacillin sodium following intramuscular injection	335
159	Physiological disposition of fenoprofen in humans, pharmacokinetic comparison of orally administered calcium and sodium salts	336
303	Pharmacokinetic comparison of two oral capsule formulations of ketoprofen–lysine salt	337
304	Bioavailability of fluoride in postmenopausal women	338
305	Pharmacokinetics of different formulations of ibuprofen and aspirin	339
134	Blood levels of isoetharine	340
306	Bioavailability of calcium from different salts	341
307	Relative bioavailability of the hydrochloride, sulfate, and ethyl carbonate salts of quinine	342
11	Absolute and relative bioavailability of lithium dosage forms in the beagle dog	343
18	Comparative bioavailability evaluation of erythromycin estolate capsules vs. enteric-coated erythromycin base tablets	344
308	Pharmacokinetics of quinidine in humans after intravenous, intramuscular, and oral administration	345
309	Intravenous pharmacokinetics and in vitro protein-binding studies of two new erythromycin salts	346
19	Comparative pharmacokinetics of zinc-65 sulfate and zinc-65 pantothenate injected intravenously in rabbits	347
154	Influence of first-pass effect on the systemic availability of propoxyphene	348
310	Rapidly dissolving tablets of theophylline	349
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[180,371] and basic drugs with sulfonic acid or carboxylic acid resinates [201]. The effects of various polymers and ion-exchange resins on drug release from hydroxypropylmethylcellulose (HPMC) [373], and polymer-drug salts [179,370,372] were studied. Most reports describe a retarded drug release because of the lower solubility of the drug–polymer or drug–resinate salt. Other papers reported on the coating of resinate–drug salts with different materials to determine the effect on the drug release rate [374–377]. Additional references on general pharmacy are given in Table 9.

Toxicological Considerations

The toxicity aspects of salts of a drug must include the pharmacological properties of the cation or anion used to form the salt as well as those of the free drug [388].

Toxicity of Salt Ion

Toxic reaction from ingestion of calcium salts of drugs is rare. If hypercalcemia occurs, calcium deposits in the kidney can cause a reduction of renal function. The principal toxic effects of lithium also involve the kidneys. Small amounts of lithium do not result in apparent damage; large amounts, however, can lead to irreversible damage.

TABLE 8 References on General Pharmacy and Pharmacological Effect

Topic	Reference
Morphine and atropine mucate	317
Naloxone mucate and morphine blockade in the mouse	356
Differential excretion of bromide and chloride ions and its role in bromide retention	357
Pharmacological study of calcium methionate	358
Synthesis and in vitro fungistatic evaluation of some N-substituted amide and amine salts of sorbic acid	359
Antiamebic studies on clamoxyquin in vitro and in experimentally infected animals	360
Adjunctive value of oral prophylaxis with the oximes pralidoxime lactate and prolidoxime methanesulfonate to therapeutic administration of atropine in dogs poisoned by inhaled sarin vapor	361
Pralidoxime methanesulfonate and atropine in the treatment of severe organophosphate poisoning	362
Efficacy and limitations of oxime-atropine treatment of organophosphorus anticholinesterase poisoning	363
Antitussive activity of enoxolone and its derivatives	364
Pharmacological properties of glycyrrhetinic acid hydrogen succinate (disodium salt)	365
Ganglionic blocking activity of diastereomeric dimethylaminobornyl acetates and their methiodides	366
A new potent nonnarcotic antitussive, 1-methyl-3-[bis(2-thienyl)methylene]piperidine; pharmacology and clinical efficacy	367
Comparison of the anticoagulant effect of the sodium and calcium salts of a new potent heparin after SC injection in beagle dogs	368
Local anesthetics: pharmacology and clinical applications	369

An apparent correlation was observed between lithium dosage and sodium intake [389]. With a low lithium dosage or high sodium intake rats were able to excrete all lithium given and sustained a reversible polyuria. Conversely, large amounts of lithium or reduced sodium intake resulted in irreversible kidney damage. Ammonium ion can be toxic in high concentrations and initiate CNS derangements. Oral toxicity of magnesium salts is rare, but may be present in the face of renal impairment. The symptoms include hypotension, muscle weakness, ECG changes, sedation, and confusion.

Sulfate ions given orally tend to be minimally absorbed and may act as a laxative. The nitrate ion is irritating to the GI tract, causing nausea and gastric distress. In addition, intestinal bacteria may convert the nitrate ion to nitrite which oxidizes hemoglobin to methemoglobin. The citrate ion can form a soluble complex with calcium which is poorly dissociable and rarely causes any toxic reactions. Tartrate ions are usually absorbed only minimally from the GI tract, but high concentrations reaching the circulation can cause renal damage. Acetate and lactate ions are normal metabolites and appear to be well tolerated in relatively large amounts. Iodide and bromide ions can produce iodism [390] and bromidism, respectively. Bromides are used as ingredients of some nonprescription preparations [391–394]. Bromide has a half-life of 12 days and tends to accumulate if taken for prolonged periods or if used by patients with low renal function.

Toxicity of Salt Form

Provided the salt-forming agents are nontoxic, the relative toxicities of a series of salts of a compound often reflect their aqueous solubility. For example, the ulcerogenic effect of five different salts of alprenolol was tested against placebo in a porcine esophageal model [395]. The salts with high water solubility, such as the hydrochloride and fumarate, produced the most serious esophageal lesions, whereas salts with lower solubility (benzoate, maleate, and sebacate) had no irritative effect. Similar reasoning has been used to explain the relative toxicities of various salts of quinapyramine [323], propoxyphene hydrochloride in rodents vs. the napsylate salt [396], salts of benzphetamine and etryptamine [397], and the sodium salt of iopanoic acid [398].

Salts of greater water solubility are not, however, always more toxic, and less soluble salts are not always less toxic. This was illustrated with methylpyridinium-2-aldoxime iodide (2-PAM iodide). In order to increase the water solubility of 2-PAM and eliminate undesirable side effects due to the iodide ion, various inorganic and organic salts were prepared [390]. Even though the aqueous solubility of most of these salts was many times higher than that of the iodide, their toxicity on a molar basis was not significantly different, with the exception of the dihydrogen phosphate salt which was 15% more toxic. Toxicity information about a representative group of oximes and their salts has been published [399]. Additional references on toxicological considerations of salt formation are given in Table 10.

Decision Tree for Salt Selection

As amply evidenced by the examples in this article, the range of salt forms available to the pharmaceutical scientist is broad and far reaching. The selection process, there-

TABLE 9 References on General Pharmacy

Topic	Reference
Relationship between salt form and biological activity of an antihypertensive	166
Effect of nonionic surfactants on the release rate of drugs from suppositories	131
Effect of metallic salts of EDTA on blood coagulation	378
Correlation between biological activity and some chromone-2-carboxylate salts	379
Hydrophobic anionic gel beads for swelling-controlled drug delivery	380
Complex formation between macromolecules and drugs; binding of drugs to the membrane in dialysis studies	381
Diffusion of salts through a lipoprotein interface	382
Influence of various counterions on the interaction of chlorhexidine with the hydrophilic contact lens polymer, poly(2-hydroxyethyl methacrylate)	383
Preparation and evaluation of directly compressed indomethacin, indomethacin sodium, and indomethacin meglumine tablets	384
Drug release from compression-molded films; preliminary studies with pilocarpine	385
Sparingly soluble salts for the preparation of oral sustained-release suspensions	386
L-649,923, the selection of an appropriate salt form and preparation of a stable oral formulation	387

TABLE 10 References on Toxicological Considerations

Topic	Reference
Toxicity of aspirin salts	400
Toxicity of polyene antibiotics	401
Toxicity and absorption of 2-sulfanilimidopyridine and its soluble sodium salt	402
Sorbic acid as fungistatic agent for foods	403
Toxicity and distribution of erythromycin	404
Further toxicological studies with erythromycin	405
Pharmacology and toxicology of erythromycin estolate	406
Erythromycin propionate, a review of case reports for side-effect data	407
New class of antibiotic salts of reduced toxicity	24
GI intolerance to oral iron preparations	408
Comparative toxicology of iron compounds	409
Influence of the dissolution rate of lithium tablets on side effects	175
Toxicity and tissue distribution studies on the hydrochloride, bismuth iodide complex, and a resinate of emetine	410
Bacitracin zinc in pharmaceutical preparations	195
New approach to quaternary ammonium compounds	203
Pharmacology of choline theophyllinate	411
Methemoglobinemia resulting from absorption of nitrates	412

fore, needs to be rational and streamlined to avoid unnecessary work. The inexperienced formulator can unwittingly request the medicinal chemist to prepare a "laundry" list of salt forms of the drug candidate for the presumed purpose of preformulation testing. Unfortunately, by the time all these salts have been isolated and characterized and the physicochemical tests performed, several valuable grams of test substance may have been consumed and valuable time may have been lost to the critical commercialization path. Similar views have been expressed by others [108,228,413,414]. Hence, there is a need for a decision tree to create a prototype thought process whereby a suitable salt form can be chosen in an efficient and timely manner with few false starts and the minimum expenditure of resources. The following decision tree (Fig. 1) is proposed to aid in this selection.

Salts of Acidic Compounds

If a hypothetical situation is chosen wherein a free acid form of a compound is selected for clinical development, it should be possible to hone in on the salt form of choice without unnecessarily burdening the development process per se.

Is a Salt Form Needed?

The first decision to be made concerns the viability of the neutral compound per se. If it is an oil, a solid form is preferred in most cases because an oil is difficult to purify and characterize, and it is difficult to maintain its potency. An oil is difficult to ship because of container compatibility and spillage; it is usually oxygen sensitive and needs to be packaged under nitrogen. Furthermore, batch-to-batch variability can create a real

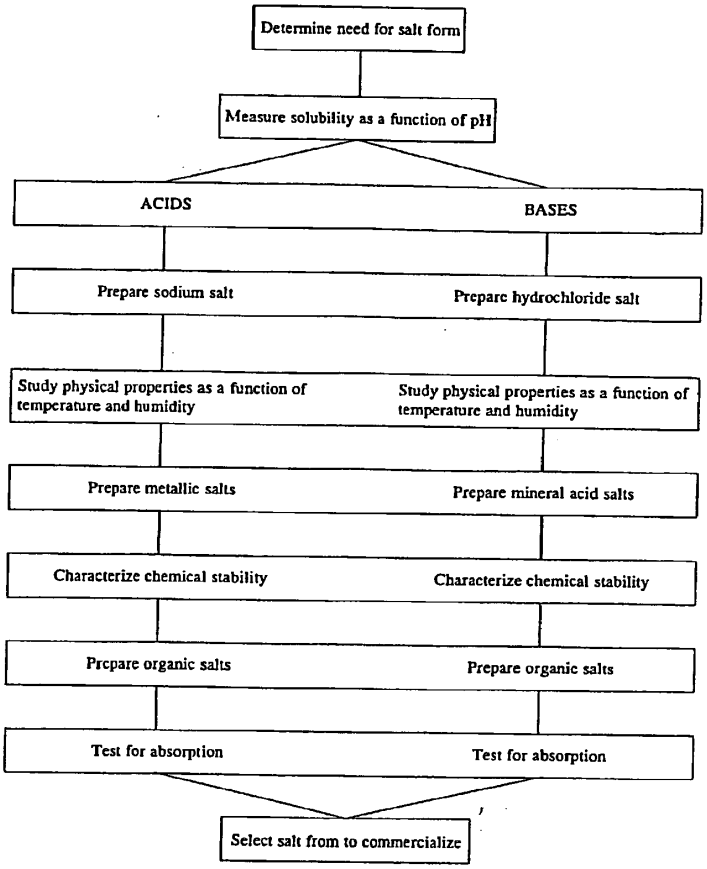

FIG. 1. Decision tree for salt selection.

problem for quality control. Exceptions include cases where a soft gelatin capsule preparation (or hard-shell starch capsule) is preferred for bioavailability purposes, for example, for an emulsion to target lymphatic absorption.

An oil may be acceptable for parenterals, if organic solvents are to be employed to facilitate administration. A cosolvent system is often used when there is no other way of enhancing the solubility of the free acid or base form [124]. Cosolvents are used for water-insoluble drugs such as taxol and cyclosporin.

Should the free acid be a high-melting water-soluble solid, there is generally no need to prepare a salt form. The importance of these criteria is discussed later.

Solubility as a Function of pH

If the free acid is a solid of reasonably high melting point (for example, >90°C), the solubility as a function of pH should be determined. To avoid the influence of buffer counterions on the solubility results, a pH-Stat to control pH is recommended. Likewise, control of ionic strength needs careful consideration if a metallic salt is employed. From a plot of log solubility vs. pH, the pKa of the substance can be calculated by the method outlined by Kramer and Flynn [124]. Should the solubility of the free acid be low (<100 μg/mL) at physiological pH (~5–8), and the therapeutic dose in the 100 mg range, bioavailability is most likely highly dissolution-rate dependent. In such cases, accepted pharmaceutical manipulation processes may need to be employed, including micronization, utilization of activated disintegrants, or a buffer to create a soluble salt form in situ during dissolution, or the addition of a solubilizing agent to the solid dosage form to improve wetting and enhance absorption (cyclodextrin or a surfactant such as a polysorbate).

Preparation of the Sodium Salt

The first salt form that should be prepared is the sodium salt if the patient does not have a restriction on sodium intake.

Unfortunately, the sodium salt of indomethacin was implicated in causing intestinal perforations when formulated in an osmotically driven device [415]. (Note that potassium bicarbonate was included as the osmotic driving agent.) Cases recording the adverse consequences of sodium salts per se are rare and the sodium salt therefore continues to be the salt form of choice.

Determination of the Physical Properties as a Function of Temperature and Humidity

If the sodium salt is sensitive to heat and moisture, its critical humidity at room temperature needs to be determined. Should the compound be hygroscopic at approximately 30% rh at room temperature, another salt form should be assessed since handling problems (stickiness, adherence to machine parts, flow, etc.) during manufacturing could be difficult and expensive to overcome. Moisture adsorption can also adversely affect stability (discussed later). Needless to say, if the salt shows no signs of crystallinity (no birefringence under polarized light), such amorphous materials are generally developed no further.

Frequently, a deliquescent or hygroscopic substance adsorbs moisture only to a stoichiometric amount. A possible strategy then is to investigate the formation of a stable hydrate by crystallizing the sodium salt from a water or aqueous alcoholic solvent system. If the water of hydration is retained at or above about 70°C in a well characterized mono- or dihydrate, this form might be a suitable candidate for commercialization. The requisite studies to characterize the resistance to moisture loss on milling or exposure to low humidity would need to be carried out before such a decision was made.

Frequently, hydrates exhibit a lower rate of dissolution than their anhydrous counterparts, and hence the possibility of reduced bioavailability would need to be assessed. In the case of piroxicam, the monohydrate exhibited a proclivity to induce ulceration in humans because of its increased GI contact time due to slower dissolution than the corresponding anhydrate.

At this point it is useful to examine the samples for a change in crystal structure, using techniques such as hot-stage microscopy, x-ray diffraction, or differential scanning calorimetry/thermogravimetric analysis (DSC/TGA). In this way, the proclivity for polymorphic transformation can be assessed early before surprises are found later in the development program. A decision can then be made to pursue the stable polymorphic form of the salt or to choose a completely new salt form.

Preparation of other Metal Salts

If the sodium salt proves to be unsuitable, a series of other metal salts may be tried, such as potassium, calcium, magnesium, or zinc. As the ionic cation potential increases, the bond distances become shorter and can result in higher melting points, and perhaps lower solubility and higher chemical stability [108,219,228]. Cations such as lithium, copper, or aluminum should be avoided because they may produce pharmacological effects.

Calcium salts are usually less hygroscopic than sodium salts and exhibit a characteristic negative heat of solution. This means that the solubility at 37°C is usually lower than at room temperature, creating a problem if the absorption is dissolution-rate dependent.

The alkalinity of metal salts can cause taste problems as well as handling problems by attacking metal machinery or leaching metals out of glass. In these cases, organic salts or other less alkaline salts should be explored.

Chemical Stability

Before a final selection is made, chemical stability under stressed heat and humidity conditions should be assessed. As a general rule, a tight, dense crystal with a high melting point tends less to react with the atmosphere or with excipients. Furthermore, in the formulation of a hydrolytically unstable compound, salts of low solubility result in less of the compound in solution and available for degradation. A suitable example is that of penicillin suspensions where procaine and benzathine salts have greater stability in aqueous vehicles than the corresponding potassium salt [218], although the thermal stability is better for the sodium or potassium salts in the dry state.

Amine salts (e.g., meglumine) have been employed with contrast agents such as diatrizoic acid to increase solubility. This cation has the particular advantage that autoclaving lowers the pH compared with the sodium salt [416,417]. This is due to the greater relative temperature sensitivity of the pKa for these amines compared to that of sodium hydroxide. As a result, there is less glass attack and less precipitation upon heat treatment.

Arginine has been used as an additive for cephalosporins where the pH of the highly concentrated solution is lower than that obtained when sodium carbonate is used for neutralization. For solutions of the same concentration, greater retention of potency is obtained after reconstitution for the arginine salt compared to the sodium salt [418].

Absorption Test

If the salts discussed so far meet the physicochemical criteria, and an absorption problem is suspected, measuring the octanol-water partition coefficient can sometimes serve as a predictor of absorption. Ultimately, there is no substitute but to measure bioavailability in an animal model.

To increase absorption, organic cations should be prepared, such as amino acids (lysine, arginine), glucoamines (meglumine), or hydroxyamines (diethanolamine or triethanolamine). For low potency compounds, an amino acid salt could result in a substantial molecular weight increase, requiring a much higher total dose, thereby negating the beneficial solubility enhancement. Their usefulness should be explored, however, because these salts usually increase solubility and therefore absorption. A good example is ibuprofen lysinate which exhibits greater aqueous solubility and better absorption than the free acid [339]. Unlike ibuprofen alone, this salt form does not need to be film-coated to prevent loss of potency on storage.

Salts are also employed to increase the absorption rate and hence speed of action, particularly for anti-inflammatory drugs (e.g., naproxen sodium vs. naproxen free acid, and diclofenac potassium vs. diclofenac sodium).

Salts of Basic Compounds

The reasons whether a salt form is preferred over the free base are the same as those for the free acid. Similarly, the reader is referred to the earlier discussion of solubility as a function of pH.

Preparation of the Hydrochloride Salt

The hydrochloride is by far the most popular salt form of basic compounds. By the simple addition of this mineral acid, most bases ionize and give a crystal adduct. The solvents for synthesis are usually selected by the medicinal chemist to accommodate the solubility of the neutral substance. The liquids employed for isolation of the salt are usually an alkane that is miscible with the reaction mixture. The range of anions available for salt formation depends on the pKa of the conjugate acid relative to the basicity of the drug itself. There should be at least one unit of separation between the pKa of the basic drug and that of the anion. Since hydrochloric acid is a very strong acid (pKa $\sim$ -6), it can form a salt with most basic drugs, in contrast with acetic acid, for example, which has a pKa of $\sim$ 4.8.

Determination of the Physical Properties as a Function of Temperature and Humidity

The hydrochlorides of weakly basic amines tend to disproportionate [166,215]. A similar phenomenon occurs during freeze-drying where the loss of hydrochloric acid is facilitated by the high vacuum applied. Thermal methods of analysis such as TGA and DSC (preferably simultaneous) are particularly useful in detecting such incompatibilities early in the development program.

Another frequent problem encountered with amine hydrochlorides is the common-ion effect. In the presence of chloride ion, such as in gastric fluid, the solubility of the

salt form is repressed because solubility is an equilibrium phenomenon, and the absolute value is controlled by the solubility product constant. Therefore, absorption rate may be low because release from the dosage form is dissolution-rate limited. This may vary from patient to patient, depending on the acidity level of the GI tract, and could lead to overdosing in conditions such as achlorhydria. In these cases, the formulator is tempted to micronize the free base and design a rapidly dissolving dosage form. Dissolution rate would, however, still be limited by the presence of chloride ion. A more favored option is to prepare a salt that has a higher innate solubility than the hydrochloride, but this does not solve the problem completely unless the drug is absorbed before the less soluble hydrochloride has a chance to form and precipitate.

The common-ion effect can also influence the the formulation of a parenteral, particularly in adjusting tonicity and osmotic pressure. Clearly, the use of sodium chloride is contraindicated when solubility is reduced because of the common ion effect. Substances such as dextrose are frequently employed as isoosmotic agents in these instances. The use of sodium chloride is not contraindicated when a "salting in" phenomenon is observed, as with some hormonal steroids. For example, normal saline increases the solubility of 17-deacetylnorgestimate 20-fold.

The strategies outlined in the section on acid salts equally apply if the hydrochloride is found to be hygroscopic or deliquescent. Because of the highly polar nature of the crystal surface, hydrochlorides frequently attract and retain moisture which can lead to decomposition and/or powder-handling problems. Metal surfaces can be attacked because the wetted powder frequently exhibits a very low pH.

Preparation of Other Mineral Acid Salts

Mineral acids other than hydrochloric, such as sulfuric, phosphoric, and hydrobromic, have been employed principally to reduce hygroscopicity and perhaps the acidity of a resulting solution. Sulfonic acids are being used more and more for various drug candidates because they can be manipulated to influence dissolution rate and reactivity. For instance, the mesylate salt is frequently highly soluble and therefore dissolves quickly. On the other hand, the hydrophobicity of the sulfonate can be increased by the incorporation of longer carbon chains (esylate, edisylate, isethionate, besylate, tosylate, pamoate (embonate), napsylate, xinafoate, and estolate). An example is propoxyphene, which with the help of the napsylate could be formulated in combination with aspirin, with which it is otherwise incompatible [419]. Apparently, the napsylate reduces wettability and hygroscopicity and therefore decreases the opportunity for the two compounds to react in a layer of moisture between them.

The use of the pamoate salt of the anthelmintic pyrantel allows the formulation of a long-acting, stable oral suspension with a local action in the intestine.

Chemical Stability

The reader is referred to the discussion on chemical stability in the section on acid salts. Again, a higher-melting crystal is chemically more stable [219]. Planar anions such as the napsylate or xinafoate are useful in raising the melting point because they impart symmetry to the crystal lattice. It therefore stands to reason that if a lower melting point is desired in order to generate an oil or a waxy material, a flexible nonsymmetrical anion such as oleate, undecylenate, or decanoate may be of some use. Salts of this nature are used in dosage forms such as oily injection depots or freon-based inhalation acrosols.

Chemical stability also correlates with wettability or interfacial tension between the crystal surface and moisture. Polyhydroxy acids are much more hydrophilic than the sulfonic acids described above and would be expected to be chemically less stable both in the neat or in the formulated state.

Preparation of Organic Salts

A reason that alternatives to mineral acid salts are sought relates to the high resulting acidity of solutions (and in liquid layers surrounding solid crystals) which can lead to container incompatibility and metal attack during processing. This acidity can cause pain on injection and precipitation in the parenteral admixture or in the vein, sometimes resulting in thrombosis. High acidity can also cause interactions with excipients and other drugs. Many of the problems can be avoided by choosing a hydroxylated conjugate acid with a pKa of about 3–4. Options include formate, acetate, glycolate, lactate, malate, gluconate, tartrate, citrate, succinate, malonate, fumarate, and maleate. Depending on the molecular weight of the parent compound, these anions rarely exhibit toxicity. However, if the total body load (insult) on the biological system is too great, toxic events eventually occur (caused by the drug or its conjugate anion); for example, maleate was identified to be responsible for causing renal failure during a toxicology study of a cannabinoid derivative.

Although formic acid is nontoxic per se, its salts are often contaminated with methyl and ethyl formate esters (reaction-solvent side products) which are toxic; this may account for its being used less than others.

Conjugate acids impart a degree of hydrophilicity to the crystal lattice with the result that the crystals are water soluble and frequently hygroscopic and have a lower melting point than the corresponding mineral acid salts. The melting point can be influenced either by size of the anion (acetate and maleic salts usually have a comparatively high melting point) or by symmetry (fumarates melt at lower temperatures than the corresponding isomer (cis-trans) maleates).

High water solubility is an advantage to the formulator since there is usually a higher dissolution rate as well as a higher rate and extent of absorption. For parenterals, organic cosolvents are to be avoided if possible, and increased aqueous solubility is a distinct advantage. By far the best salt to increase water solubility is the lactate. For parenterals it can be formed in situ during processing, and many lactates are used for injectables. Such salts, however, are invariably very hygroscopic and exhibit a low melting point. Thus, for solid dosage forms, they are cohesive and difficult to mill and process, and a compromise must be made in which another hydroxy acid, such as the acetate, is chosen.

Organic salts do not necessarily eliminate drug–excipient interactions. For example, microcrystalline cellulose has been shown to increase degradation of enalapril maleate [420] by reducing heats of fusion. Such a finding, however, is fairly unusual and the decision to examine drug–excipient compatibility can usually be postponed until the final selections are made [421].

Absorption Test

Absorption may be improved by selecting a salt form with greater aqueous solubility. Since absorption is a kinetic rather than an equilibrium phenomenon, the controlling

factor is the rate at which dissolved molecules are presented to the absorbing membranes. Soluble salt forms influence the dissolution rate and the kinetic phenomenon usually to a greater extent than reduction of particle size or the addition of a surfactant to an insoluble free base compound.

Absorption can be impaired when a hydrochloride precipitates in gastric juice due to the common-ion effect. In such cases, a hydroxy acid anion is clearly advantageous since dissolved molecules are more likely to be absorbed before the solubility product is exceeded for the chloride. Similarly, if a salt is insoluble at, say, pH 5(c.g., maleate pKa_2 is 6.3), absorption is likely to be compromised. Often, however, the situation is helped considerably when the dissociated anion acts as a buffer species with the salt and maintains the pH at a sufficiently low value that dissolution occurs in the localized cybotactic region. The anion is apparently diluted by GI fluids before precipitation can occur or ion exchange causes the formation of insoluble salts.

Absorption via the lymphatic system can also be enhanced by the formation of lipid soluble salts such as the oleate. The salt can be dissolved in oils or monoglycerides to assist in forming a microemulsion which is optimal for lymphatic transport. Long-acting depot injections can be enhanced by the formation of insoluble salts.

Utility of the Decision Tree

The decision tree shown in Fig. 1 was designed with the efficiency of the pharmaceutical scientist in mind. Although choice of a salt form is frequently beyond the formulator's control due to cost, toxicity, level of impurities, number of polymorphs, and yield, it is the pharmacist who is burdened with the selection responsibility. There is a choice. Formulators can either approach the matter arbitrarily in an empirical fashion or use a rigorous scientific method. Furthermore, they need to be efficient since this decision lies fairly and squarely on the critical path of the drug's development schedule, and any unnecessary delays due to indecision or a lack of predefined rational selection criteria can be seen as incompetence by peers in both discovery and in project management. Additionally, the criteria for selection must be linked directly to the clinical use situation, that is, the criteria differ quite substantially for a parenteral vs. a powder for inhalation.

Clearly, the selection process is an iterative one and the formula for success is to be able to keep the number of iterations to a minimum. With a cadre of counterions at their disposal, the formulator or medicinal chemist can easily screen for enhanced solubility at physiological pH, employing only a modicum of drug substance. (In the region of pH-independent solubility on the pH-solubility profile, the differences observed are due to the effect of the couterion only.) Then it is a matter of preparing only several salt form for experimentation, and these should be selected scientifically, based on knowledge of physicochemical constants derived from the free acid or base form. It thus becomes a compromise situation, balancing the desirable attributes vs. the undesirable ones and making the decision process transparent to all. An excellent case history where a similar philosophy was employed in selecting the arginine salt of an HMG-CoA reductase inhibitor was recently reported [413]. The decision tree as outlined in this article gives formulators some guidance in the requisite thought processes so that they can be successful in minimizing false starts and can rationally choose a suitable salt for clinical trials and eventual commercialization.

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Continuous Subcutaneous Infusion of Treprostinil, a Prostacyclin Analogue, in Patients with Pulmonary Arterial Hypertension

A Double-blind, Randomized, Placebo-controlled Trial

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Pulmonary arterial hypertension is a life-threatening disease for which continuous intravenous prostacyclin has proven to be effective. However, this treatment requires a permanent central venous catheter with the associated risk of serious complications such as sepsis, thromboembolism, or syncope. Treprostinil, a stable prostacyclin analogue, can be administered by a continuous subcutaneous infusion, avoiding these risks. We conducted a 12-week, double-blind, placebo-controlled multicenter trial in 470 patients with pulmonary arterial hypertension, either primary or associated with connective tissue disease or congenital systemic-to-pulmonary shunts. Exercise capacity improved with treprostinil and was unchanged with placebo; the between treatment group difference in median six-minute walking distance was 16 m ($p = 0.006$). Improvement in exercise capacity was greater in the sicker patients and was dose-related, but independent of disease etiology. Concomitantly, treprostinil significantly improved indices of dyspnea, signs and symptoms of pulmonary hypertension, and hemodynamics. The most common side effect attributed to treprostinil was infusion site pain (85%) leading to premature discontinuation from the study in 8% of patients. Three patients in the treprostinil treatment group presented with an episode of gastrointestinal hemorrhage. We conclude that chronic subcutaneous infusion of treprostinil is an effective treatment with an acceptable safety profile in patients with pulmonary arterial hypertension.

Keywords: treprostinil; prostacyclin analogue; primary pulmonary hypertension; pulmonary arterial hypertension associated with connective tissue disease; pulmonary arterial hypertension associated with congenital systemic-to-pulmonary shunts

Despite recent major therapeutic advances, pulmonary arterial hypertension remains a life-threatening disorder (1, 2). Continuous intravenous infusion of epoprostenol (prostacyclin) has been shown to improve exercise capacity, hemodynamics, and quality of life in primary pulmonary hypertension (3, 4) as well as in other forms of pulmonary arterial hypertension complicating scleroderma (5, 6) and congenital systemic-to-pulmonary shunts (7, 8). In addition, improved survival with epoprostenol has been demonstrated in one unblinded, randomized study of patients with severe primary pulmonary hypertension (3). However, despite these favorable outcomes, continuous intravenous infusion of epoprostenol is far from ideal as a treatment for severe pulmonary arterial hypertension due to its very short half-life (one to two minutes) requiring a continuous intravenous infusion. This delivery method is associated with frequent severe and potentially serious side effects (3, 5, 9). In addition, it is very costly (10). Thus, other modes of prostacyclin delivery are being considered using stable prostacyclin analogues administered orally (11, 12), subcutaneously (13), or by inhalation (14, 15).

Treprostinil, a stable prostacyclin analogue, shares pharmacological actions similar to epoprostenol (16, 17), with similar acute hemodynamic effects (18). However, in contrast to epoprostenol, treprostinil is chemically stable at room temperature and neutral pH and has a longer half-life (three to four hours) permitting continuous subcutaneous infusion rather than continuous intravenous infusion, avoiding the risks of severe infection and thrombosis (19). The objective of this study was to assess the effects of subcutaneous treprostinil on exercise capacity, disease symptoms, hemodynamics, and quality of life in patients with severe pulmonary arterial hypertension, including primary pulmonary hypertension as well as pulmonary arterial hypertension associated with connective tissue disease and congenital systemic-to-pulmonary shunts.

METHODS

Patients

Between November 1998 and October 1999, 470 patients were randomized from 24 centers in North America (Canada, Mexico, and the United States), and from 16 centers in the rest of the world (Australia, Austria, Belgium, France, Germany, Israel, Italy, Poland, Spain, UK). Eligible patients had pulmonary arterial hypertension in accordance with the inclusion and exclusion criteria summarized in Table 1. Patients with connective tissue disease had no pulmonary parenchymal disease as evidenced by lung function tests and a high-resolution computed tomography (CT) scan. Patients with congenital heart disease (left-to-right shunts) had either pulmonary arterial hypertension that developed a variable number of years after surgical correction, or presented with an inversion of the shunt due to the development of

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The members of the Treprostinil Study Group are listed in the online data supplement.

This article has an online data supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

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TABLE 1. MAIN INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria
Primary pulmonary hypertension or pulmonary hypertension associated with connective tissue diseases or associated with congenital systemic to pulmonary shunts
Age between 8 and 75 yr
New York Heart Association (NYHA) functional class II, III, or IV
Significant pulmonary hypertension defined by
Mean pulmonary arterial pressure ≥ 25 mm Hg at rest
Mean pulmonary capillary wedge pressure ≤ 15 mm Hg
Pulmonary vascular resistance > 3 mm Hg/L/min
Ventilation perfusion lung scan or pulmonary angiography not indicative of thromboembolic disease
Exclusion criteria
Significant parenchymal pulmonary disease as evidenced by pulmonary function tests or high resolution CT scan
Porto pulmonary hypertension or HIV-associated pulmonary hypertension
Uncontrolled sleep apnea
History of left side heart disease
Other diseases associated with pulmonary hypertension (e.g., sickle cell anemia, shistosomiasis)
Baseline exercise capacity of less than 50 m or greater than 450 m walked in 6 min
Any new type of chronic therapy for pulmonary hypertension added within the last month
Any pulmonary hypertension medication discontinued within the last week except anticoagulants
Any use of prostaglandin derivatives within the past 30 d

Definition of abbreviations: CT = computed tomography; HIV = human immunodeficiency virus.

pulmonary hypertension and associated increase in pressures of the right heart (Eisenmenger complex). All patients gave written informed consent. The protocol was approved by the local ethics committee at each participating center.

Randomization and Treatment

Patients were randomly assigned to receive either continuous subcutaneous infusion of treprostinil (Remodulin; United Therapeutics Corporation, Research Triangle Park, NC) plus conventional therapy or continuous infusion of placebo (vehicle solution without treprostinil) plus conventional therapy. All patients had conventional therapy optimized for at least one month before enrollment. Conventional therapy could include oral vasodilators, oral anticoagulants, diuretics, and/or digitalis (20). Randomization was based on a permuted block design stratified on the basis of baseline exercise capacity and etiology of pulmonary arterial hypertension.

Treprostinil or placebo was administered using a positive pressure, microinfusion pump (MiniMed, Sylmar, CA). The infusion catheter was placed by the patient in the subcutaneous tissue of the abdominal wall. Chronic study drug infusion was initiated at the dose of 1.25 ng/kg/min. During the 12-week study, doses were increased to a maximum dose at which pulmonary hypertension signs and symptoms were improved while achieving an acceptable side effect profile. At Week 12, the maximum allowable dose was 22.5 ng/kg/min. These doses were selected on the basis of approximately equipotent pulmonary hemodynamic effects compared with those of prostacyclin (18).

Outcome Measures

The primary measure of efficacy was exercise capacity as defined by the maximum distance a patient could walk in six minutes (21). The unencouraged six-minute walk test was administered by a "blinded" tester not involved in the patient's daily care and unaware of the patient's treatment assignment. Each patient performed at least one practice walk test before the baseline assessment conducted before randomization. The walk test was then repeated at Weeks 1, 6, and 12.

Principal reinforcing endpoints of efficacy were signs and symptoms of pulmonary hypertension using a composite score including 16 signs or symptoms recorded at baseline and Weeks 1, 6, and 12; the Dyspnea Fatigue Rating (22) assessed at baseline and Weeks 1, 6, and 12; and the number of deaths, lung transplantations, or discontinuations for clinical deterioration. Secondary endpoints were assessment of shortness of breath immediately after the six-minute walk test using the Borg Dyspnea Scale (23) at baseline and Weeks 1, 6, and 12; cardiopulmonary hemodynamics measured by right heart catheterization at baseline and Week 12; global, physical, and emotional quality of life using the "Minnesota Living with Heart Failure Questionnaire" (24) assessed at baseline and Weeks 6 and 12.

Safety was assessed by comparison of adverse experiences in the two treatment groups and by laboratory assessments (including hemoglobin level, platelet count, leukocyte count, serum creatinine concentration, blood urea nitrogen, alkaline phosphatase, and alanine aminotransferase) at baseline and Week 12. An independent data safety monitoring board reviewed serious adverse events and deaths after 20%, 40%, and 60% of patients had completed the study.

Statistical Analysis

Changes in the distance walked in six minutes from baseline to Week 12 were compared between treatment groups using an intention-to-treat, nonparametric analysis of covariance, prespecified as the primary analysis. A least squares regression analysis was applied to calculate the six-minute walk distances as linear functions of baseline walk, vasodilator use, etiology, and study center. The standardized mid-ranks of the residuals from these linear regression analyses were then determined. Patients who discontinued the study due to death, clinical deterioration, or transplantation before Week 12 were assigned a standardized rank of 0. For patients who discontinued before Week 12 for any other reason, the standardized mid-rank from the last available assessment was carried forward. The ranks were then compared between treatment groups using the extended Cochran–Mantel–Haenszel test. Changes from baseline to Week 12 in the composite score of signs and symptoms of pulmonary hypertension, Dyspnea-Fatigue Rating, Borg Dyspnea Score, and Quality of Life scores were compared between treatment groups using the Wilcoxon rank sum test without imputation. Between treatment group changes in hemodynamic variables were compared using parametric analysis of covariance adjusting for baseline value without imputation. In analyses of possible treatment interactions, a significance level of $\alpha = 0.1$ was considered suggestive of a treatment effect.

RESULTS

Baseline Characteristics and Patient Disposition

Baseline demographic and hemodynamic characteristics of the two groups are shown in Tables 2 and 3, respectively. The two groups were well matched with respect of severity of pulmonary hypertension, duration of illness, and etiology of

TABLE 2. DEMOGRAPHIC CHARACTERISTICS AT BASELINE

Characteristic	Treprostinil (*n = 233*)	Placebo (*n = 236*)
Age, yr	44.6 ± 1.0	44.4 ± 0.9
Sex, n (%)		
Male	36 (16)	51 (22)
Female	197 (85)	185 (78)
Ethnic group, n (%)		
Black	13 (6)	8 (3)
White	198 (85)	198 (84)
Other	22 (9)	30 (13)
NYHA functional class, n (%)		
II	25 (11)	28 (12)
III	190 (82)	192 (81)
IV	18 (8)	16 (7)
6-min walk distance, m	326 ± 5	327 ± 6
Etiology of pulmonary hypertension, n (%)		
Primary pulmonary hypertension	134 (58)	136 (58)
Connective tissue disease	41 (17)	49 (20)
Congenital systemic to pulmonary shunts	58 (25)	51 (22)
Years since pulmonary hypertension diagnosis	4.3 ± 0.5	3.3 ± 0.5

Definition of abbreviation: NYHA = New York Heart Association.

TABLE 3. HEMODYNAMIC VARIABLES AT BASELINE

Variables	Treprostinil (N = 233)	Placebo (N = 236)
Heart rate, beats/min	82 ± 1	82 ± 1
Mean right atrial pressure, mm Hg	10 ± 0.4	10 ± 0.4
Mean pulmonary artery pressure, mm Hg	62 ± 1	60 ± 1
Mean pulmonary capillary wedge pressure, mm Hg	10 ± 0.3	9 ± 0.2
Cardiac index, L/min/m^2	2.4 ± 0.1	2.3 ± 0.1
Pulmonary vascular resistance index, units/m^2	26 ± 1	25 ± 1
Mean systemic artery pressure, mm Hg	90 ± 1	91 ± 1
Systemic vascular resistance index, units/m^2	38 ± 1	39 ± 1
Mixed venous oxygen saturation, %	62 ± 1	60 ± 1
Arterial oxygen saturation	92 ± 0.5	91 ± 0.5

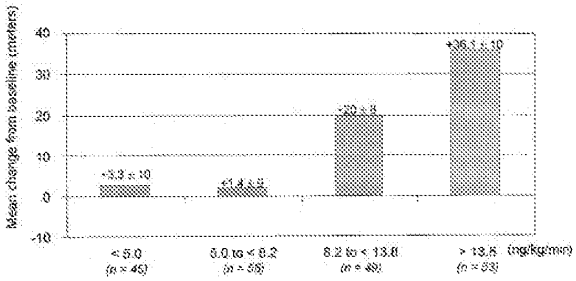

Figure 1. Mean change in the six-minute walk distance from baseline to Week 12 versus Week 12 trepostinil dose quartile.

pulmonary hypertension; 233 patients were randomized to treprostinil and 237 patients to placebo.

Primary Endpoint

Exercise capacity. All but one of the 470 randomized patients were included in the analysis of the primary endpoint; this patient, assigned to receive placebo, did not receive any study drug. The distance walked in six minutes improved at Week 12 in the treprostinil group by a median change of 10 m (−24 to +47 m; 25th–75th percentile) and remained essentially unchanged in the placebo group with a median change of 0 m (−44 to +32 m; 25th–75th percentile). The difference in median distance walked between the two groups at Week 12 was 16 m (95% CI, 4.4 m to 27.6 m, Hodges–Lehmann estimate of the median difference, p = 0.006).

Neither baseline demographic covariates nor disease etiology showed significant interaction with the change in exercise capacity. In contrast, a treatment interaction was observed with the baseline walking distance (p = 0.03), baseline New York Heart Association (NYHA) functional class (p = 0.11), and baseline mixed venous oxygen saturation (p = 0.07). Patients who were more compromised at baseline had a greater improvement in exercise capacity from baseline to Week 12. Severely ill patients who walked less than 150 m at baseline had an estimated treatment effect of +51 ± 16 m (p = 0.002) and less sick patients who walked more than 351 m at baseline had no substantial estimated treatment effect (−2 ± 12 m, p = 0.869). In addition, there was a relationship between the treprostinil dose achieved at Week 12 and the change in the 6-min walk distance. When patients were grouped by quartile of the dose achieved at Week 12, the highest quartile dose had the greatest improvement in six-minute walk distance, and the first and second quartile dose had small improvements (p = 0.03) (Figure 1).

Principal Reinforcing Endpoints

Signs and symptoms of pulmonary arterial hypertension. The signs and symptoms composite score improved in the treprostinil group from 7.6 ± 0.5 at baseline to 8.5 ± 0.5 at Week 12 compared with the placebo group, in which it worsened from 7.5 ± 0.4 at baseline to 7.4 ± 0.2, p < 0.0001 for the comparison between the treatment groups.

Dyspnea-Fatigue Rating. The Dyspnea-Fatigue Rating improved from 4.2 ± 0.1 at baseline to 5.4 ± 0.2 at Week 12 in the treprostinil group, whereas it worsened in the placebo group from 4.4 ± 0.1 to 4.3 ± 0.1, p = 0.0001 for the comparison between the treatment groups.

Death, transplantation, or clinical deterioration. Fourteen patients died while receiving the study drug (seven in each group). Five additional patients (two in the treprostinil group and three in the placebo group) died during the 12-week study period but after withdrawal of the study drug. Six patients in each group discontinued the study due to clinical deterioration; five patients receiving treprostinil and four receiving placebo were transitioned to continuous intravenous epoprostenol therapy. One placebo patient underwent lung transplantation and was alive at the end of the 12-week study period. The total number of deaths, transplantations, or discontinuations due to clinical deterioration was 13 patients in the treprostinil group versus 16 patients in the placebo group.

Secondary Endpoints

Borg Dyspnea Score. In the treprostinil group, patients had an improvement in the Borg Dyspnea Score from 4.3 ± 0.2 at baseline to 3.2 ± 0.2 at Week 12, versus an improvement in the placebo group from only 4.4 ± 0.2 to 4.2 ± 0.2, p < 0.0001 for the comparison of the treatment groups.

Cardiopulmonary hemodynamics. Changes in hemodynamic variables are shown in Table 4. Comparison of treatment groups showed that treprostinil-treated patients had significant improvement in mean right atrial pressure, mean pulmonary artery pressure, cardiac index, pulmonary vascular resistance, and mixed venous oxygen saturation.

Quality of life. Patients treated with treprostinil experienced a significant improvement in their physical dimension score at Week 12 (p = 0.0064) with a trend toward improvement in the global dimension score (p = 0.17) as compared with the placebo group.

Tolerability, Dose, Safety

No clinically significant changes in hematologic or biochemical variables were observed in either group. The most common adverse events are shown in Table 5. Infusion site pain was common in both treatment groups but was more common in the treprostinil group, 85% versus 27%, respectively. Eighteen patients (8%) in the treprostinil group discontinued their study treatment due to intolerable abdominal infusion site pain versus one in the placebo group. Adverse events classically related to the use of prostacyclin, such as diarrhea, jaw pain, flushing, and lower limb edema occurred more often in the treprostinil group. There were no reports of infusion site infections in either group.

By the end of the 12-week study period, the mean dose of the study drug received was 9.3 ng/kg/min versus 19.1 ng/kg/min in the placebo group (p < 0.001). Infusion system malfunctions were common, reported in 55 patients of the treprostinil group (24%) and in 77 patients of the placebo group (33%). Adverse events resulting from these dysfunctions were rare (four patients in each group) and had no clinically serious adverse consequences.

In addition, three patients in the treprostinil group presented with a gastrointestinal hemorrhage; each patient experienced

TABLE 4. CARDIOPULMONARY HEMODYNAMICS: CHANGE FROM BASELINE TO WEEK 12

	Treprostinil	Placebo	p Value
Heart rate, beats/min	−0.5 ± 0.8	−0.8 ± 0.7	ns
Mean right atrial pressure, mm Hg	−0.5 ± 0.4	+1.4 ± 0.3	0.0002
Mean pulmonary artery pressure, mm Hg	−2.3 ± 0.5	+0.7 ± 0.6	0.0003
Cardiac index, L/min/m^2	+0.12 ± 0.04	−0.06 ± 0.04	0.0001
Pulmonary vascular resistance index, units/m^2	−3.5 ± 0.6	+1.2 ± 0.6	0.0001
Mean systemic artery pressure, mm Hg	−1.7 ± 0.9	−1.0 ± 0.9	ns
Systemic vascular resistance index, units/m^2	−3.5 ± 0.9	−0.8 ± 0.8	0.0012
Mixed venous oxygen saturation, %	+2.0 ± 0.8	−1.4 ± 0.7	0.0001

melena and one patient experienced a small hematemesis and rectal bleeding. Two of these patients presented with excessively increased INR (4.0 and 3.14), one of whom had taken naproxen, a nonsteroidal antiinflammatory drug. Two of these patients required transfusion of one and three units of packed red blood cells, respectively. All three gastrointestinal hemorrhage episodes subsided spontaneously without adverse clinical consequences.

DISCUSSION

This study is the first double-blind, placebo-controlled trial conducted in pulmonary arterial hypertension; it is also the largest clinical trial with worldwide participation. The results show that continuous subcutaneous infusion of treprostinil, a stable prostacyclin analogue, is effective therapy in patients with primary pulmonary hypertension as well as in patients with pulmonary arterial hypertension associated with either connective tissue disease or congenital systemic-to-pulmonary shunts. Compared with continuous subcutaneous infusion of placebo, treprostinil consistently improved exercise capacity, indices of dyspnea, signs and symptoms of pulmonary arterial hypertension, cardiopulmonary hemodynamics, and the physical dimension of quality of life.

The distance walked in six minutes has been previously shown to be an independent predictor of mortality in primary pulmonary hypertension (3, 25). After 12 weeks, the difference in median distance walked between the two treatment groups was 16 m. Although significant, this difference appears moderate compared with previous results obtained with intravenous epoprostenol (3, 5). The relatively limited increase in the six-minute walk distance after three months of subcutaneous treprostinil may be explained by the inclusion of less compromised patients, and by the fact that the most important improvement in exercise capacity was observed in the sickest patients. Actually, in the sicker patients, the magnitude of the exercise capacity improvement was similar to that obtained with epoprostenol therapy (3, 5). In addition, in a proportion of patients, only a relatively low dose of treprostinil was achieved by Week 12 due to local infusion site pain. This likely limited the improvement in the six-minute walking distance, as the present results also show a relationship between the dose of treprostinil achieved and increase in six-minute walk distance. However, although limited in magnitude, the increase in the six-minute walk distance in treprostinil-treated patients really reflected clinical improvement as supported by the improvements in the Dyspnea-Fatigue Rating, signs and symptoms scores, and the Borg Dyspnea Score measured at the end of the exercise test indicating increased exercise with less dyspnea. The effectiveness of treprostinil is further supported by a significant improvement in the hemodynamic variables previously shown to be associated with mortality in primary pulmonary hypertension (26). These hemodynamic changes observed after 12 weeks of treprostinil therapy, although small, with average differences between study groups of only 3 mm Hg in mean pulmonary artery pressures and 0.3 L/min/m^2 in cardiac index, were of the same order of magnitude as those previously reported in randomized controlled trials of 12 weeks of intravenous epoprostenol in patients with primary pulmonary hypertension (3) or with pulmonary hypertension secondary to connective tissue disease (5). Chronic epoprostenol or treprostinil may induce more important improvements in exercise hemodynamics, accounting for the observed clinical improvement in patients with pulmonary arterial hypertension.

TABLE 5. MOST FREQUENT ADVERSE EVENTS

Event	Treprostinil (*n* = 233) n (%)	Placebo (*n* = 236) n (%)	p Value
Infusion site pain	200 (85)	62 (27)	< 0.0001
Infusion site reaction	196 (83)	62 (27)	< 0.0001
Infusion site bleeding/bruising	79 (34)	102 (44)	ns
Headache	64 (27)	54 (23)	ns
Diarrhea	58 (25)	36 (16)	0.009
Nausea	52 (22)	41 (18)	ns
Rash	32 (14)	26 (11)	ns
Jaw pain	31 (13)	11 (5)	0.001
Vasodilatation	25 (11)	11 (5)	0.01
Dizziness	21 (9)	19 (8)	ns
Edema	21 (9)	6 (3)	0.002
Vomiting	12 (5)	14 (6)	ns

The large placebo-control group in the present trial offered a unique opportunity to observe the spontaneous evolution, over a period of three months, of stable NYHA class III patients with pulmonary arterial hypertension under optimal medical treatment but without prostacyclin therapy. It appears that such patients are remarkably stable, at least over a three-month period of time, with modest changes in exercise capacity, and a mortality of only 3%. This mortality rate after a relatively short observation period of only three months was actually expected on the basis of both the six-minute walk distances (25) and the hemodynamic profile (26) of the included patients. Much larger numbers of patients with the same disease severity and longer periods of observation would be necessary to show a treatment effect on mortality. The mortality rates in the present study are similar to those reported in a recent randomized, controlled trial of intravenous epoprostenol in patients with pulmonary arterial hypertension secondary to connective tissue disease (5). Intravenous epoprostenol was previously reported to decrease mortality in a randomized, controlled trial that differed from the present study by the inclusion of a smaller number of more severely ill patients, with lower exercise capacity and worse NYHA functional class (3).

In terms of safety, continuous subcutaneous infusion of treprostinil presents several advantages over continuous intravenous infusion of epoprostenol. Due to its short half-life (one to two minutes) and chemical instability, epoprostenol can be given only intravenously, and requires a permanently implanted cen-

tral venous catheter and a portable infusion pump, as well as refrigeration during administration. Sepsis, thrombosis, paradoxical embolism, and interruptions of treatment due to accidental occlusions, perforations, and dislodgments of the catheter, and pump malfunction all have been reported under intravenous epoprostenol. Additionally, any interruption of intravenous delivery of epoprostenol may be associated with syncope, and even death, from an acute pulmonary hypertensive crisis (3–8). None of these life-threatening complications of intravenous epoprostenol therapy was observed in the present study.

Infusion site pain was the most common side effect attributed to treprostinil. Its mechanism remains unclear. It did not appear to be dose-related but seems correlated to the rate of dose increase. It often improved after several months of treatment and could be minimized by moving the infusion site every three days as opposed to every day. Topical cold and hot packs, topical and oral analgesics, and antiinflammatory drugs were variably effective.

There were three episodes of gastrointestinal hemorrhage reported in the treprostinil group. These serious adverse events were attributed to concomitant administration of anticoagulant therapy as indicated in severe pulmonary hypertension (2) in two patients, and the use of a nonsteroidal antiinflammatory drug in one patient with the known platelet antiaggregatory effects of prostacyclin. All of these gastrointestinal hemorrhage episodes rapidly resolved without adverse clinical consequences, after adjustment of anticoagulant therapy, and withdrawal of the antiinflammatory drug, but required a transfusion in two patients.

During the 12-week study period, five patients receiving subcutaneous treprostinil and discontinuing the study due to clinical deterioration were transitioned to intravenous epoprostenol.

In conclusion, chronic subcutaneous treprostinil is an effective therapy with an acceptable safety profile in patients with pulmonary arterial hypertension. Further clinical experience with chronic subcutaneous treprostinil will define its place as an alternative to intravenous epoprostenol in patients with pulmonary arterial hypertension.

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(57) Abstract

Disclosed is an osmotic pharmaceutical delivery system comprising (a) a semipermeable wall that maintains its integrity during pharmaceutical delivery and which has at least one passage therethrough; (b) a single, homogeneous composition within said wall, which composition consists essentially of (i) a pharmaceutically active agent; (ii) at least one non–swelling solubilizing agent which enhances the solubility of the pharmaceutically active agent; (iii) at least one non–swelling osmotic agent; and (iv) a non–swelling wicking agent dispersed throughout the composition which enhances the surface area contact of the pharmaceutical agent with the incoming aqueous fluid.

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SOLUBLE FORM OSMOTIC DOSE DELIVERY SYSTEM

The present invention relates to the field of osmotic pharmaceutical dose delivery systems and preparations, particularly preparations which can be administered orally.

Theeuwes *et al.*, U.S. Patent No. 3,916,899, discloses a drug delivery preparation that is said to release the pharmaceutical agent through openings in the wall of the tablet or capsule by the osmotic pressure differential that is set up between the concentration of pharmaceutical agent in the tablet or capsule interior and the exterior fluid environment of the patient when the medicament is taken orally. See, also, Theeuwes *et al.*, U.S. Patent No. 3,845,770 which discloses another preparation for osmotic pressure differential delivery of a pharmaceutical agent. In this original type of approach the interior of the tablet had a hydrophobic core surrounded by a hydrophilic layer within the tablet wall. As such, water entering the tablet remained in the hydrophilic layer and so very little drug was actually released.

It has been believed that this approach did not deliver the pharmaceutical agent as completely or efficiently as had previously been thought. Therefore, a different approach to releasing the pharmaceutical agent was developed. In this approach the interior of the tablet or capsule is characteristically of two layers, one

containing the pharmaceutical agent (again to be released through openings in the wall of the tablet or capsule) and the other being a layer of material that swells when coming into contact with water. These materials that swell or expand to an equilibrium state when exposed to water or other biological fluids are referred to as "osmopolymers". This volume expansion is used to physically force the pharmaceutical agent out through openings which have been formed in the wall, shell or coating during manufacture. The pharmaceutical agent is primarily released as insoluble particles, which therefore have limited bioavailability. This has commonly been referred to as the "push/pull" approach. See, for example, U.S. Patent Nos. 5,422,123; 4,783,337; 4,765,989; 4,612,008; and 4,327,725. The patent literature has taught that this approach was necessary to deliver adequate doses, at controlled rates and for extended times, of a broad variety of drugs. Other "osmotic delivery systems have also been described. See, for example, U.S. Patent Nos. 4,609,374; 4,036,228; 4,992,278; 4,160,020; and 4,615,698. The osmopolymers used in these types of systems are components whose functions are to swell when they interact with water and aqueous fluids. This swelling effect is defined in these patents as a property of embibing fluid so is to expand to a very high degree, usually exhibiting a 2 to 50 fold volume increase.

Summary of the Invention

In arriving at the present invention it has been discovered that it is possible to efficiently deliver therapeutically effective doses, at controlled rates and for extended times, of a broad variety of drugs without the need for polymers that swell or expand within the tablet wall so as to physically force the medicament particles out into their intended environment of use. As used herein the term "swell", i.e. that property which the present invention has been able to avoid, is used so as to have the same definition as in the patents described above. Further, the invention makes it possible to deliver agents which have limited aqueous solubility.

In accordance with the preferred invention, there is provided an osmotic delivery system, preferably in the form of a tablet, which dispenses a therapeutic agent having a limited solubility in water or physiological environments without the

use of osmopolymers or swelling agents to deliver the therapeutic agents. Further in accordance with the present invention, the therapeutic agent is incorporated into a composition which is capable of solubilizing the therapeutic agent whereby the therapeutic agent is delivered in a predominantly solubilized form.

In a preferred embodiment, the invention has combined appropriate solubilizing agents and, throughout the composition containing the solubilizing and pharmaceutical agent(s), a "wicking" agent which provides enhanced flow channels for the pharmaceutical agent which has been made predominantly into its solubilized form by the solubilizing agent(s) while still within the tablet or capsule. Thus, the drug is delivered out through passages in the coating wall by true osmosis predominantly in its solubilized form, rather than by physical force on a particulate form.

Accordingly, in one aspect, the invention provides an osmotic pharmaceutical delivery system comprising (a) a semipermeable wall that maintains its integrity during pharmaceutical delivery and which has at least one passage therethrough; (b) a single, homogeneous composition within said wall, which composition contains (i) a pharmaceutically active agent, (ii) at least one non-swelling solubilizing agent which enhances the solubility of the pharmaceutically active agent; (iii) at least one non-swelling osmotic agent and (iv) a non-swelling wicking agent dispersed throughout the composition which enhances the surface area contact of the pharmaceutical agent with the incoming aqueous fluid. The pharmaceutical agent is thus released in a predominantly soluble form.

Preferred non-swelling solubilizing agents include (i) agents that inhibit crystal formation of the pharmaceutical or otherwise acts by complexation therewith; (ii) a high HLB (hydrophilic-lipophilic balance) micelle-forming surfactant, particularly non-ionic and/or anionic surfactants; (iii) citrate esters; and combinations thereof, particularly combinations of complexation agents with anionic surfactants. Preferred non-swelling osmotic agents include sugars with ten or fewer rings, preferably five or fewer rings and most preferably two rings. Examples include fructose, lactose, xylitol and sorbitol. Preferred wicking agents include colloidal

silicon dioxide and polyvinyl pyrrolidone and sodium lauryl sulfate can also function as wicking agents.

Brief Description of the Drawings

The invention will now be further described by reference to a brief description of each of the accompanying drawings. The brief description and the drawings are in no way a limitation of the invention.

Figure 1A schematically illustrates the elementary osmotic dose delivery system of the prior art.

Figure 1B schematically illustrates the osmotic dose delivery system of the prior art.

Figure 2 schematically illustrates the osmotic dose delivery system of the present invention.

Figure 3 diagramatically shows the percent of nifedipine released by dosage forms of the invention containing formulations 1G (30 mg); 1C (30 mg); as shown on Table 1 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 4 diagramatically shows the percent of nifedipine released by dosage forms of the invention containing formulations 2B (47 mg); 2C (47 mg); and 2D (47 mg) as shown on Table 2 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 5 diagramatically shows the percent of nifedipine released by dosage forms of the invention containing formulations 3C (30 mg); 3H (30 mg); as shown on Table 3 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 6 diagramatically shows the percent of nifedipine released by forms of the invention containing formulations 4H (30 mg); 4C (90 mg); as shown on Table 4 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 7 diagramatically shows the percent of nifedipine released by forms of the invention containing formulations 5G (60 mg); 5H (60 mg); as shown on Table 5 as compared to Procardia XL®(Pfizer, Inc.; 60 mg).

Figure 8 diagramatically shows the percent of nifedipine released by forms of the invention containing formulations 6E (60 mg); 6F (60 mg); as shown on Table 6 as compared to Procardia XL®(Pfizer, Inc., New York; 60 mg).

Figure 9 diagramatically shows the percent of nifedipine released by forms of the invention containing formulation 6F (60 mg) with a 1% ethylcellulose seal coat as shown on Table 6 as compared to Procardia XL®(Pfizer, Inc., New York; 60 mg).

Detailed Description of Preferred Embodiments

The invention will now be described in more detail with respect to numerous embodiments and examples in support thereof.

The semipermeable wall of the elementry osmotic delivery system is composed of a polymeric material cast or sprayed onto the tablet to give a 2 - 15% coating weight. One example of a polymeric material includes, but is not limited to, cellulose acetate. The use of such polymeric material requires plasticizers for increased flexibility, durability, and stability. In the case of cellulose acetate, examples of suitable plasticizers are triethyl citrate (TEC), propylene glycol (PG), a mixture of TEC and PG in ratios ranging from 25% TEC plus 75% PG to 75% TEC plus 25% PG, Tween 80 or other polyoxyethylene sorbitan esters, triacetin, diethyl phthalate, polyethylene glycol, mineral oil, tributyl sebacate, and glycerol. The plasticizers are included as a weight ratio of cellulose acetate suitable for creating a semipermeable wall to achieve retainment of the bioactive substance while permitting water permeation to the core of the tablet.

The semi-permeable wall of the tablet can contain at least one passageway communicating the contents of the core with the exterior of the device, delivering the beneficial drug through the passageways from the elementary osmotic device. The size of an individual passageway can range from 100 microns to 1000 microns, more preferred 300 to 900 microns, most preferred 500 to 850 microns. One or

multiple passageways can be present to communicate the contents with the exterior of the tablet.

A wicking agent, defined as any material with the ability to draw water into the porous network of a delivery device is included in the core of this type of tablet formulation. A wicking agent can do this with or without swelling, but those used in the present invention are non-swelling wicking agents. Some materials can both wick water and swell, others can function as wicking agents only. The wicking agents are characterized by having the ability to undergo physisorption with water. Physisorption is defined as a form of adsorption in which the solvent molecules can loosely adhere to surfaces of the wicking agent via van der Waals interaction between the surface of the wicking agent and the adsorbed molecule. In the case of a drug delivery device, the adsorbed molecule is primarily water or other biological fluid which is mainly composed of water. A wicking agent that attracts water will ultimately have a volume that is essentially composed of the volume of wicking agent and the volume of water attracted to it. A material that swells will have a volume that is esentially composed of the volume of wicking/swelling agent, the volume of water attracted to it, and an additional volume created by steric and molecular forces.

The wicking agent included in the formulations described in this invention creates channels or pores in the core of the tablet. This facilitates the channeling of water molecules through the core of the tablet by physisorption. The function of the wicking agent is to carry water to surfaces inside the core of the tablet, thereby creating channels or a network of increased surface area. For the purposes of this invention, these wicking agents do not swell to any appreciable degree. For bioactive agents with low solubility in water, the wicking agent aids in the delivery of partially solubilized bioactive agent through the passageway in the semipermeable coating. Materials suitable for acting as wicking agents include, but are not limited to, colloidal silicon dioxide, kaolin, titanium dioxide, fumed silicon dioxide, alumina, niacinamide, sodium lauryl sulfate, low molecular weight polyvinyl pyrrolidone, m-pyrol, bentonite, magnesium aluminum silicate, polyester, polyethylene. Materials particularly suitable for the purpose of this invention include

the non-swellable wicking agent, examples of which are sodium lauryl sulfate, colloidal silicon dioxide, and low molecular weight polyvinylpyrrolidone.

Preferred non-swelling solubilizing agents include (i) agents that inhibit crystal formation of the pharmaceutical or otherwise acts by complexation therewith; (ii) a high HLB (hydrophilic-lipophilic balance) micelle-forming surfactant, particularly anionic surfactants; (iii) citrate esters; and combinations thereof, particularly combinations of complexation agents with anionic surfactants. Examples of the agents that inhibit crystal formation of the pharmaceutical or otherwise acts by complexation therewith include polyvinylpyrrolidone, polyethyleneglycol (particularly PEG 8000),α, β and δ cyclodextrins and other modified cyclodextrins. Examples of the high HLB, micelle-forming surfactants include non-ionic and/or anionic surfactants, such as Tween 20, Tween 60 or Tween 80; polyoxyethylene or polyethylene-containing surfactants, or other long chain anionic surfactants, particularly sodium lauryl sulfate. Examples of citrate ester derivatives that are preferred are the alkyl esters, particularly triethyl citrate. Combinations of these types of non-swelling solubilizing agents are especially effective. Preferred among such types of combinations are combinations of complexation agents and anionic surfactants. Particularly preferred examples of such combinations are polyvinylpyrrolidone with sodium lauryl sulfate and polyethyleneglycol with sodium lauryl sulfate.

Lubricants are also added to assure proper tableting, and these can include, but are not limited to: magnesium stearate, calcium, stearate, stearic acid, polyethylene glycol, leucine, glyceryl behenate, and hydrogenated vegetable oil. These lubricants should be present in amounts from 0.1-10% (w/w), with a preferred range of 0.3-3.0% (w/w).

Preferred lubricants for tableting include but are not limited to sodium stearyl fumarate, magnesium stearate, calcium stearate, zinc stearate, stearic acid, glycerol behenate, sodium lauryl sulfate, polyethylene glycol and hydrogenated vegetable oil. Particularly preferred lubricants are those which are soluble in water or gastric fluids or are readily emulsified. Combinations of lubricants are especially effective.

Lubricant combinations which are preferred are a small amount of hydrophobic lubricant with a larger amount of soluble or emulsifiable lubricant. The rate of use for lubricants extends from 0.25 to 10.0% with a preferred range of 1 to 4%.

The delivery system of the invention can be used to provide controlled release of any of a broad variety of therapeutically active agents. Examples include the following: cough suppressants, such as dextromethorphan hydrobromide and codeine; antihistamines such as chlorpheniramine maleate, brompheniramine maleate, loratidine, astemizole, diclofenac sodium and terfenadine; decongestants such as pseudoephedrine and phenylephrine; antihypertensives such as nifedipine, verapamil, enalapril and salts thereof, metoprolol, metoprolol succinate, metoprolol fumarate, metoprolol tartarate; calcium channel blockers such as verapamil, diltiazam, nifedipine, nimodipine, felodipine, nicardipine, isradipine and amlodipine; antidiabetic agents such as glipizide and ibromectin; proton pump inhibitors such as omeprazole; H2 receptor antagonists such as cimetidine, ranitidine, famotidine, nizatidine; carbamazepine; anti-Parkinson agents such as selegiline, carbidopa/levodopa, pergolide, bromocriptine, amantadine, trihexyphenidyl HCl; antiviral agents including antiherpesvirus agents such as acyclovir, famciclovir, foscarnet, ganciclovir; antiretroviral agents such as didanosine, stavudine, zalcitabine, zidovudine; and others such as amantadine, interferon alpha, ribavirin, rimantadine; and other therapeutic agents such as cimetidine, propiomazine, phenytoin, tacrine, propiazam, proplazam. The system of the present invention is particularly applicable to therapeutic agents which are insoluble or poorly soluble in water or aqueous environments at physiological pH.

In a preferred embodiment the system of the present invention is employed for dispensing nifedipine. In such a preferred embodiment, the composition is free of agents which prevents solubilization of the nifedipine such as the Group I and Group II metals and salts thereof. In such compositions preferred osmotic agents are sugars.

Example 1

Nifedipine Granulation/Tableting/Coating

(TEC) or another suitable wetting agent is added to enough water to produce a good dispersion which will atomize and pump well. Add between 50 to 100% of the PEG 8000. Next add between 50 to 100% of the nifedipine to the dispersion. Finally add between 25 to 75% of the Cab-o-Sil® to the binder dispersion. Mix for ~20 minutes before spraying. Also, other ingredients can be added to or removed from the dispersion as necessary. A dispersion is also not necessary, the binder may be a solution of PVP, PEG, surgar or other binder. The solution may be aqueous or organic. In some cases, a hot melt method of granulating may be preferred. In this case, the binder may be a molten wax, wax mixture or other material.

Charge a fluid bed bowl with osmagents (xylitol, sorbitol lactose, fructose, inositol, etc.). Add between 50 to 100% of the SLS, add the remaining PEG 8000, and add between 50-100% of the PVP K-25, add all or the remaining amount of Nitedipine and other ingredients as required.

Spray the dispersion onto the powder bed with a spray rate of 20-50 g/min which will produce granules of an adequate size for tableting. (Spray rate will vary with batch size.) Inlet air flow rate and temperature are adjusted to keep powder bed from over-granulating or becoming overly wet. (Typical range 100-250 CMH and 40-60°C, depending on batch size.)

Discharge granulation and add remaining sodium lauryl sulfate (SLS), polyvinyl pyrrolidone (PVP K-25), osmagents, polyethylene glycol (PEG), nifedipine and Cab-o-Sil® (colloidal silicon dioxide; Cabot Corporation) and mix in a V-blender or appropriate mixer for 2-5 minutes or as necessary. Add suitable lubricant such as Magnesium Stearate (approximately 0.5-1.5%) and blend 2-5 minutes or as necessary.

Discharge final blend from mixer and tablet on suitable tablet press. Coat tablets in pan coater or fluid bed dryer with spray rate of 30-100 g/min or higher (depending on batch size). The coating solution is prepared by dissolving ~5%

cellulose Acetate, NF (National Formulary) in Acetone or other suitable solvent then adding 25-45% plasticizers such as TEC or PG or mixture thereof.

Process may also be done by direct compression, high shear granulation, dry compression or slugging.

In some cases it may be desirable to modify the solubility characteristics of the osmagents, solubilizers, granulation or other ingredient to achieve a desired release profile.

One method for modifying the release profile is to use a hydrophobic coating method. Initially, all ingredients could be granulated together with a 0-20% PVP K 25 or PEG 8000 or other binder aqueous or organic solution to ensure that drug, sugars, and solubilizers are evenly distributed throughout the granules. Following this procedure, a coating agent such as hydrogenated castor oil, hydrogenated vegetable oil, type I, ethyl cellulose, glyceryl monostearate, Gelucire® or carnauba wax at 1-20% of the total formulation weight could be applied to 5-50% of the total granulation. The coating agent may be applied in a fluid bed by top spray, wurtser column coating, or rotor application; a pan coater equipped with a screen for coating granules may also be utilized. The hydrophobic agent could be applied in a melted state or dissolved in a suitable solvent in which it would be sprayed onto the granules. Both parts of the granulation, immediate and sustained release, could then be blended thoroughly by using a V-Blender before tableting.

Alternatively, the method presented above may be applied to a component or combination of components of the formulation. One or more of the osmagents may be granulated alone or in combination with other osmagents, solubilizers or other components of the core. These granules may then be coated alone or in combination with any other component of the core with the materials and methods described above. The coated granules can then be added to the rest of formulation by dry blending, or they may actually be granulated with the remainder of the formulation.

Alternatiavely, a hydrophobic granulation method may be utilized. In this method powdered wax is mixed together with the portion of the granulation to be coated (in the same percentage ranges already stated). Non-powdered wax may be utilized by milling the wax to a fine particle size. Wax mixtures may be formed by melting the wax, adding the desired component, allowing the mixture to congeal and then screening or milling the wax mixture to a fine particle size. The powdered wax or wax mixture is then added to the fluid bed with the portion of the granulation to be coated. The materials are granulated by increasing and controlling the inlet temperatures of the fluid bed (inlet temperature ~60-80°C, outlet temperature ~40-60°C), to cause the melting/congealing steps involved in the granulation process. In other instances a jacketed device could be used to granulate. Here, however, the temperature ranges would apply to the substance used in heating and cooling the device, such as steam, hot oil or water.

For sustained release agents which are not waxes, the granulation process can be carried out utilizing standard granulation techniques such as aqueous moist granulation or solvent granulation (in the same percentage ranges already stated). The sustained release agent may be dissolved or suspended in the granulating fluid or it may be dispersed with the powders to be granulated. The granules are formed and dried and finally added to the remainder of the formulation.

Again, the above granulation techniques may be applied to a portion of the entire formulation or any component or mixture of components in the formulation. The sustained release granules may then be combined with the remainder of the formulation by techniques previously discussed.

Finally, a matrix technique may be utilized. This technique involves adding a powdered wax at 5-30% of the total formulation weight, such as hydrogenated castor oil, glyceryl palmitostearate, glyceryl behenate, Gelucire®, PEG 8000 or any other non-swellable matrix forming agent known to one skilled in the art to the formulation. The wax may be granulated with any component or combination of components of the formulation with a 0-20% PVP K25 or PEG 8000 or other binder solution, or a roller compaction or slugging method may be used in the formation

of the granules. The granules are then added to the remainder of the formulation using the methods stated earlier.

The modified release osmagents, solubilizers or granulation may then be tableted after addition of a suitable lubricant. A single layer tablet would have all components of the formulation blended together and compressed. One or more holes may be provided to give the proper release. One or more holes may be provided on the tablet. It may be beneficial for a tablet to have a hole on both sides of the tablet so that the optimum release rate is achieved. One or more holes may be provided to achieve the desired release characteristics.

It is possible that any of the previously discussed excipients in combination with the tablet core may lower the melting point. The temperatures that the tablet should be exposed to in an aqueous color coating process may be extreme enough (~60°C) to partially melt the core and change the physico-chemical behavior of the tablet in dissolution or stability. To avoid this change, a solvent-based color coat was formulated at Shire Laboratories Inc., consisting of a 1:1 mixture of hydroxypropyl cellulose and HPMC, and 1% of a colored aluminum lake dispersed in a 70:30 IPA:Water solution. Because the color coat is solvent-based, the temperature that the tablets will be exposed to in the coating process is significantly lower (~35-40°C).

A one to two hour delay before the onset of dissolution may be beneficial. In order to provide this lag time a seal coat may be added to the tablet. The seal coat should provide a water impermeable barrier for no longer than two hours. Some polymers which would provide this type of coating include ethylcellulose, shellac, Eudragit RS. Other ingredients may be added to the polymers in order to modify the coating to achieve the desired lag time. A 1-10% weight gain should be applied to the tablets. The coating is applied as an aqueous or organic solvent solution or dispersion. The coating is typically applied in a coating pan or fluid bed equipped with a wurster column.

Example 2

Nifedipine Forulations

The following are examples of formulations of the single, homogeneous composition within the tablet wall of the dosage form of the invention.

Table 1

Ingredients	1A	1B	1C	1D	1E	1F	1G	1H
Fructose		43.5	21.5	49.6	44.5	37.2	20.5	18.5
Lactose 315	17	18	17	17	17	32	17	17
Sorbitol	43.5		21				21	19
PVPK25	15	15	15	12.7	15	12.5	15	10
PEG8000	10 (5*)	10 (5*)	10 (5*)	8.5 (4.2*)	10 (5*)	8.4 (4.2*)	10 (5*)	20 (10*)
TEC	1*	1*	1*	1.7*	1*	0.84*	1*	1*
SLS	3	3	3	3	3 (1.5*)	1.95 (1.25*)	3	3
Cab-o-Sil®	2.0 (0.5*)	1.0 (0.5*)	1 (0.5*)	0.92 (0.42)	1 (0.5*)	1.1 (0.4*)	1 (0.5*)	0.5
Nifedipine	8*	8*	8*	6.8*	8*	6.7	8*	8*
Mg Stearate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3
K Sorbate							3	
Total	100	100	100	100	100	100	100	100

* Indicates in dispersion

Table 2

Ingredients	2A	2B	2C	2D	2E
Fructose					
Lactose 315					
Sorbitol	15.5	15.5	5.5	23.25	12.5
Xylitol	23.25	23.25	23.25	15.5	6.5
Mannitol					
PVPK-12PF					
PVP-K25	35	35	35	35	50
PEG8000	10(5*)	10*	20(10*)	10(5*)	10(5*)
SLS	5	5	5	5	10
Cab-o-Sil®	1(0.5*)	1(0.5*)	1(0.5)	1(0.5*)	
Nifedipine	8.25*	8.25*	8.25	8.25*	10
TEC	1*	1*	1*	1*	
Mg Stearate	1	1	1	1	1
Total	100	100	100	100	100

* Indicates in dispersion

Table 3

Ingredients	3A	3B	3C	3D	3E	3F	3G	3H
Fructose	21.5	20.5	19.5	16.5	18.5	17.5	17.5	16.5
Lactose 315	17	17	17	17	17	17	17	14.5
Sorbitol	23	21	23	21	21	20	20	18.5
PVPK25	15	15	15	15	15	15	15	15
PEG8000	10 (5*)	10 (5*)	10 (5*)	10 (5*)	10 (5*)	10 (5*)	10 (5*)	10 (5*)
TEC	1*	1*	1*	1*	1*		1*	1*
SLS	3	3	5	5	3	5	5	5
Cab-o-Sil®	1(0.5*)	1(0.5*)	1(0.5*)	1(0.5*)	1(0.5*)	1(0.5*)	1(0.5*)	1(0.5*)
Nifedipine	8*	8*	8*	8*	8*	8*	8*	8*
Mg Stearate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
K Sorbate		3		5	5			
Glycerol						5		
Cremophor EL**							5	
Inositol								10*
Total	100	100	100	100	100	100	100	100

* Indicates in dispersion

** Polyethylene glycol castor oil derivative (other suitable derivatives of castor oil are disclosed by the International Cosmetic Ingredient Dictionary (5th Ed.), Cosmetic Fragrance and Toiletry Association, Washington, D.C. (1993), *e.g.* at pages 479-481)

Table 4

Ingredients	4A	4B	4C	4D	4E	4F	4G	4H
Fructose	13	13	4.5	17.5	18.5	15.25	15.675	15.675
Lactose 315	10.5	10.5	4	17	17.5	30	16.15	16.15
Sorbitol	16	16	6	20	22		19.95	19.95
PVPK25	35	15	35	15	15	15	14.25	14.25
PEG8000	10 (5*)	35 (5*)	35 (5*)	10 (5*)	10 (5*)	10	9.5(4.75*)	9.5(4.75*)
TEC	1*	1*	1*	6*			0.95*	0.95*
SLS	5	5	5	5	5	5	5	5
Cab-o-Sil®	1.(0.5*)	1.0 (0.5*)	1.0 (0.5*)	1.0 (0.5*)	1.0 (0.5*)	1	.975 (0.475*)	.975 (0.475*)
Nifedipine	8*	8*	8*	8*	8*	8.25	7.6*	7.6*
Mg Stearate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
K Sorbate							4.75	4.75
Glycerol					2.5			
Cremophor EL						10		
Inositol							5	
Calcium Sulfate								5
Total	100	100	100	100	100	100	100	100

* Indicates in dispersion

Table 5

Ingredients	5A	5B	5C	5D	5E	5F	5G	5H
Xylitol	15.5	5	32.5		31.5	24.0	16.9	20
Sorbitol	15.0	5			15.5	15.5	15	18.75
Fructose		7.5		17.5				
Lactose		5						
PEG 8000	10	10	20	10		10(5*)		10
PVP K-25	35	35	15	15	35	35	50	35(5*)
TEC	1	1	1	1.0	1*	1*	1*	1*
Cab-o-Sil®	1.0	1	1		1 (0.5)	2 (1*)	1(0.5*)	1(0.5*)
Nifedipine	17	25	25	50.0	10	10	10.1	8.25
Mg Stearate	0.5	0.5	0.5	0.5	1	1	1	1
SLS	5.0	5	5	3		5	5	5
K Sorbate				3				
Total	100	100	100	100	100	100	100	100

* Indicates in dispersion

Table 6

Nifedipine Formulations

	6A	6B	6C	6D	6E	6F	6G	6H
Xylitol	27.5	27.5	25.5	30.8	28.5	32.5	34.5	25.5
Sorbitol	25	25	26	28.5	29	30	30	26
SLS	5	5	4.5	4.8	5	5	5	4.5
PVP K25	15(3*)	15(3*)	13.5 (2.7*)	14.2 (2.8*)	15(3*)	15(3*)	5	13.5
Nifedipine	15	15	18	14.2	20	15	17.5	18
Stearic Acid	1	11	1	1	1	1	2	1
Mag. Stearate	1	1	1	1	1	1	0.5	1
Cab-o-sil®	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glycerol Behenate	10							
Stearic Acid (Binder)							5.0	
Stearic Acid Coated Xylitol			10	5				
Stearic Acid Coated 6E								10

Example 3

Comparative Percentage of Nifedipine Release

This example reports experiments which compared the percentage of nifedipine released by certain of the above formulations in dose delivery forms of the invention as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Materials and Methods

Dose delivery forms of the invention are placed in a Vankel Dissolution Apparatus containing simulated gastric fluid without enzymes and dissoluted for 20 to 24 hours. Samples of the dissolution media are taken periodically and analyzed by high performance liquid chromatography for nifedipine concentration. The calculated percent release is plotted versus time. Dose delivery forms of the invention and procardia XL tablets are tested in the same manner to produce effective comparisons.

Results

Figure 3 diagramatically shows the percent of nifedipine released by dosage forms of the invention containing formulations 1G (30 mg); 1C (30 mg); as shown on Table 1 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 4 diagramatically shows the percent of nifedipine released by dosage forms of the invention containing formulations 2B (47 mg); 2C (47 mg); and 2D (47 mg) as shown on Table 2 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 5 diagramatically shows the percent of nifedipine released by dosage forms of the invention containing formulations 3C (30 mg); 3H (30 mg); as shown on Table 3 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 6 diagramatically shows the percent of nifedipine released by forms of the invention containing formulations 4H (30 mg); 4C (90 mg); as shown on Table 4 as compared to Procardia XL®(Pfizer, Inc.; 30 mg).

Figure 7 diagramatically shows the percent of nifedipine released by forms of the invention containing formulations 5G (60 mg); 5H (60 mg); as shown on Table 5 as compared to Procardia XL®(Pfizer, Inc.; 60 mg).

Figure 8 diagramatically shows the percent of nifedipine released by forms of the invention containing formulations 6E (60 mg); 6F (60 mg); as shown on Table 6 as compared to Procardia XL®(Pfizer, Inc., New York; 60 mg).

Figure 9 diagramatically shows the percent of nifedipine released by forms of the invention containing formulation 6F (60 mg) with a 1% ethylcellulose seal coat as shown on Table 6 as compared to Procardia XL®(Pfizer, Inc., New York; 60 mg).

What Is Claimed Is:

1. An osmotic pharmaceutical delivery system comprising (a) a semipermeable wall that maintains its integrity during pharmaceutical delivery and which has at least one passage therethrough; (b) a single, homogeneous composition within said wall, which composition consists essentially of (i) a pharmaceutical agent, (ii) at least one non-swelling solubilizing agent which enhances the solubility of the pharmaceutical agent; (iii) at least one non-swelling osmotic agent and (iv) a non-swelling wicking agent dispersed throughout the composition

2. The pharmaceutical delivery system of claim 1 wherein the pharmaceutical agent is released through said at least one passage.

3. The pharmaceutical delivery system of claim 1 wherein the wall has a plurality of passages therethrough.

4. The pharmaceutical delivery system of claim 1 wherein the non-swelling solubilizing agent is selected from the group consisting of (i) agents that inhibit crystal formation of the pharmaceutical or otherwise act by complexation therewith; (ii) a high HLB (hydrophilic-lipophilic balance) micelle-forming surfactant, particularly anionic surfactants; (iii) citrate esters; and combinations thereof.

5. The pharmaceutical delivery system of claim 4 which comprises the combinations of at least one complexation agent with at least one anionic surfactant.

6. The pharmaceutical delivery system of claim 5 wherein the combination is selected from the group consisting of (i) a polyvinylpyrollidone and sodium lauryl sulfate and (ii) a non-swellable polyethyleneglycol and sodium lauryl sulfate.

7. The pharmaceutical delivery system of claim 1 wherein the at least one non-swelling osmotic agent is a sugar.

8. The pharmaceutical delivery system of claim 7 wherein the sugar has no more than ten rings.

9. The pharmaceutical delivery system of claim 8 wherein the sugar has no more than five rings.

10. The pharmaceutical delivery system of claim 9 wherein the sugar is selected from the group consisting of monosaccharides, disaccharides and trisaccharides.

11. The pharmaceutical delivery system of claim 9 wherein the sugar is selected from the group consisting of fructose, lactose, xylitol, inositol and sorbitol.

12. The pharmaceutical delivery system of claim 11 wherein the sugar is coated with a hydrophobic material.

13. The pharmaceutical delivery system of claim 1 wherein the non-swelling wicking agent is selected from the group consisting of colloidal silicon dioxide, polyvinyl pyrrolidone and sodium lauryl sulfate.

FIG. 1A
Prior Art

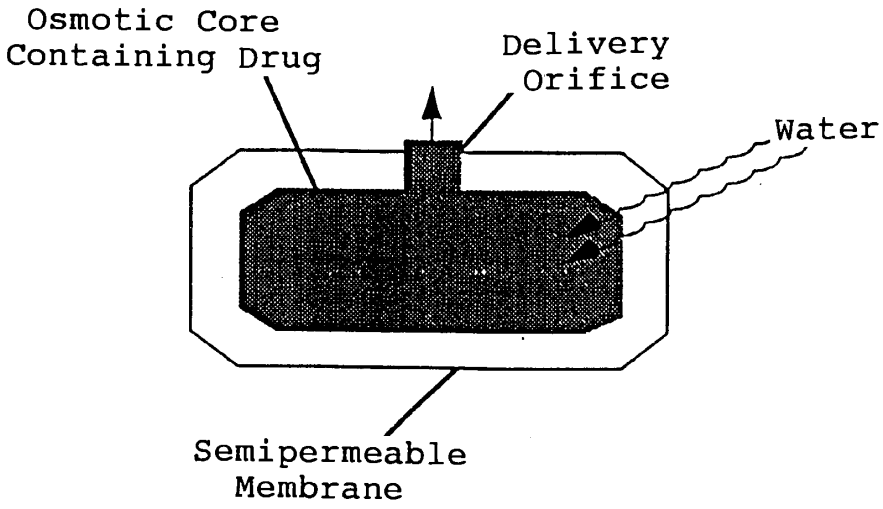


FIG. 1B
Prior Art

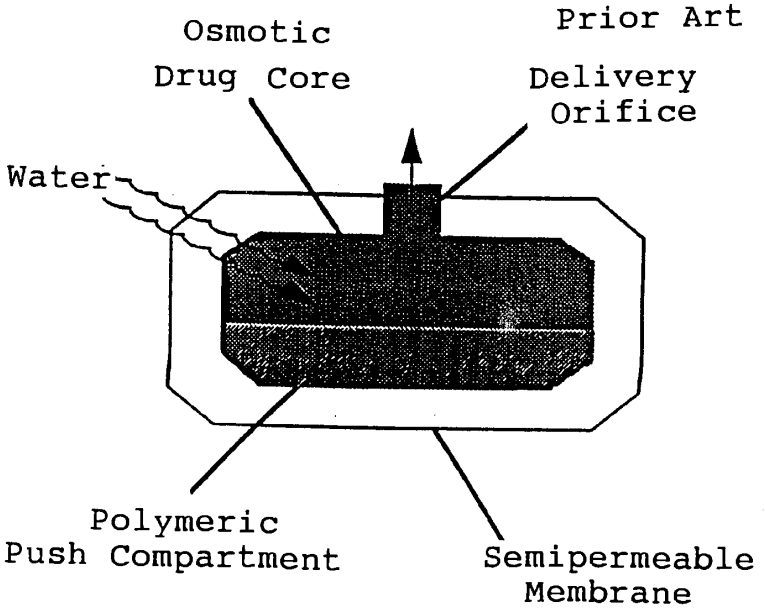


FIG. 2

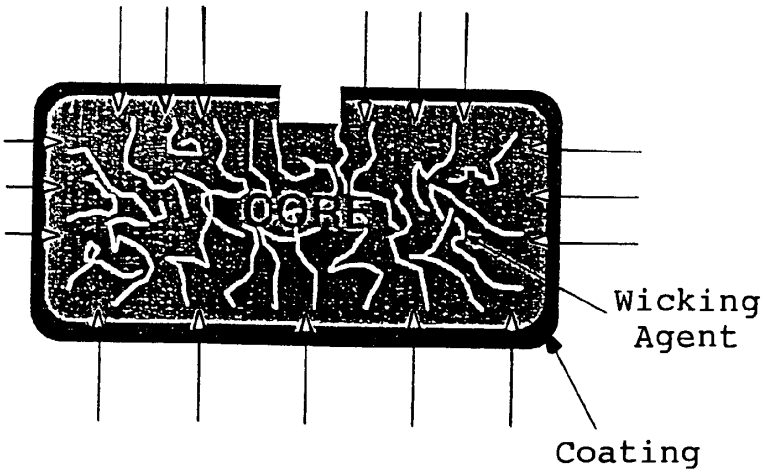


FIG. 3

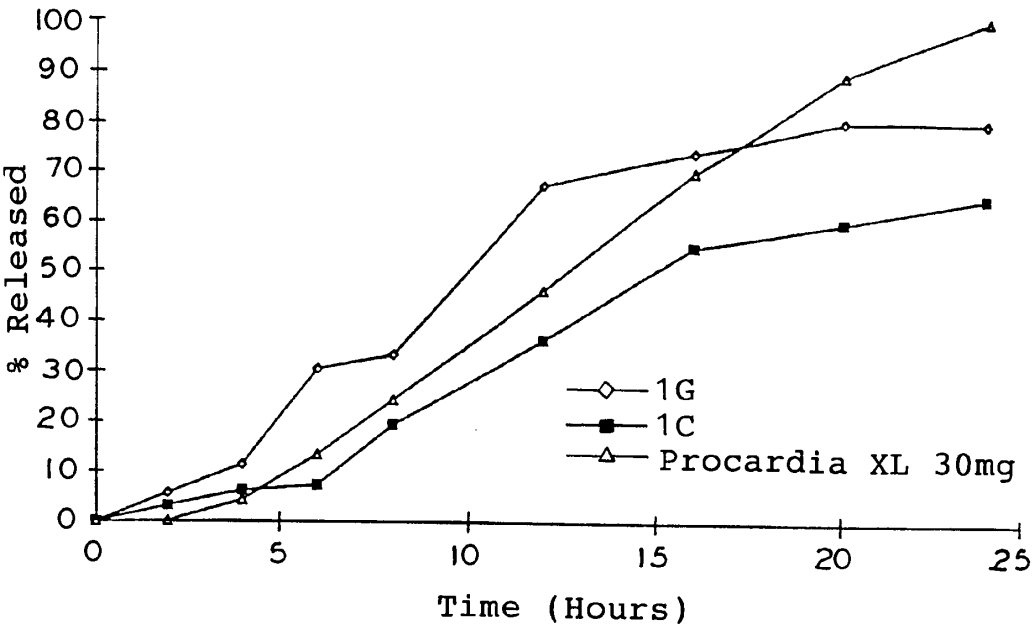


FIG. 4

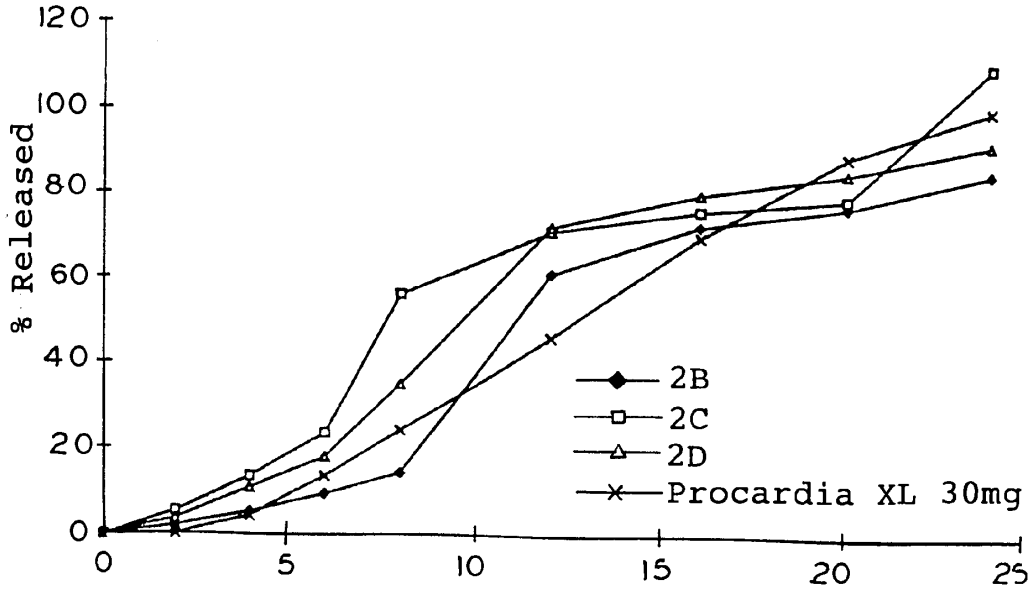


FIG. 5

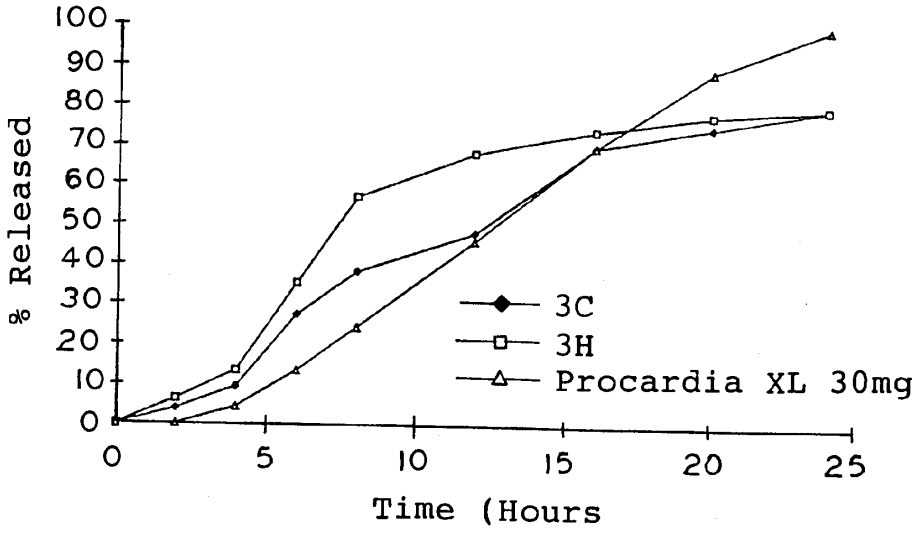


FIG. 6

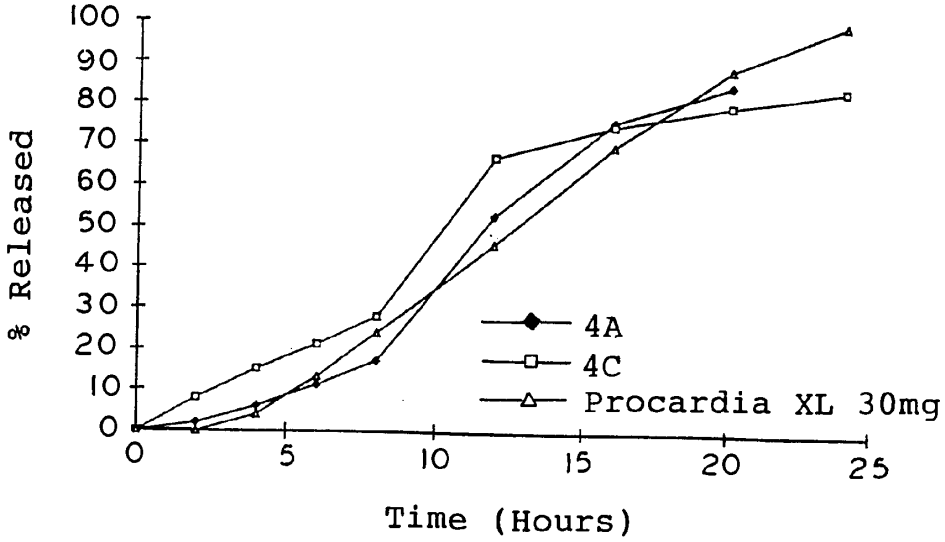


FIG. 7

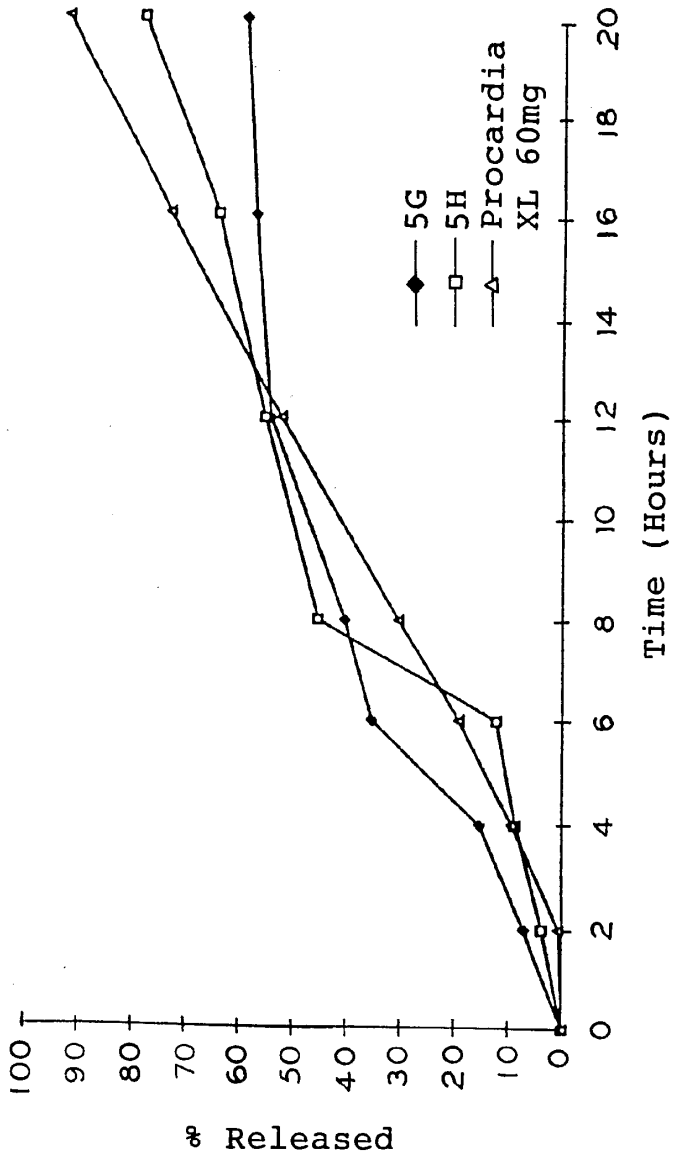


FIG. 8

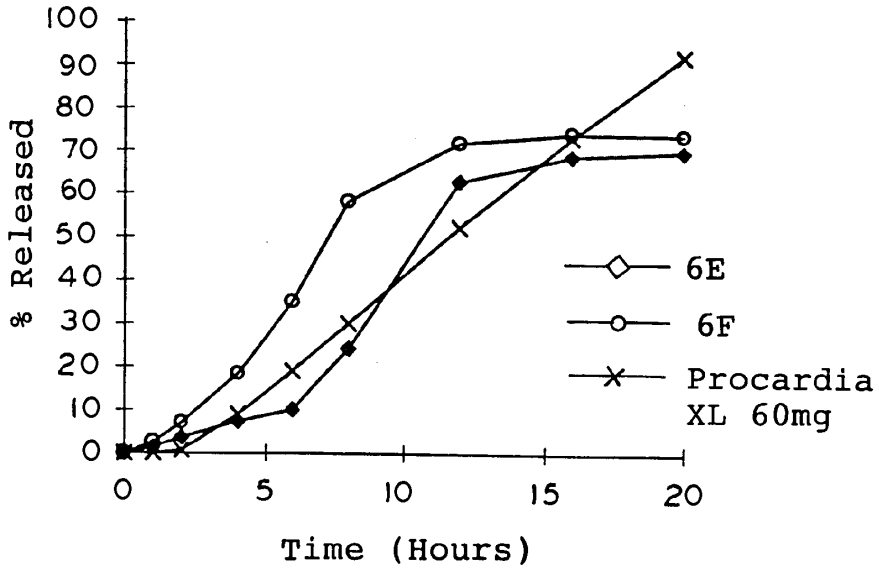
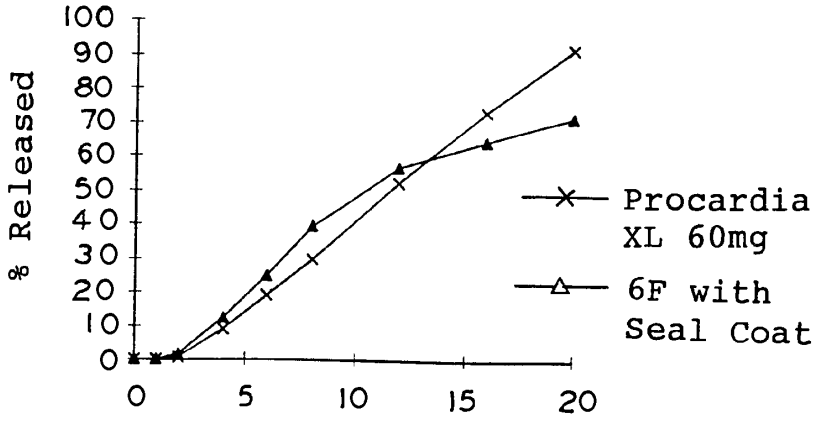


FIG. 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/18912

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/22, 9/44
US CL :424/468, 473, 474

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/468, 473, 474

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,654,005 A (CHEN et al.) 05 August 1997 (05/08/97), see column 3, line 30 through column 5, line 48.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 JANUARY 1998	28 JAN 1998

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	JAMES M. SPEAR
Facsimile No. (703) 305-3230	Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)★

Electronic Patent Application Fee Transmittal

Application Number:	14754932
Filing Date:	30-Jun-2015
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN2
First Named Inventor/Applicant Name:	Hitesh Batra
Filer:	Stephen Bradford Maebius/Karen Strawderman
Attorney Docket Number:	080618-1550

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt	
EFS ID:	25052492
Application Number:	14754932
International Application Number:	
Confirmation Number:	1865
Title of Invention:	PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN2
First Named Inventor/Applicant Name:	Hitesh Batra
Customer Number:	22428
Filer:	Stephen Bradford Maebius/Karen Strawderman
Filer Authorized By:	Stephen Bradford Maebius
Attorney Docket Number:	080618-1550
Receipt Date:	29-FEB-2016
Filing Date:	30-JUN-2015
Time Stamp:	16:12:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$180
RAM confirmation Number	3317
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		IDS.pdf	170736 ce89cc36fa2d77e015df0bcbfbf987f7ee81ebe6	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	2	
	Information Disclosure Statement (IDS) Form (SB08)		3	3	
Warnings:					
Information:					
2	Non Patent Literature	Bighley.pdf	2623108 a302f7171ebdcd21bc35ebb4204bdac7ce97ceb9	no	49
Warnings:					
Information:					
3	Non Patent Literature	Simonneau.pdf	87871 2515adbd0748403b78c5ff7b1b13c94c58954171	no	5
Warnings:					
Information:					
4	Foreign Reference	WO9818452.pdf	1072536 6fbfe44c04082973ab341afba4af852cf6d4e707	no	32
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Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	31031 5a3125e950d3d50c72209110649969b47e4c1f67	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	3985282		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Application No.:	14/754932
Filing Date:	6/30/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation No.:	1865

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR §1.56

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant submits herewith documents for the Examiner's consideration in accordance with 37 CFR §§1.56, 1.97 and 1.98.

Applicant respectfully requests that each listed document be considered by the Examiner and be made of record in the present application and that an initialed copy of Form PTO/SB/08 be returned in accordance with MPEP §609.

The submission of any document herewith is not an admission that such document constitutes prior art against the claims of the present application or that such document is considered material to patentability as defined in 37 CFR §1.56(b). Applicants do not waive any rights to take any action which would be appropriate to antedate or otherwise remove as a

competent reference any document submitted herewith. However, in accordance with MPEP § 609.04(a)(I), Applicant hereby states that for items for which the date of publication supplied does not include the month of publication, the year of publication is sufficiently earlier than the effective U.S. filing date and any foreign priority date so that the particular month of publication is not in issue.

TIMING OF THE DISCLOSURE

The listed documents are being submitted in compliance with 37 CFR §1.97(c), before the mailing date of any of a final action under 37 CFR §1.113, a notice of allowance under 37 CFR §1.311, or an action that otherwise closes prosecution in the application.

FEE

Fees in the amount of $180.00 to cover the fee associated with an information disclosure statement are being paid by credit card via EFS-Web.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this submission under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account Number 19-0741.

Respectfully submitted,

Date Feb. 29, 2016

By /Stephen B. Maebius/

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name:	Hitesh BATRA
Title:	AN IMPROVED PROCESS TO PREPARE TREPROSTINIL, THE ACTIVE INGREDIENT IN REMODULIN®
Appl. No.:	14/754932
Filing Date:	6/30/2015
Examiner:	Yevgeny Valenrod
Art Unit:	1672
Confirmation Number:	1865

NOTIFICATION OF RELATED PROCEEDINGS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant hereby updates the Office concerning the status of a related proceeding styled *Steadymed Ltd. (Petitioner), v. United Therapeutics Corporation (Patent Owner)*, Case IPR2016-00006, US Patent 8,497,393, which involves the issued parent of the above-captioned patent application. Other documents from the above-identified Inter Partes Review (IPR) were submitted in the present application with an Information Disclosure Statement filed on December 8, 2015, for the Examiner's consideration. The purpose of this notice is to provide a copy of Patent Owner's Preliminary Response Under 35 U.S.C. § 313 and 37 C.F.R. § 42.107, Patent Owner Exhibit List and Exhibits 2002 and 2007-2016 as filed on January 14, 2016 from

the IPR proceeding. Certain information in the Preliminary Response is redacted and certain exhibits are not provided due to their filing under seal in the IPR proceeding.

Respectfully submitted,

Date Feb. 18, 2016

By /Stephen B. Maebius/

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Telephone: (202) 672-5569
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Stephen B. Maebius
Attorney for Applicant
Registration No. 35,264

Paper _____

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

STEADYMED LTD.,

Petitioner,

v.

UNITED THERAPEUTICS CORPORATION,

Patent Owner.

Case IPR2016-00006
Patent 8,497,393

Patent Owner Preliminary Response Under 35 U.S.C. § 313 and 37 C.F.R. § 42.107

4827-9995-8060.1

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TABLE OF AUTHORITIES

CASES

STATUTES

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REGULATIONS

I. INTRODUCTION

United Therapeutics Corporation ("Patent Owner") submits this preliminary response under 35 U.S.C. § 313 responsive to the petition for *inter partes* review ("IPR") of claims 1-22 of U.S. Patent No. 8,497,393 ("the '393 patent") filed by SteadyMed LTD. ("Petitioner"). This preliminary response is timely filed within three months of the Board's notice, Paper 3 mailed October 14, 2015, indicating that the petition was accorded a filing date. For the reasons set forth herein and in the accompanying exhibits, Petitioner's petition for IPR should be denied.

II. DEVELOPMENT OF REMODULIN®

Patent Owner holds approved New Drug Application No. 21-272 for Remodulin® (treprostinil) Injection, which Patent Owner markets and sells as Remodulin®. Remodulin® is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1), a rare, fatal disease affecting the pulmonary vasculature. Remodulin® was the second drug to receive FDA approval for the treatment of PAH. Ex. 2002.

When a compound exists in multiple stereoisomeric forms, "[i]t is extremely important to the proper biological function of a drug" to obtain the specific stereoisomer that produces the desired activity, as "other stereoisomers may have

no biological effect or a deleterious biological effect." Ex. 2013[1] at p. 15, ll. 8-17. Treprostinil, the active ingredient of Remodulin®, is a complex prostacyclin analogue compound containing five chiral centers; consequently, thirty-two stereoisomers of the molecule are possible. Ex. 2013, at p. 11, l. 18 – p. 12, l. 18. Only one particular stereoisomer, treprostinil, is able to mimic the function of a natural hormone, prostacyclin, because it has the same configuration at the five chiral centers as the natural hormone prostacyclin. Ex. 2013, at p. 15, ll. 1-8, p. 19, ll. 14-25.

No sample of treprostinil is 100% pure. Ex. 2013, at p. 12, ll. 16-17, p. 41, ll. 22-25. Each sample of treprostinil carries with it characteristic impurities, including other stereoisomers, arising, *inter alia*, from the synthetic process used to form it. Ex. 2013, at p. 132, l. 21- p. 133, l. 2.

[1] In May of 2014, Dr. Williams and Dr. Aristroff provided expert testimony on behalf of United Therapeutics Corporation in *United Therapeutics Corp. v. Sandoz, Inc.*, 3:13-cv-00316-PGS-LHG (D.N.J.), and Ex. 2013 comprises the trial transcript relating to that testimony. While the '393 patent itself was not at issue in that case, Dr. Williams and Dr. Aristoff offered opinions concerning certain subject matter that is relevant to the Board's consideration of the '393 patent.

The '393 patent, entitled "Process To Prepare Treprostinil, The Active Ingredient in REMODULIN®," appears in FDA's Orange Book for Remodulin® and also other products. The claims of the '393 patent are product-by-process claims that resulted from the inventors' discovery that a combination of processes unexpectedly provides a physically different and improved final product with significantly reduced overall impurities and a distinct and unexpected impurity profile.

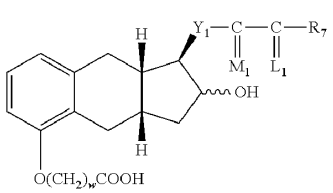
In fact, when the FDA approved the Patent Owner's implementation of the new process steps covered by the '393 patent, the FDA adopted a higher purity specification reflecting the physically changed nature of the product as explained below in Section IX.

III. THE '393 PATENT

The '393 patent issued from U.S. Patent Application No. 13/548,446, filed July 13, 2012, which is a continuation of U.S. Patent Application No. 12/334,731 (now U.S. Patent No. 8,242,305; "'305 patent;" Ex. 2007) filed December 15, 2008, which claims priority to U.S. Provisional Patent Application No. 61/014,232, filed December 17, 2007. Ex. 2008. The '393 patent contains twenty-two product-by-process claims, including two independent claims, directed to an improved treprostinil product.

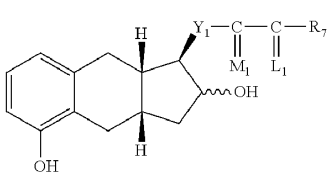
Claim 1 of the '393 patent recites:

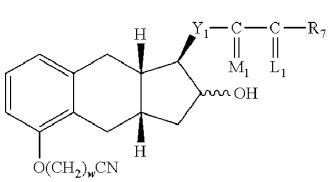
A product comprising a compound of formula I



or a pharmaceutically acceptable salt thereof, wherein said product is prepared by a process comprising

(a) alkylating a compound of structure II with an alkylating agent to produce a compound of formula III,

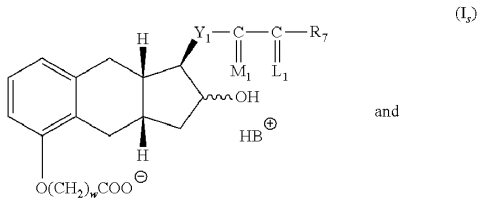




wherein [recitation of Markush groups for the specified structures]…,

(b) hydrolyzing the product of formula III of step (a) with a base,

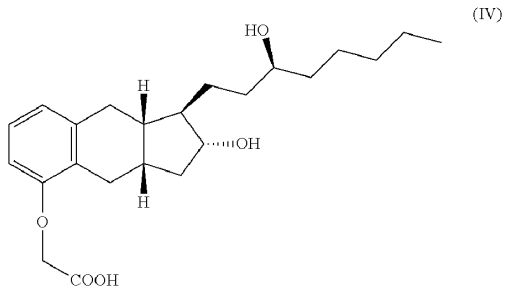
(c) contacting the product of step (h) [sic: (b)][2] with a base B to form a salt of formula I_5.



(d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula I.

Claim 9 of the '393 patent recites:

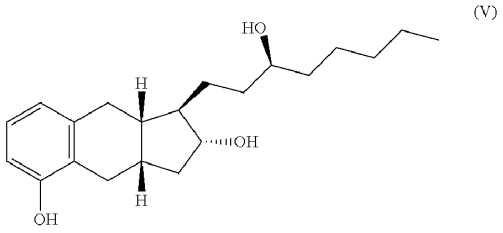
A product comprising a compound having formula IV



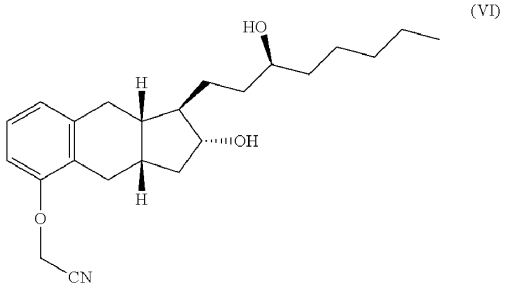
[2] The reference to step "(h)" is an obvious typographical error in claim 1 the '393 patent. The "(h)" should have been "(b)", as indicated in the specification of the '393 patent which correctly recites "(b)". Ex. 1001, p. 4, left column. A person of ordinary skill in the art would recognize that the claims should be read as reciting step (b), as Petitioner's expert has expressly acknowledged (Ex. 1009, ¶ 51) which acknowledgement the Petition has adopted (Pet. at pp. 24-25, and 27, citing ¶ 51).

or a pharmaceutically acceptable salt thereof, wherein the product is prepared by the process comprising

(a) alkylating a compound of formula V with an alkylating agent to produce a compound of formula VI,



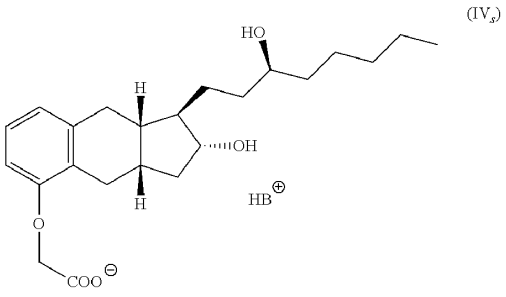
(V)



(VI)

(b) hydrolyzing the product of formula VI of step (a) with a base,

(c) contacting the product of step (h) [sic: (b)][3] with a base B to form a salt of formula IV_s, and



(IV_s)

[3] See preceding footnote.

(d) optionally reacting the salt formed in step (c) with an acid to form the compound of formula IV

As the specification of the '393 patent explains, there was a need "for an efficient process to synthesize treprostinil compounds on a large scale suitable for commercial production." Ex. 1001. Col. 1 ll.58-61. The '393 patent claims are directed to improved treprostinil products with higher purity that are novel and nonobvious to a person of ordinary skill in the art ("POSA"). Ex. 1001. Col. 5 ll. 46-47.

IV. THE PROSECUTION HISTORIES OF THE '393 PATENT AND RELATED APPLICATIONS ALREADY ADDRESS AND REJECT PETITIONER'S ARGUMENTS

A. Moriarty and Phares were both considered by the Examiner during prosecution of the '393 patent

Petitioner relies heavily on Moriarty (Moriarty *et al.*, J. Org. Chem. 2004, 1890-1902; Ex. 1004) and Phares (International Publication No. WO 2005/007081; Ex. 1005), but these references already were considered by the Examiner during original prosecution. Specifically, the Examiner considered these references in the context of rejections under 35 U.S.C. § 102 and 35 U.S.C. § 103, which were overcome, as discussed in more detail below. This is reflected on the face of the patent, which includes Moriarty (Ex. 1004) and Phares (Ex. 1005) as References Cited, and in the prosecution history of the '393 Patent and related patents.

The Examiner's prior consideration of these references undermines any reasonable likelihood that Petitioner will prevail in this IPR with respect to any claim. The Examiner has already considered these references and found neither anticipation nor obviousness. By now asking the Board to consider very similar arguments about the same references in the hopes they will reach the opposite conclusion, Petitioner places unjustified and redundant demands upon the Office's resources. The additional references cited by Petitioner (Kawakami and Ege) add no significant teachings, and the basic arguments underlying the petition already have been thoroughly addressed by the Office.

B. The Examiner considered Moriarty during prosecution of the '393 patent and ultimately found no anticipation

As originally filed, the application resulting in the '393 patent contained 21 claims. Ex. 1002. The Examiner rejected originally filed claims 1-21 as anticipated by Moriarty. Ex. 1002 at pp. 293-296. The Examiner initially found that Moriarty discloses a "compound 7" that has the same structure as the claimed product and a purity of 99.7%. Ex. 1002 at p. 295. According to the Examiner, "[s]ince the claims were product by process claims, the patentability of the product did not depend on the method of its production." *Id.* Applicants submitted a response explaining that the impurity profile of the claimed invention was different as compared to Moriarty's product. The Examiner issued a final action

maintaining the rejection because Applicants evidence was not presented as a declaration. Ex. 1002 at pp. 325-330.

In response, Patent Owner submitted "a declaration under 37 C.F.R. § 1.132 by Dr. David Walsh." Ex. 1002, at pp. 346-350. Dr. Walsh provided data from representative Certificates of Analysis with impurity profiles for treprostinil prepared according to the process corresponding to Moriarty, treprostinil diethanolamine prepared according to the process specified in claim 1 or 10 [issued claim 9], and treprostinil as the free acid prepared according to the process specified in claim 1 or 10 [issued claim 9]. *Id.* Dr. Walsh concluded that the claimed treprostinil differed from the treprostinil produced by Moriarty:

> "[E]ach of treprostinil as the free acid and treprostinil diethanolamine prepared according to the process specified in claim 1 or 10 [issued claim 9] of the present application is physically different from treprostinil prepared according to the process of 'Moriarty' at least because neither of them contains a detectable amount of any of benzindene triol, treprostinil methyl ester, 1AU90 treprostinil stereoisomer and 2AU90 treprostinil stereoisomer, each of which were present in detectable amounts in treprostinil produced according to the process of 'Moriarty.'"

Ex. 1002, at pp. 347-349. He further noted that in these representative examples, the "treprostinil diethanolamine prepared according to claims 1 or 10 of the present

application has only one impurity, treprostinil stereoisomer 3AU90, in a detectable amount" and that "treprostinil as the free acid prepared according to claims 1 or 10 of the present has only three impurities, treprostinil ethyl ester, treprostinil dimers 750W93 and 751W93." Ex. 1002, at pp. 348-349.

The Examiner subsequently allowed claims 1-23 (claim 8 was later canceled by Patent Owner), which issued as claims 1-22 in the '393 patent. Ex. 1002, at pp. 359, 370-376. Thus, the Examiner fully considered Moriarty's impact on patentability and determined that the claims are patentable over Moriarty.

C. The Examiner considered Phares combined with Moriarty during prosecution of the '393 patent and ultimately found no obviousness

The Examiner also considered Phares during prosecution of the application leading to the '393 patent and asserted an obviousness rejection based upon the combination of Moriarty in view of Phares. Ex. 1002, at pp. 122-123. Specifically, the Examiner stated that "[t]he instant invention amounts to addition of a purification step via crystallization." Ex. 1002, at p. 123. This is effectively the same as one of Petitioner's arguments. Petition at section VIII.B.2. Patent Owner provided a detailed response to this rejection, which is detailed below in this Preliminary Response. *See* Ex. 1002, at pp. 176-183; *see infra* Section IV.D.

D. The Examiner considered Phares alone and in combination with Moriarty during prosecution of a related continuation application and found no anticipation or obviousness

Phares was also cited under 35 U.S.C. §§ 102 and 103 during prosecution of a continuation application of the '393 patent, U.S. Patent Application No. 13/910,583, filed June 05, 2013 (now U.S. Patent No. 8,748,657; "the '657 patent;" Ex. 2009). The '657 patent contains seven product-by-process claims, including one independent claim.

Claim 1 of the '657 patent recites:

> A process for producing a pharmaceutical composition comprising treprostinil, comprising providing a starting batch of treprostinil having one or more impurities resulting from prior alkylation and hydrolysis steps, forming a salt of treprostinil by combining the starting batch and a base, isolating the treprostinil salt, and preparing a pharmaceutical solution from the isolated salt comprising treprostinil or a pharmaceutically acceptable salt thereof from the isolated treprostinil salt, whereby a level of one or more impurities found in the starting batch of treprostinil is lower in the pharmaceutical composition, and wherein said alkylation is alkylation of benzindene triol.

The Examiner asserted that "Phares discloses a method of producing a pharmaceutical composition comprising combining a starting batch of treprostinil

which comprises treprostinil, ethanol and water with diethanolamine to produce treprostinil diethanolamine salt … Since the process steps of claim 1 are the same as the process steps described by Phares et al, the purity of the Phares salt is inherently the same as the instantly claimed purity of claims 3 and 10." Ex. 2010, at pp. 164-167 (Office Action mailed July 19, 2013).

In response, Patent Owner amended claim 1 to recite "the starting batch of treprostinil has one or more impurities resulting from prior alkylation and hydrolysis steps." Patent Owner explained that "Phares neither anticipates nor renders obvious amended claim 1 or any claim depending from it because Phares discloses more than one process for providing a starting batch of treprostinil and because Phares does not provide evidence of whether salt formation can remove any impurities from a given type of treprostinil starting material." Ex. 2010, at pp. 149-155. At that time, Patent Owner explained to the Examiner that Phares provides at least two routes for producing the diethanolamine salt of treprostinil. One of those routes prepares treprostinil without prior alkylation and hydrolysis as recited in steps (a) and (b) of the '393 independent claims, and the other route employs prior alkylation and hydrolysis. Ex. 2010, at pp. 151-154.

Patent Owner further explained why Phares does not inherently result in the claimed product :

> [T]here are several different processes for preparing a starting batch of treprostinil, only one of which leads to treprostinil having one or more impurities resulting from prior alkylation and hydrolysis steps. Therefore, Phares does not inherently and necessarily result in a process in which the same kind or amount of impurities are present in the starting batch and in which the level of one or more such impurities resulting from prior alkylation and hydrolysis steps is reduced in the final product as required by claim 1. For this reason alone, Phares cannot anticipate the present claims based on inherency.

Ex. 2010, at p. 153. The "Examiner agree[d] with the applicant that the amended claims are not anticipated by Phares" (Final Office Action mailed August 20, 2013). Ex. 2010, at pp. 138-144.

Having conceded novelty over Phares, the Examiner then asserted that the pending claims were unpatentable under 35 U.S.C. § 103 over Phares in view of Moriarty. Ex. 2010, at p. 140. Although the claims of the '657 patent and the '393 patent are not identical, the issues raised by the Petitioner for the '393 patent with regard to Phares and Moriarty are indistinguishable from the issues raised by the Examiner during the prosecution of the '657 patent.

In response, the Patent Owner stated in a Request for Continued Examination (RCE) that Phares does not teach forming a salt intermediate in a

process that allows reduction of one or more impurities resulting from prior alkylation and hydrolysis steps. Ex. 2010, at pp. 39-43. Since Moriarty does not teach or suggest forming an intermediate salt to remove impurities, even if Phares and Moriarty were combined, Patent Owner noted there still would have been no motivation to perform the claimed process. *Id.* Following an agreement to cancel certain product claims, the Examiner then directly issued a Notice of Allowance for remaining process claims 1-7 based on the earlier arguments. Ex. 2010, at pp. 5-8.

E. The Board should exercise its discretion to decline to institute trial

Because Petitioner's arguments are a rehashed version of issues already considered and rejected by the Office, the Board should exercise its discretion under 35 U.S.C. § 325(d) to decline to institute trial. *See* 35 U.S.C. § 325(d); *Prism Pharma Co., Ltd. v. Choongwai Pharma Corp.*, IPR No. 2014-00315 (Paper 14, July 8, 2014) (Because "[t]he same prior art . . . and arguments substantially the same as Petitioner's current contention . . . were presented previously to the Office" during prosecution, Examiner considered, the same prior art and substantially the same arguments" during prosecution, the Board chose to "exercise [its] discretion and deny the Petition under 35 U.S.C. § 325(d)"); *Universal Remote Control, Inc. v. Universal Electronics, Inc.*, IPR No. 2014-01084 (Paper 26, December 18, 2015) ("[I]n determining whether to institute an *inter partes* review

we may take into account whether a prior art reference was presented previously to the Office and have discretion to deny a petition on that basis, *see* 35 U.S.C. § 325(d)….").

Phares and Moriarty were considered and applied by the Examiner during original prosecution of the '393 patent in the same way presented by Petitioner and Patent Owner distinguished those references. Petitioner does not cast Phares or Moriarty in a new light or present any persuasive evidence to supplement the record that was previously in front of the Office. Consequently, the facts in this case are like those in *Funai Electric* and the result should be the same – exercising the Board's discretion under § 325(d) and declining to institute trial. *Funai Electric Co. v. Gold Charm Ltd.*, IPR No. 2015-01491 (Paper 15 at 19-20, December 28, 2015).

V. CLAIM CONSTRUCTION

Patent Owner disagrees with Petitioner's proposed construction of the term "product" found in claims 1, 9, and 22, "A product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof" in claims 1 and 9, and "A process comprising" and "the process comprising" in claims 1 and 9. "[O]nly those terms need be construed that are in controversy, and only to the extent necessary to resolve the controversy." *Vivid Tech., Inc. v. Am. Sci. & Eng'g., Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999); *Eli Lilly and Company., v. Los Angeles*

Biomedical Research Institute At Harbor-Ucla Medical Center, IPR2014-00693 (Paper 45, October 22, 2015). Accordingly, Patent Owner focuses claim construction on the contested terms. *Gechter v. Davidson*, 116 F.3d 1454, 1460 (Fed. Cir. 1997); *accord Aero Prods. Int'l, Inc. v. Intex Rec. Corp.*, 466 F.3d 1000, 1012 n.6 (Fed. Cir. 2006) (appropriate to focus on disputed limitations), *citing Scripps Clinic & Research Found. v. Genentech, Inc.*, 927 F.2d 1565, 1580 (Fed. Cir. 1991).

A. Legal Standard

"[C]laim construction begins with, and remains focused on, the language of the claims." *Biagro W. Sales, Inc. v. Grow-More, Inc.*, 423 F.3d 1296, 1302 (Fed. Cir. 2005) (internal citations omitted). Claim terms are generally given their plain and ordinary meaning. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (*en banc*). "[T]he ordinary and customary meaning of a claim term is the meaning that the term would have to a [POSA] in question at the time of the invention, i.e., as of the effective filing date of the patent application." *Id.* at 1313. Indeed, there is "a 'heavy presumption' that a claim term carries its ordinary and customary meaning." *Teleflex, Inc. v. Ficosa N. Am. Corp.*, 299 F.3d 1313, 1325 (Fed. Cir. 2002); *DSW, Inc. v. Shoe Pavilion, Inc.*, 537 F.3d 1342, 1347 (Fed. Cir. 2008) ("[A]bsent contravening evidence from the specification or prosecution history, plain and unambiguous claim language controls the construction

analysis."). Absent a disclaimer of subject matter (*i.e.*, a clear or unmistakable surrender of subject matter in the patent specification or prosecution history) or lexicography explicitly defining a claim term, the plain meaning of the claim controls. *Toshiba Corp. v. Imation Corp.*, 681 F.3d 1358, 1369 (Fed. Cir. 2012).

If a claim term is construed, the Board should construe claim terms such that they have the "broadest reasonable construction in light of the specification of the patent in which it appears." 42 C.F.R. § 42.100(b).

B. "Product"

Petitioner proposed that the term "product" be construed as "a chemical composition." Patent Owner, however, submits that "product" means "a substance resulting from a chemical reaction," which is the "broadest reasonable construction in light of the specification of the patent in which it appears." 42 C.F.R. § 42.100(b). Moreover, this definition is also consistent with the plain and ordinary meaning of the term as used in the context of the '393 patent as well as the intrinsic and extrinsic evidence. In particular, the claims and specification of the '393 patent consistently use the word "product" to refer to a substance resulting from a chemical reaction. Ex. 1001, at Col. 5:45-46 ("the product of the process according to the present invention has higher purity"); Col. 7:16-20 ("a compound of formula XI, which is a cyclization product of a compound of formula X"); Col. 17:37-40 ("This process provides better quality of final product."); and claims 1-22. This

usage is in line with how a POSA would understand the term "product," as the real world product of a chemical reaction, particularly in the context of these product-by-process claims.

In the prosecution history, Patent Owner distinguished the "product" of the claimed invention from the prior art on the basis that both the chemical process steps recited in the claims "and the products resulting from those steps are different than the chemical process and product of" the prior art, noting specifically that the "product" of the claims lacks certain impurities found in the product prepared by the prior art process. Ex. 1002, at p. 315. The Examiner found that the prior art and specification lacked sufficient evidence about impurities in question to support a finding "that the process by which the instantly claimed product is prepared results in a product that is different from the product of" the prior art. Ex. 1002, at p. 328. The Examiner stated that evidence of "data demonstrating the difference between the two products" should be presented in the form of a declaration. Ex. 1002, at pp. 328-329. Accordingly, Dr. David Walsh submitted a declaration providing evidence from representative "product batch[es]" to show that the product of the '393 claims is physically different from treprostinil produced according to the prior art process at least because the product of the '393 claims lacks certain impurities found in treprostinil made by the prior art process. Ex. 1002, at pp. 346-350. Thus, during prosecution, the Patent Owner and Examiner

explicitly discussed the "product" of the claims as a real world substance that results from employing a specific chemical process, as differentiated from the substance obtained from employing a different chemical process. Such usage is consistent with the plain and ordinary meaning of this term and with Patent Owner's proposed construction.

Patent Owner's construction also comports with how a POSA would understand these terms in the context of the '393 patent. Indeed, well-known chemistry textbooks specifically define "product" as "a substance resulting from a chemical reaction; it is shown to the right of the arrow in a chemical equation." Ex. 2011, Zumdahl, *Chemistry*, pp. A25, A36 (1986); *see also* Ex. 2012, Brown, et al., *Chemistry: The Central Science*, pp. G-2, G-10 (9th ed. 2003). Several other references also similarly define or describe a "product" to indicate it is the result of a chemical reaction. Ex. 2014, Suchocki, et al., *Conceptual Chemistry*, p. G-6 (2001).

Simply put, the "product" claimed in a product-by-process claim is necessarily a substance that results from the process specified in that claim. In the case of the '393 patent, wherein the claims specify the process of a certain chemical reaction, the claimed "product" must be understood to be "a substance resulting from a chemical reaction." Patent Owner's proffered definitions, for both the term

"product" and for other related terms that contain this word, comport with this understanding.

Petitioner's definition of "product" as "a chemical composition" is unreasonably broad, as it erroneously removes from the term its identity as a product made by a specified process (*i.e.*, a chemical reaction). In doing so, Petitioner's definition disregards both the intrinsic evidence and the nature of a product-by-process claim. Under Petitioner's over-expansive construction, the term "product" would refer not only to the substance created by a chemical reaction, but also the starting materials, solvents, catalysts, and glassware involved in performing that reaction. Such an unreasonable definition would render the claims nonsensical. The invention of the claims of the '393 patent is not merely "a chemical composition" but a specific, real world substance that results from the chemical process specified in the claims and thus possesses the characteristics that result from employing that process.

C. "A product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof"

Petitioner proposed that the term "A product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof" be construed as "a chemical composition that includes, but is not limited to, a compound of Formula I, or a pharmaceutically acceptable salt thereof, and that may also include other non-

mentioned substances (including impurities), additives, or carriers, without limitation as to the types of or relative amounts thereof." Petition at p. 11.

Patent Owner submits that this term means "a substance resulting from a chemical reaction constituted primarily of formula I/IV or a pharmaceutically acceptable salt thereof." This construction is consistent with how a POSA would understand the term in the context of the patent. The '393 patent is directed toward prostaglandin products, including specifically treprostinil, which are made by a process that results in both high yield and high purity. Ex. 1001. Col. 1:58-60 ("Because Treprostinil, and other prostacyclin derivatives are of great importance from a medicinal point of view, a need exists for an efficient process to synthesize these compounds on a large scale suitable for commercial production."), Ex. 1001. Col. 5:36-49. Indeed, as detailed above with respect to the term "product," the very high purity of the claimed product, which constituted an improvement over the prior art product, was explicitly identified during the prosecution of the '393 patent. *See* section V.B. In light of this, a POSA would understand that the substances described by this term would be substances primarily constituted, respectively, of formula I and formula IV.

Petitioner's proposed construction should be rejected, as it is not a reasonable construction of the term. With respect to the term "product," which is nested within this term, the points made above apply here with equal force, rendering Petitioner's

construction unreasonably overbroad. *See* section V.B. With respect to the remainder of this term, Petitioner's construction is both unreasonable and improperly overbroad. As an initial, matter, Petitioner seeks to construe two terms – one term containing a reference to "Formula I" and the other containing a reference to "Formula IV" – with a single construction that would only refer to "Formula I," inexplicably reading Formula IV out of the term entirely. This is nonsensical and thus inherently unreasonable.

Moreover, Petitioner's construction is unreasonable in that it seeks to broaden the term to encompass "other non-mentioned substances (including impurities), additives, or carriers, *without limitation as to the types or relative amounts thereof*" [emphasis added]. This construction is contradicted by the nature of the claims themselves and finds no support in the specification. The claims at issue are product-by-process claims that claim a real-world product created by a specified chemical process. Thus, the claimed product must have the characteristics, including the characteristic types and amount of impurities that result from the claimed process. During prosecution, Patent Owner pointed to these characteristics as distinguishing the claimed subject matter from the prior art. Ex. 1002, at pp. 315, 344. Petitioner's construction contradicts this inherent limitation of the claims. Additionally, the word "comprising" is defined in the specification as simply meaning "including but not limited to." It does not provide any support for

Petitioner's contradictory construction of these terms as encompassing other substances "without limitation as to the types or relative amounts thereof." Ex. 1001. Col. 4, ll. 22-25. By attempting to introduce the phrase "without limitation as to the types or relative amounts thereof" into these claims terms, Petitioner's construction would render the term unreasonably broad, contradicting the inherent limitations of product-by-process claims that would be understood by one of ordinary skill in the art. Accordingly, Petitioner's proposed construction should be rejected.

D. "A process comprising" and "the process comprising"

Petitioner proposed that the terms "A process comprising" and "the process comprising" be construed as "a process that includes, but is not limited to, the recited process steps, and may include, without limitation, any other non-recited steps." Petitioner's proposed construction repeats the words "a process," and thus effectively only seek to construe the word "comprising." Yet the word "comprising" is a well-understood term with no ambiguity. The Federal Circuit has noted that "[i]n the patent claim context the term 'comprising' is well understood to mean 'including but not limited to.'" *CIAS, Inc. v. Alliance Gaming Corp.,* 504 F.3d 1356, 1360 (Fed. Cir. 2007). This well-understood meaning is consistent the patent specification's definition of "comprising" to mean "including but not limited to." Ex. 1001. Col. 4, ll. 22-25. Accordingly, Patent Owner submits that the terms

"a process comprising" and "the process comprising" have an indisputable meaning: "a/the process including but not limited to."

Petitioner asserted that its construction was supported by Patent Owner's definition of "comprising" as meaning "including but not limited to" and that "other non-mentioned … steps may be present." Petition at p. 16. Yet Petitioner's proposed phrase "and may include, without limitation, any other non-recited steps" is unsupported by the patent specification. Petition at p. 17. In particular, the phrase "without limitation" in Petitioner's proposed construction threatens to swallow the essential elements of the claim. As such, Petitioner's proposed construction should be rejected.

The Board should adopt the Patent Owner's construction of the terms "Product"; "A product comprising a compound of formula I/IV or a pharmaceutically acceptable salt thereof"; and "A process comprising" and "the process comprising," by applying the "broadest reasonable construction in light of the specification of the patent in which it appears" standard. 42 C.F.R. § 42.100(b).

VI. STANDARD OF REVIEW

The Board may not grant a petition for IPR unless the Board "determines that the information presented in the petition filed under section 311 and any

response filed under section 313 shows that there is a reasonable likelihood that the petitioner would prevail." 35 U.S.C. § 314(a).

Importantly, § 314(a) requires the Board's determination to be based on "information presented in the petition." Likewise, the petitioner has a statutory obligation under § 312(a)(3) to identify "with particularity, each claim challenged, the grounds on which the challenge to each claim is based, and the evidence that supports the grounds for the challenge to each claim." Thus, it the responsibility of the Petitioner in the first instance, not the Board, to present information adequate to justify institution on any grounds.

Equally important is § 314(a)'s requirement that the Board's determination take into account "information presented in . . . any *response* filed under section 313." (emphasis added) In other words, the Board's determination must be based on the totality of the written evidence presented at the pre-trial stage.

Ultimately, the focus of the inquiry under § 314(a) is whether the petitioner "would prevail"—i.e., *win on the merits* based exclusively on the "information presented in the petition . . . and any response."

VII. THE PETITION SHOULD BE DENIED BECAUSE IT RAISES ISSUES ALREADY ADDRESSED IN PROSECUTION

As indicated above, the core issues presented in the Petition have been extensively addressed in prosecution of applications related to the '393 patent. The

Board should use its discretion under 35 U.S.C. § 325(d) to deny some or all of the Grounds in the Petition because the same or substantially the same issues were addressed during prosecution.

For Grounds 2 and 3, the first alternative (*i.e.,* over Moriarty and Phares, and for Ground 3, Ege) should be denied under § 325(d) because the same or substantially the same prior art was considered by the Office during prosecution. *See* IPR2015-00525, Paper No. 12, p. 17 (denying institution of petition that raised arguments already considered by Examiner); IPR2015-01491, Paper 15, pp. 19-20 (same). The USPTO explicitly considered Moriarty and Phares in combination, and by Petitioner's own admission, Ege is nothing more than a first-year organic chemistry textbook. Thus, the same or substantially the same prior art has already been considered. Furthermore, while Patent Owner acknowledges that the Board has in the past declined to exercise the discretion afforded by § 325(d) when Petitioner submits evidence in the form of a declaration along with previously-considered prior art, in the case of at least claims 6, 15, 21, and 22, Petitioner relies on nothing more than conclusory statements in three paragraphs of the Winkler declaration. As stated in 37 C.F.R. § 42.65(a), "testimony that does not disclose the underlying facts or data on which the opinion is based is entitled to little or no weight." Thus, for at least these claims, Petitioner has provided no evidence of

probative value that is any different than what was already before the Patent Office during prosecution.

VIII. GROUND 1: THE PETITION SHOULD BE DENIED BECAUSE PETITIONER HAS FAILED TO PROVIDE ANY EVIDENCE THAT A SINGLE EMBODIMENT OF PHARES WOULD INHERENTLY RESULT IN THE SAME PRODUCT AS THAT CLAIMED IN ANY OF CLAIMS 1-5, 7-9, 11-14 OR 16-20 OF THE '393 PATENT

Petitioner asserts in Ground 1 that Phares anticipates claims 1-5, 7-9, 11-14, and 16-20 of the '393 patent under 35 U.S.C. 102(b). Petitioner's arguments are misplaced for two primary reasons.

First, Petitioner is unable to identify a single embodiment in Phares that would anticipate any claim of the '393 patent. Instead, Petitioner cobbles together disclosure from four disparate portions of Phares covering multiple distinct embodiments (pp. 24, 41-42, 85-93, and 99 of Ex. 1005). At the same time, Petitioner selectively ignores other portions in the Phares disclosure that suggest the four disparate portions of Phares should not be cobbled together to a single allegedly anticipatory embodiment. Petition at pp. 22-24 and 33-34. Of the four regions, three (pp. 24, 85-93, and 99) relate to step (c), and only one (pp. 41-42) relates to steps (a)-(b) of claims 1 and 9. This patchwork approach to anticipation is improper because "[t]he identical invention must be shown in as complete detail as is contained in the …claim." *Richardson v. Suzuki Motor*, 868 F.2d 1226 (Fed. Cir. 1989). To anticipate, "[the] reference must clearly and unequivocally

disclose the claimed [invention] or direct those skilled in the art to the [invention] without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited reference". *In re Arkley*, 455 F.2d 586, 587 (CCPA 1972) (emphasis in original), *see also Sanofi-Synthelabo v. Apotex Inc.*, 550 F.3d 1075 (Fed. Cir. 2008)(*citing Arkley*). Moreover, if the teachings of the prior art can be practiced in a way that yields a product lacking the allegedly inherent property, the prior art in question does not inherently anticipate. *See Glaxo Inc. v. Novopharm Ltd.,* 52 F.3d 1043, 1047–48 (Fed. Cir. 1995).

Second, even if Phares is effectively rewritten as argued by Petitioner, Phares does not teach each and every element of the challenged claims. Specifically, Petitioner is forced to rely on inherency because Petitioner concedes that Phares lacks express disclosure of certain claim elements. *E.g.*, Petition at 24-25 and 28. Proving inherency, however, is a high burden. "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic." *In re Rijckaert*, 9 F.3d 1531 (Fed. Cir. 1993). In the context of an IPR, the Board requires more than conclusory statements in an expert declaration that something is inherent, and the Board has declined to institute when petitioner has not carried its burden to show a result is inherent based on objective evidence. *See* IPR2014-01116, Paper No. 12, p. 10 (quoting 37 C.F.R. § 42.65(a): "testimony that does not disclose the

underlying facts or data on which the opinion is based is entitled to little or no weight"). As shown below, Petitioner has not provided such objective evidence of inherency in this case.

A. Petitioner cannot pick and choose from unrelated portions of Phares to establish anticipation

In attempting to show inherent anticipation, Petitioner cites four different portions of Phares, Ex. 1005, as teaching the combined elements of claims 1 and 9. Inherent anticipation requires that the undisclosed feature must "necessarily and inevitably" flow from practice of what is disclosed in the prior art reference. *Schering Corp. v. Geneva Pharms., Inc.*, 339 F.3d 1373, 1378 (Fed. Cir. 2003). Yet Petitioner does not, and indeed cannot, show that these elements in Phares "necessarily and inevitably" give rise to the treprostinil synthesis required by claims 1 and 9 of the '393 patent: first, because there would be no reason to combine the disparate portions of Phares identified by Petitioner, which cover different subject matter; and second, because Phares explicitly points to methods of practicing its teachings which would not lead to the invention of the '393 patent. While Petitioner cites three portions of Phares, specifically Ex. 1005, at pp. 24, 85-93, and 99, as teaching a salt formed by step (c) (discussed in detail below), Petitioner only cites one portion of Phares, specifically Ex. 1005, at pp. 41-42, as teaching steps (a) and (b). Yet Phares (Ex. 1005) at pp. 41-42 is not concerned

with the synthesis of treprostinil itself, but instead with the synthesis of *the enantiomer* of treprostinil. Ex. 1005, at pp. 41-42 (Specifically noting that "*(-)-treprostinil* can be synthesized as follows" and then summarizing that "the *enantiomer* of the commercial drug (+)-Treprostinil was synthesized." [emphasis added]). Accordingly, there is no reason to connect this portion of Phares, which discloses a synthesis for the *enantiomer* of treprostinil, with the other portions relied upon by Petitioner, which all relate to treprostinil itself. Further, Petitioner does not provide any evidence that this same synthesis was in fact used to make the starting treprostinil material associated with any of the three portions of Phares Petitioner cites as disclosing step (c).

Moreover, Petitioner ignores other parts of Phares that disclose different ways of making treprostinil that do *not* include steps (a) and (b). For example, Phares states that "[c]ompounds of the present invention can also be provided by modifying the compounds found in US Patent Nos. 4,306,075 and 5,153,222 in like manner." Ex. 1005, at p. 11. The method of making treprostinil disclosed in U.S. Patent No. 4,306,075 is diagrammed in Moriarty at "Scheme 2." Ex. 1004, at p. 4 (see footnote 26 citing "US 4306075"). This scheme does not involve steps (a) and (b) of claims 1 and 9 of the '393 patent. Thus, Phares explicitly provides that treprostinil can be made by methods that do not include steps (a) and (b). When "the teachings of the prior art can be practiced in a way that yields a product

lacking the allegedly inherent property, the prior art in question does not inherently anticipate." *United Therapeutics Corp. v. Sandoz, Inc.*, 2014 U.S. Dist. LEXIS 121573, *80-81 (D.N.J. Aug. 29, 2014) (*citing Glaxo Inc. v. Novopharm Ltd.*, 52 F.3d 1043, 1047-48 (Fed. Cir. 1995)). Likewise here, these disparate portions of Phares cannot be combined to inherently anticipate the invention of the '393 patent. As discussed above, this same issue of combining disparate portions was addressed in a similar way during prosecution of an application related to the '393 patent and ultimately led to the Examiner's decision to withdraw an anticipation rejection over Phares.

B. Phares does not anticipate step (c) of the '393 patent claims

The first portion in Phares cited by Petitioner for teaching step (c) is p. 24 of Ex. 1005, which reads: "Treprostinil acid [of unknown origin] is dissolved in a 1:1 molar ratio mixture of ethanol:water and diethanolamine is added and dissolved. The solution is heated and acetone is added as an antisolvent during cooling." This describes an example of how to make treprostinil diethanolamine from a starting material of treprostinil acid. This example, however, provides no detail whatsoever about how the starting treprostinil acid was made or where it comes from. Consequently, this lack of disclosure fails to demonstrate inherent anticipation for lack of evidence. Nothing on Phares' p. 24 shows that the treprostinil acid was made using steps (a) and (b).

The second portion in Phares cited by Petitioner for teaching step (c) is pp. 85-93 of Ex. 1005. This portion of Phares relates to a clinical study of sustained release capsules and tablets of treprostinil diethanolamine and to a polymorph characterization study of treprostinil diethanolamine. Again, there is no indication in this portion of Phares what process was actually used to make the starting "treprostinil acid" for the treprostinil diethanolamine that is the subject of these pages.

The third portion in Phares cited by Petitioner for teaching step (c) is p. 99 of Ex. 1005, which is claim 49 directed to the diethanolamine salt of treprostinil. Again, there is no indication in this portion of Phares what process was used to make the starting treprostinil acid leading to the treprostinil diethanolamine that is the subject of the claim.

Because the portions of Phares relied upon by Petitioner do not necessarily describe a treprostinil acid made using steps (a) and (b), these portions of Phares cannot constitute an anticipatory disclosure. Indeed, as discussed below and as disclosed by Phares itself, treprostinil acid can be made in a variety of ways, which in turn, impact the composition of the final product.

C. Petitioner has not shown that step (c) would necessarily lead to the same final product if made from different starting treprostinil materials

As stated in the '393 patent itself, treprostinil free acid can be made in different ways. Treprostinil was first disclosed in U.S. Patent No. 4,306,075 ("the '075 patent") and was produced by a method that does not include steps (a) and (b). This alternative process of the '075 patent for making a starting treprostinil material is one of the references cited in Phares. This method resulted in a mixture of diastereomers and a physically different form of treprostinil. Ex. 1004, at pp. 3-4. Later in U.S. Patent No. 4,668,814 ("the '814 patent" Ex. 2015), a slightly better scheme for synthesizing an impure treprostinil product was developed. This new route included an alkylation and hydrolysis step similar to steps (a) and (b) of the '393 patent (Ex. 2015, at Col. 30-32), producing a physically different form of treprostinil, though this scheme also resulted in a mix of diastereomers and could not be scaled up. Ex. 2013, at p. 178, ll. 8-22, p. 193, l. 21-p. 195, l. 8; p. 197, l. 20-p. 198, l.3; p. 219, l. 15-p. 220, l. 4; p. 338, l. 14-p. 339, l. 2. As referenced above, Moriarty was a synthesis that reduced impurities, increased yields, and resulted in a different impurity profile. Ex. 1002, at pp. 346-350. Each method was an improvement over the previous one and each resulted in a different product with different impurity profiles. Thus, at the time of the '393 patent, there existed at least three prior art methods to obtain an impure treprostinil product.

During prosecution, Patent Owner demonstrated that the final treprostinil product from the '393 patent is physically different than that of Moriarty. Thus, even if the Moriarty treprostinil was used for Phares, Petitioner has failed to provide any evidence that the final Phares treprostinil product would necessarily be the same as the products claimed in the '393 patent. Specifically, the declaration of Dr. Walsh submitted during original prosecution shows that certain impurities in representative examples are reduced below detectable amounts by step (c), while others are still present in detectable amounts, such as treprostinil stereoisomer 3AU90. Ex. 1002, at pp. 346-350. Both the type of impurity, as well as the relative amount of that impurity in the starting treprostinil material, may impact the impurity profile of the final product after step (c), yet there is absolutely no disclosure of any specific impurities or total amount of impurities in Phares and Petitioner has failed to provide any further evidence on this point.

Phares simply does not disclose what starting treprostinil material is used. Thus, Phares cannot inherently anticipate the final treprostinil product of the '393 patent because each method would result in a distinct impurity profile. Indeed, the particular process by which a treprostinil product is made will affect the impurity profile and total amount of impurities in the final product, and thus each process may result in a structurally different treprostinil product. Ex. 2013, at p. 207, ll. 19-24. Petitioner has failed to show that performing step (c) on a starting

treprostinil material with a different impurity profile than a starting treprostinil material made a different way would *necessarily* lead to an identical product after step (c). For this reason alone, there can be no inherent anticipation based on a teaching of a treprostinil salt product that does not identify the source of its starting treprostinil material, as is the case with Petitioner's reliance on each of the three separate portions of Phares discussed above.

Additionally, Petitioner fails to identify any specific purity in Phares that would anticipate any claim of the '393 patent. Instead, Petitioner relies on melting point alone as a proxy for a certain purity, but melting point does not disclose any specific impurity level and instead may demonstrate a different form, or polymorph, of treprostinil diethanolamine altogether. Petition at pp. 27-28. Just because Phares discloses a higher melting point does not mean that it is necessarily a "higher purity" or even necessarily the same polymorph of treprostinil diethanolamine. While Petitioner admits that Phares and the '393 patent cite different melting point ranges for treprostinil diethanolamine, it ignores the fact that different melting points can result in different forms or polymorphs of treprostinil diethanolamine. *Id.*; *see also* Ex. 1005, pp.88-93; Ex. 1001, Example 3 and 4. Given that both Phares and the '393 patent provide different melting points for the treprostinil diethanolamine polymorph, Petitioner has not carried its burden to show that these materials are the same form, let alone the same purity.

Moreover, despite providing an expert declaration, Petitioner provides no additional evidence regarding the purity, melting point, or polymorph structure of either Phares or the '393 patent. *Glaxo Inc.*, 52 F.3d at 1047-48 (finding no inherent anticipation where testing evidence demonstrated that the prior art example could yield crystals of either the claimed polymorph or a different polymorph). On this additional basis, Phares cannot inherently anticipate the claims of the '393 patent. For these reasons, Petitioner's petition for IPR should be denied as to Ground 1.

IX. THE FDA ACCEPTED A NEW PURITY SPECIFICATION WHEN PATENT OWNER IMPLEMENTED CLAIMS 1 AND 10 OF THE '393 PATENT

Ex. 2004 is a process validation report (Protocol No. "VAL-00131"), which states at p. 3 that it applies to "production of treprostinil diethanolamine intermediate (UT-15C-I), a chemical intermediate used for the production of active pharmaceutical ingredients treprostinil (UT-15) and treprostinil diethanolamine (UT-15C)." Ex. 2004, at pp. 5-7 shows that [redacted].

Ex. 2005 is a Process Optimization Report that provides results for batches resulting from step (d) of claims 1 and 10 in the '393 patent, which was performed on specific batches of the diethanolamine salt intermediate produced by steps (a)-(c) [redacted].

Ex. 2005, at p. 3 states that "[redacted]" The percent yield and purity levels of the final treprostinil product are compared to the former process in a chart on Ex. 2005, at p. 3, further demonstrating the differences that result in the final treprostinil product when all of steps (a)-(d) of claims 1 and 10 of the '393 patent are performed.

Ex. 2006 is a letter from Patent Owner to the FDA, which references the VAL-00131 report of Ex. 2004 and states as follows:

[redacted]

Ex. 2006, at p. 2. In addition, Ex. 2006, at p. 3 states as follows:

In all lots, the total unidentified impurity level (%AUC) decreased from triol to UT-15C intermediate. [redacted]

Finally, Ex. 2006, at pp. 3-4 states that, when the new process was implemented, "it was observed that the purity of the treprostinil improved close to 100%", and the letter proposes that "the range of the specification for the HPLC assay for treprostinil be shifted from [redacted]% to [redacted]% [redacted]."

The FDA subsequently approved the Patent Owner's proposed implementation of the '393 process and the increased purity standard. Ex. 2003.

X. GROUNDS 2 AND 3 OF THE PETITION SHOULD BE DENIED BECAUSE THE TRANSLATOR'S DECLARATION FOR KAWAKAMI IS INSUFFICEINT

All Grounds relying on Kawakami should be denied because Petitioner relies on an improper translation of the Japanese-language reference. Ex. 1011, at p. 1. When a party relies on a document or is required to produce a document in a language other than English, a translation of the document into English and an affidavit attesting to the accuracy of the translation must be filed with the document. 37 C.F.R. § 41.154(b). However, the declarant, Boris Levine, states that another individual (James Dowdle) carried out the translation. The declaration does not indicate that the declarant understands Japanese, but rather that someone else, James Dowdle, knows that language. The actual translator did not submit

declaration. Thus, the declaration is objectionable under Federal Rule of Evidence 602 because the declarant lacks personal knowledge of the relevant facts, e.g., the accuracy of the translation. The declaration is also objectionable as irrelevant under Federal Rule of Evidence 402 because the accuracy of the translation cannot be determined. The Levine declaration is of no probative value because the declarant has no personal knowledge regarding the accuracy of the translation, and any belief the declarant may have is not founded on evidence because he had no way of determining whether Mr. Dowdle's translation was accurate. *See*, 37 C.F.R. § 1.68. Petitioner's use of this declarant also means that the real translator is shielded from cross-examination, contrary to what is required by the IPR rules and Trial Practice Guide. For these reasons, the translation of Kawakami, and therefore Kawakami itself, should not be included in evidence and Grounds 2 and 3 should be denied on this basis alone.

XI. GROUND 2 SHOULD BE DENIED BECAUSE IT FAILS TO ESTABLISH A REASONABLE LIKELIHOOD OF SUCCESS THAT ANY OF CLAIMS 1-5, 7-9, 11-14, OR 16-20 WOULD HAVE BEEN OBVIOUS

Petitioner asserts that claims 1-5, 7-9, 11-14, and 16-20 are rendered obvious under 35 U.S.C. § 103 when considering Moriarty in view of either Phares or Kawakami. Petition at p. 4. Petitioner, therefore, has asserted Ground 2 in the alternative. Either Moriarty in view of Phares, or Moriarty in view of Kawakami is

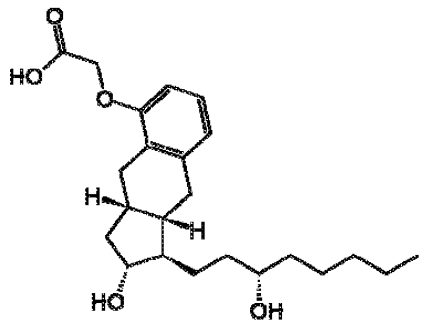
asserted against claims 1-5, 7-9, 11-14, and 16-20. Patent Owner addresses the deficiencies of each alternative in turn below.

A. Petitioner fails to establish a motivation to combine Moriarty with Kawakami with a reasonable expectation of success

Even if the Board accepts the defective declaration for Kawakami, Petitioner has failed to establish that the '393 patent would have been obvious in view of any Kawakami combination. Simply put, Kawakami is directed to entirely different compounds with entirely different impurity profiles. Nothing in Kawakami comes close to addressing the treprostinil product of the '393 patent much less how a POSA would or would not go about synthesizing or purifying the product. Thus, a POSA would have no motivation to combine Moriarty with Kawakami and no reasonable expectation of success of obtaining the same high purity treprostinil product of the '393 patent.

As previously described, Moriarty fails to disclose the high purity treprostinil product of the '393 patent, much less the same impurity profile. Kawakami is asserted as allegedly remedying these deficiencies. However, Petitioner does not establish a motivation or reasonable expectation of success of forming a salt of the compounds in Moriarty with a purity profile of the products in the present claims. Petitioner fails to establish that Kawakami provides a reasonable expectation that the purity profile of the products in the present claims

can be obtained. The Petition relies on the Winkler declaration to provide motivation to combine, but the relied-upon portion of the declaration merely states that Kawakami discloses prostacyclin compounds, and treprostinil is a prostacyclin compound.



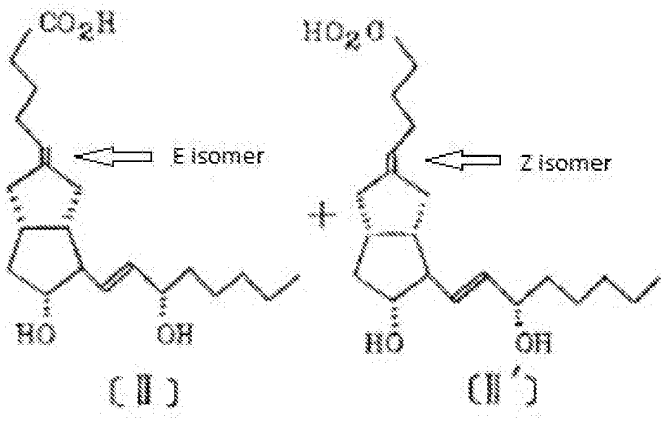
Treprostinil

"prostacyclin compound" in Kawakami

There are myriad "prostacyclin compounds" with widely-varying structures. Treprostinil's core structure and side chains are entirely different from those found in the "prostacyclin compounds" in Kawakami. Indeed, the alleged "prostacyclin compound" disclosed in Kawakami is a two ring structure, yet the core three ring structure of treprostinil is key to its pharmaceutical usefulness (Ex. 2013, at pp. 15, ll. 1-17) and is also present in every structure of every step of the '393 patent. *See, e.g.*, '393 patent claim 1. Other than the Winkler Declaration's conclusory statement that Kawakami's compounds are "prostacyclin compounds (of which treprostinil is an example)," Petitioner offers no basis from which to draw any

conclusion about whether an impurity reduction step in Kawakami would possibly have any relevance to a process to synthesize and or purify a totally different structure such as treprostinil.

To illustrate this point further, Kawakami is directed to purifying E- and Z-isomers of "prostacyclin compounds" from one another. In order for the E- and Z-isomers to exist, the "prostacyclin compound" must have an alkene. For example, Kawakami discusses separating a mixture of the following compounds:



Treprostinil, on the other hand, contains no mixture of E/Z isomers. In fact, it cannot because it does not contain an alkene capable of E/Z isomerization. Petitioner has failed to provide a factual basis as to how or why the separation of E/Z isomers of an alkene would provide a motivation to combine or reasonable expectation of success in a compound not containing an alkene capable of E/Z isomerization, such as treprostinil.

For these reasons, a POSA would have no motivation to look at Kawakami in order to arrive at the claimed invention of the '393 patent.

B. Moriarty in view of Phares does not render the '393 patent obvious

As explained above for Ground 1, Phares fails to disclose the synthetic route or purity of the claimed treprostinil product. Moriarty adds nothing to cure these deficiencies. Moriarty was considered during prosecution and disclosed multiple routes to synthesize the treprostinil formula. (Ex. 1004) Those same routes were also disclosed on the face of the '393 patent itself. Specifically, Moriarty discloses three distinct routes. *See, supra*, Section VIII.C. While Moriarty itself was an improvement over the previous methods by reducing the level of total impurities, reducing specific types of impurities and increasing the yield of the treprostinil product (Ex. 2013, at pp. 177, ll. 24–pp. 178, ll. 5, pp. 196, ll. 3–11, pp. 197, ll. 20–23, pp. 218, ll. 21–pp. 219, ll. 9, pp. 311, ll. 15–17), the '393 patent unexpectedly reduced the impurity level in the claimed treprostinil product even more. Petitioner, however, ignores this evidence, and states that "Moriarty discloses the synthesis (Ex. 1004, at p. 6) of treprostinil which is Formula 7 on p. 3" and that "formation of salts by the reaction of carboxylic acids with bases is a common reaction in organic chemistry." But this misses the point. The claimed invention is not the discovery that carboxylic acids react with bases, but rather that

compounds of Formula (I), and in particular treprostinil or a salt thereof, can be obtained with a superior purity profile compared to the prior art.

Specifically, Patent Owner discovered that performing step (c) on a product that resulted from steps (a) and (b) provided a product with reduced impurities. This discovery was not disclosed or suggested in Moriarty and resulted in a significant improvement in the treprostinil product. *See, supra*, Section IV.D.

Moreover, Patent Owner established at that time that, when "treprostinil acid made by the type of process disclosed in Moriarty 2004 was analyzed by the applicants, it was found to contain small amounts of 4 different impurities in a representative sample (benzindene triol, treprostinil methyl ester, and 2 different stereoisomers of treprostinil)." Each of these impurities, however, is reduced or eliminated in a product produced by the process according to claims 1 or 9 of the '393 patent. *See supra*, Section IV.D, Ex. 1002, at pp. 346-350. Petitioner argues that the salt formation step would have been obvious to reduce or remove acidic or basic impurities, but these reduced or removed impurities are neither strongly acidic nor basic as they are either diastereomers of treprostinil – which is very weakly acidic – or similarly neutral ester and triol impurities. The '393 patent therefore not only reduced the weakly acidic impurities present from the already improved Moriarty process, but also unexpectedly reduced or eliminated non-acidic impurities as well. Thus, even under Petitioner's broad and erroneous

understanding of the standard for obviousness, it was unexpected that the salt formation step would remove these additional impurities.

XII. GROUND 3 SHOULD BE DENIED BECAUSE THE PETITION FAILS TO ESTABLISH THAT IT WOULD HAVE BEEN OBVIOUS TO COMBINE THE REFERENCES WITH A REASONABLE EXPECTATION OF SUCCESS

Petitioner asserts that claims 6, 10, 15, 21 and 22[4] are rendered obvious under 35 U.S.C. § 103 over Moriarty with Phares or Kawakami, and in further combination with Ege. Petition, p. 53. Petitioner has asserted Ground 3 in the alternative. Either Moriarty in view of Phares and in further combination with

[4] These claims require that the product is produced with the optional step (d), and claim 22 requires that the product is produced with an additional step after step (d) wherein the product comprises a pharmaceutically acceptable salt formed from the product of step (d). Thus, claims 6, 10, 15, and 21 require the free acid of a compound of Formula (I) or treprostinil, while claim 22 recites a salt form of a compound of Formula (I) that has been purified through the salt-formation step (c) followed by the acid-formation step (d). Thus, in addition to the many reasons why these claims are not rendered obvious as described herein, these additional steps are similarly not disclosed in any of the prior art references asserted by Petitioner and for this additional reason would not have been obvious over the prior art.

Ege, or Moriarty in view of Kawakami and in further combination with Ege is asserted against claims 6, 10, 15, 21 and 22. Thus, each of the combinations asserted in Ground 3 requires Ege. Ege, however, does not disclose any of the missing claim elements from these previously addressed obviousness combinations.

A. Ege is not relevant to the '393 patent

Ege provides no additional support for any of these alleged obviousness combinations as it is merely an undergraduate chemistry textbook with only generalized descriptions of carboxylic acids and related synthetic procedures. Ege discloses nothing about any prostacyclin derivative, much less treprostinil free acid. Indeed, Ege fails to disclose anything about the synthesis of pharmaceuticals. Ege merely shows it was known to form a free acid from treatment of the corresponding carboxylate salt with a strong acid. But this fact alone provides no reason why one of ordinary skill in the art, based on any reference, would conduct a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step with a reasonable expectation of obtaining the claimed product.

In fact, Ege actually suggests this "carboxylate salt formation and regeneration of the neutral carboxylic acid" step would be relatively useless as a means for purifying treprostinil:

> Carboxylic acids that have low solubility in water, such as benzoic acid, are converted to water-soluble salts by reaction with aqueous base (p. 95). Protonation of the carboxylate anion by a strong acid regenerates the water-insoluble acid. These properties of carboxylic acids are *useful in separating them from reaction mixtures containing neutral and basic compounds*.

Ex. 1008, p. 8 (emphasis added). However, other compounds containing carboxylic-acids are not "neutral and basic compounds." Thus, Ege would not create an expectation of success for separating one carboxylic-acid compound (*e.g.,* treprostinil free acid) from other carboxylic-acid containing compounds (*e.g.,* different stereoisomers of treprostinil free acid). If anything, Ege would teach away or discourage the use of salt formation for purifying a mixture of compounds that includes other carboxylic-acid containing compounds as impurities.

B. Moriarty in view of Phares with Ege Fails To Establish Obviousness

In the first alternative for Ground 3 (Moriarty, Phares and Ege), Petitioner fails to establish a reasonable likelihood that claims 6, 10, 15, 21, and 22 are unpatentable as obvious.

1. Petitioner fails to provide a motivation to combine Moriarty, Phares, and Ege or an expectation of success for obtaining the free-acid product of claims 6, 10, 15, and 21

Claims 6, 10, 15, and 21 are to the free acid of Formula (I) or treprostinil. As mentioned above, out of Moriarty, Phares and Ege, only Moriarty discloses free acid treprostinil with any particularity. However, the free acid treprostinil in Moriarty was analyzed by Patent Owner, and representative samples were found to contain small amounts of four different impurities, including two different stereoisomers of treprostinil.

As explained previously, the claimed free-acid compounds, including treprostinil, produced by the processes of claims 6, 10, 15, and 21 provided a new product that induced FDA to adopt a new purity standard for treprostinil free acid due to the excellent purity of the final product. Furthermore, Patent Owner demonstrated that treprostinil free acid made by the claimed methods provided a compound without many of the impurities included in the free acid treprostinil of the Moriarty process, including the two different stereoisomers of treprostinil.

Neither Phares nor Ege provide a reason that a POSA would include a "carboxylate salt formation and regeneration of the neutral carboxylic acid" step. *See* Petition, p. 54. Phares merely discloses forming a salt from treprostinil free acid of undisclosed origin. *See* Section VIII.B, *supra*. There is no suggestion that