54. Moreover, the examples above underscore the fact that efficacy of a given drug administered by a given route of dosing (e.g., intramuscular) cannot be known until appropriate comparative studies are performed in a suitable animal model. For some drugs, the desired effect might be achieved following a particular route of dosing, but for other drugs it might not. The rate and extent of drug absorption, and the associated pharmacodynamics (e.g., the achievement of a desired drug effect) may differ greatly depending on the properties of the drug, the choice of an animal model, and the site of drug administration.

55. Consequently, one of ordinary skill in the art having the very limited experimental subcutaneous data from *McLeskey* would not have had an expectation that the intramuscular administration of fulvestrant using the *McLeskey* castor oil composition would have been effective following intramuscular administration, such as in the method described in the claims. This is especially true because *McLeskey* did not disclose plasma or blood levels of fulvestrant in mice after subcutaneous administration of the formulation, nor any information regarding the rate and/or extent of absorption of fulvestrant from the subcutaneous injection site. Additionally, the claims recite achieving a given therapeutic plasma concentration for at least four weeks, and there is no information in any of the references cited in the Office Action that would have suggested that such long-term efficacy associated with a single dose would be exhibited using the *McLeskey* castor oil composition by any route of administration.

56. Thus, one of ordinary skill in the art would not have had an expectation that the castor oil composition disclosed in *McLeskey*, which was administered

subcutaneously to mice, would have been therapeutically effective upon intramuscular administration of fulvestrant, for example, by following the method described in the claims.

The composition of a formulation can have a significant effect on the efficacy observed when the formulation is administered

57. Where a dosage form of a drug is being developed for intramuscular administration in humans, one of ordinary skill in the art typically relies upon the results of intramuscular dosing studies in suitable animal models where pharmacokinetic data are collected to characterize the absorption of the drug from its dosage form.

58. Typically, during the development of an intramuscular dosage form for administration of a drug in humans, one would have carried out, among other tasks, formulation studies to determine suitable compositions in which the drug of interest is dissolved, as well as initial intramuscular dosing experiments in animals (e.g., mice, rabbits, and/or dogs) under various conditions (e.g., different compositions, different solvents, varying the proportion of the components of the composition, different drug concentrations, etc.) in order to gain an understanding of the pharmacokinetics of fulvestrant before attempting human administration. The very existence of this generalized approach highlights the lack of expectation of success with respect to the extrapolation of the *McLeskey* disclosure of subcutaneous administration to mice, lacking any pharmacokinetic information, to human intramuscular administration.

59. With respect to the importance of formulation studies, I have read the Declaration Under 35 U.S.C §1.132 of Dr. Paul Gellert filed on August 2008 ("the Gellert

Declaration", cited as "Gellert Decl." and enclosed here as **Exhibit 16**). I understand that the Gellert Declaration was submitted to the U.S. Patent and Trademark Office in Application No. 10/872,784 (as indicated by the caption on the first page of the declaration).

60. As part of the discussion of the development of methods of treatment involving the administration of fulvestrant, the Gellert Declaration states that "the experienced formulator would want to minimize the amount of co-solvents and excipients in any injectable formulation." Gellert Decl. at ¶ 22.

61. Thus, even if the *McLeskey* castor oil composition had been considered as a potentially useful formulation in the development of a method of treatment for humans, one of ordinary skill in the art would have performed additional formulation studies to obtain a composition with suitable characteristics for the desired route of administration. The Gellert Declaration explains one of the rationales to perform those additional studies:

> Ideally, it is best to select and use solvents that would maximize the solubility of the compound. Maximizing the solubility of a compound in a particular cosolvent system would result in lower total levels of the non-aqueous solvent(s) being administered to the patient, thereby lowering the chance for potential side effects.

Gellert Decl. at ¶ 22 (quoting directly from P.K. Gupta and G.A. Brazeau (eds), "Injectable Drug Development: Techniques to Reduce Pain and Irritation" Chapter 11, p. 217, Interpharm Press, Denver, Colorado (1999)).

62. Regardless of how high or low the cosolvent concentrations are in a given formulation, the preparation of formulations in which a drug such as fulvestrant can be

solubilized is not sufficient to ensure the desired therapeutic effect when such formulation is administered to patients. As explained in the '887 application "[s]imply solubilising fulvestrant in an oil based liquid formulation is not predictive of a good release profile or lack of precipitation of drug after injection at the injection site." **Exhibit 7** at ¶ [0054]. Thus, suitable experiments are needed to determine the pharmacokinetic performance of any candidate formulation(s).

63. In that regard, it is understood that an animal model for drug dosage form performance may provide some discrimination among candidate dosage forms in development. Thus, the plasma concentration profile should reflect changes in the release characteristics of the drug from the formulation. That type of pharmacokinetic data could be used to characterize important variables in the development of a suitable method of treatment. For drugs that are difficult to formulate, such as fulvestrant (**Exhibit 7**, at ¶ [0014]), the pharmacokinetic data could be useful to investigate the most promising formulation for the desired route of administration.

64. For example, for fulvestrant, PCT Application Publication No. WO 03/006064 ("WO 03/006064", attached here as **Exhibit 17**) shows pharmacokinetic results of intramuscular administration of fulvestrant to rabbits. Figure 1 shows differences in results when seven different formulations of fulvestrant, each containing 100 mg/ml of the drug, but having different co-solvent compositions, were dosed intramuscularly in rabbits. The table related to Example 4 on page 30 of WO 03/006064 reports the composition of each formulation, labeled F1 to F7. As can be seen, all of these fulvestrant formulations contained ethanol, benzyl alcohol, and benzyl benzoate in

a castor oil vehicle; these are the same components of the fulvestrant composition recited in the claims, but with different proportions for each component.[2]

65. WO 03/006064 reports that "[p]lasma levels were more variable than Control over the first 30 days" following intramuscular administration of fulvestrant. WO 03/006064 at 30, l. 23. WO 03/006064 explains that "some differences in profiles were noted over the first 30 days such that they were divided into 2 groups (with Formulation F7 showing intermediate behaviour)." *Id.* at 30, ll. 29-30. According to WO 03/006064, Group A demonstrates "rapid release early time points", corresponding to formulations containing high benzyl benzoate and low castor oil concentrations, while Group B shows a "lower release, flatter profile" corresponding to formulations containing lower benzyl benzoate and higher castor oil concentrations. *Id.* at 30, ll. 31-34. WO 03/006064 replotted the data from Figure 1 corresponding to those formulations in Group A as part of Figure 2A and the data corresponding to those formulations in Group B as part of Figure 2B.

66. Therefore, based on WO 03/006064's own characterization of the differences in the pharmacokinetic profile of different fulvestrant formulations, higher benzyl benzoate concentrations in the formulation resulted in a more rapid initial release of fulvestrant, whereas lower benzyl benzoate concentrations resulted in a lower initial release, and a flatter plasma level profile. Depending on the overall objective of the administration of fulvestrant, some of the fulvestrant formulations tested in

[2] The right-hand column in this table appears to indicate the % w/v composition of castor oil. All the entries in this column should more properly be "to 100%", as they are in the Tables provided in the preceding Examples 2 and 3.

WO 03/006064's study would be more desirable than others for that given purpose and, based on the relevant pharmacokinetic profiles, one of ordinary skill in the art would be able to select one of those fulvestrant formulations for further development and/or testing.

67. However, one of ordinary skill in the art would not have been able to determine whether a given fulvestrant formulation injected intramuscularly as in WO 03/006064 would have had the desired pharmacokinetic profile until such *in vivo* pharmacokinetic studies were carried out. The testing of various formulations having different compositions, as portrayed in Figures 1, 2A and 2B, would typically be undertaken during the development of a dosage form in order to ensure an optimal method of treatment using a drug that is difficult to formulate. Such studies would be expected to demonstrate differences in the blood plasma concentrations of a test drug, and would allow the investigators to identify factors that would enhance the performance of the formulation.

68. Therefore, when considering the differences in pharmacokinetic profiles demonstrated in the example from WO 03/006064, it becomes clear that one of ordinary skill in the art knowing only the composition of a given formulation administered subcutaneously, but having no pharmacokinetic data following its intramuscular administration, would have had no expectation, one way or another, that the formulation would be effective when administered intramuscularly in a given method of treatment.

69. In particular, one of ordinary skill in the art would not have had a reasonable expectation that the *McLeskey* castor oil composition would have been

effective when given as an intramuscular injection, such as in the method of treatment recited in the claims.

70. I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

January 13, 2012
Date:

Ronald J. Sawchuk

Exhibit List for Declaration Under 37 C.F.R. § 1.132 of Ronald J. Sawchuk

Exhibit No.	Description
1	Curriculum Vitae of Ronald J. Sawchuk
2	Office Action for U.S. Patent Application No. 12/285,887 dated September 16, 2011
3	McLeskey et al., "Tamoxifen-resistant fibroblast growth factor-transfected MCF-7 cells are cross-resistant in vivo to the antiestrogen ICI 182,780 and two aromatase inhibitors", *Clinical Cancel Research* 4:697-711 (1998) ("*McLeskey*")
4	European Patent Specification No. EP 0 346 014, naming Michael Dukes as inventor ("*Dukes*")
5	Osborne et al., "Comparison of the effects of a pure steroidal antiestrogen with those of tamoxifen in a model of human breast cancer", *J. National Cancer Institute*, 87(20):746-750 (1995) ("*Osborne*")
6	Abstract for Wakeling et al., "ICI 182,780, a new antioestrogen with clinical potential", *J. Steroid Biochemistry & Molecular Biology*, 43(1-3):173-177 (1992) ("*Wakeling*")
7	U.S. Patent Publication No. 2010/0152149
8	Pending claims in U.S. Application No. 12/285,887, with proposed amendments
9	U.S. Patent No. 3,164,520
10	Riffkin et al., "Castor oil as a vehicle for parental administration of steroid hormones", J. Pharma Sci., 53(8):891-895 (1964) ("*Riffkin*")
11	Nema et al., "Excipients and their use in injectable products", *PDA J Pharma Sci Tech.*, 51(4):166-171 (1977) ("*Nema*")
12	The Merck Index, 12th Ed., Merck & Co., Inc. (1996) ("the Merck Index")
13	Guerrini et al., "Pharmacokinetics of probenecid in sheep", *J Vet Pharmacol Ther.*, 8:128-135 (1985) ("*Guerrini*")
14	Lavy, et al., "Pharmacokinetics of clindamycin HC1 administered intravenously, intramuscularly and subcutaneously to dogs:, *J Vet Pharmacol Ther.*, 22(4)261-265 (1999) ("*Lavy*")
15	Ismail, "Disposition kinetics of difloxacin after intravenous, intramuscular and subcutaneous administration in calves", *Vet Res Commun.*, 31(4):467-476 (2007) ("*Ismail*")
16	Declaration Under 35 U.S.C §1.132 of Dr. Paul Gellert filed on August 2008 in U.S. Application No. 10/872,784 ("the Gellert Declaration")
17	PCT Application Publication No. WO 03/006064

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	12/285,887
				Filing Date	October 15,2008
				First Named Inventor	John R. EVANS
				Art Unit	1628
				Examiner Name	HUI, San Ming R.
Sheet	1	of	1	Attorney Docket Number	11285.0056-00000

U.S. PATENTS AND PUBLISHED U.S. PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

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 Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[6]
	1	WO 03/006064	23-JAN-2003	Astrazeneca AB		

NONPATENT 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.	Translation[6]
	2	The Merck Index, 12th Ed., Merck & Co., Inc., pgs. xiv, 189-190, 641-642 and 1715 (1996).	
	3	Guerrini, et al., "Pharmacokinetics of probenecid in sheep", J Vet Pharmacol Ther., 128-135 (1985).	
	4	Lavy, et al., "Pharmacokinetics of clindamycin HCl administered intravenously, intramuscularly and subcutaneously to dogs", J Vet Pharmacol Ther., 22(4):261-265 (1999).	
	5	Ismail, "Disposition kinetics of difloxacin after intravenous, intramuscular and subcutaneous administration in calves", Vet Res Commun., 31(4):467-476 (2007).	
	6	Documents from the prosecution of European Application No. 01900186.6 (EP 1 250 138) from August 27, 2009 to December 15, 2011.	
	7	Documents from the prosecution of European Application No. 10180667.7 (EP 2 266 573) from November 23, 2010 to December 19, 2011.	
	8	Documents from the prosecution of European Application No. 10180661.0 (EP 2 286 818) from January 19, 2011 to December 19, 2011.	
	9	Declaration Under 35 U.S.C §1.132 of Dr. Paul Gellert filed in August 2008 in U.S. Application No. 10/872,784.	
Examiner Signature		Date Considered	

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

PATENT
ATTORNEY DOCKET NO.: **056291-5004-01**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:	)	Confirmation No. **2093**
EVANS et al.	)	
Application No.: **10/872,784**	)	Group Art Unit: **1617**
Filed: **June 22, 2004**	)	Examiner: **Hui, San-Ming R**
FOR: **FORMULATION**	)	

DECLARATION UNDER 35 U.S.C. § 1.132
OF PAUL RICHARD GELLERT

PAUL RICHARD GELLERT of AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK declares:

1. I graduated from the University of Oxford in Chemistry in 1984. I undertook postgraduate research with Professor Brian Howard in the Physical Chemistry Laboratory at the University of Oxford leading to the award of a D.Phil in 1988. From February 1988 until the present I have been employed by AstraZeneca, (formerly Zeneca and ICI) initially as a Senior Research Scientist and subsequently as a Team Leader/Manager, Principal Scientist and, since 2004, a Senior Principal Scientist.

2. I have worked in the formulation and drug delivery area throughout my career with AstraZeneca, where my research and development work has covered a range of formulation types including sustained released injections, including fulvestrant.

3. During the course of my study of the subject application (hereinafter "the Evans Application") and the underlying data, I have become aware of several transcription or other errors between certain disclosures of the subject application and the underlying laboratory notebook data. One purpose of this Declaration is to point out the existence

and nature of these errors and to report further testing that has been carried out under my guidance to obtain additional data (paragraphs 4-10 below and Attachments A-D). A further purpose of this Declaration is to set out and document the manner in which an experienced formulator would likely have approached the task of developing a sustained release injectable formulation suitable for human use for a steroidal compound such as fulvestrant in about early 2000, which I understand is when the priority applications supporting the Evans Application were filed (paragraphs 11 - 25 below and Attachment E). Citations to literature and patent references in this Declaration will be in the format Lead Author (Date), and the full citations are given in the Table of References at the end of this Declaration. A copy of each cited reference (or cited portions of the longer references) is included in Attachment F under the Tab number noted in the Table of References.

4. In Table 2 of the Evans Application, the solubility of fulvestrant in castor oil appears to have been transcribed incorrectly from the original source, the laboratory notebook. The value in the latter is 24.5 mg/ml and not 20 mg/ml. In other experiments to determine the solubility of fulvestrant in castor oil and also in benzyl benzoate, some variability was observed.

5. In Table 3 of the Evans Application, the given solubility values were generated at 4°C and not at 25°C as is stated in the title of Table 3. For fulvestrant formulations, it is preferable that the fulvestrant remains completely in solution at both 4°C and 25°C. The 4°C temperature corresponds to the storage temperature (2°C to 8°C in the FDA approved label for Faslodex), and the 25°C temperature corresponds to the administration temperature (ambient temperature). In addition, the specified solubility values on this Table 3 are mean values calculated from analysis of replicate samples from one or more trials. The individual values are shown in handwriting in the amended version of Table 3 in Attachment A. In addition, it appears that the mean values for the last three compositions have been incorrectly calculated. The corrected mean values, together with the correction of the temperature from "25°C" to read "4°C", are also shown in handwriting in the amended version of Table 3 in Attachment A.

6. I have evaluated the transcription and other errors against the original application disclosures and conclude that these do not change the ultimate conclusions made from the data as originally reported. The addition of 15% w/v benzyl benzoate to compositions having total alcohol concentrations in castor oil of 10%, 15%, 20% and 30% w/v unexpectedly provides a positive effect on fulvestrant solubility, significantly increasing the solubility of fulvestrant in the compositions despite fulvestrant having a lower solubility in benzyl benzoate than in either alcohol or castor oil.

7. An additional set of experiments has been conducted at 25°C under my guidance to obtain consistent data with reduced variability from a single set of rigorously controlled solubility experiments and to demonstrate that the unexpected increase of solubility of fulvestrant by adding benzyl benzoate into compositions containing ethanol, benzyl alcohol and castor oil, is present across the broader range of composition encompassed by the claims being presented with this Declaration. The solubility of fulvestrant in benzyl benzoate and in castor oil was also measured in the same set of experiments using the same batch of benzyl benzoate and the same batch of castor oil as were used to make up the compositions. The Experimental Test Procedure is described in Attachment B.

8. The results from these solubility experiments are shown in the table in Attachment C. These results show that the solubility of fulvestrant in castor oil alone (21.4 mg/ml) is significantly greater than the solubility of fulvestrant in benzyl benzoate alone (3.8 mg/ml) and demonstrate the unexpected increase in fulvestrant solubility on the addition of 10, 15 and 25% w/v benzyl benzoate, in place of an equivalent amount of castor oil, to compositions having total alcohol concentrations in castor oil of 10%, 15%, 20%, 25% and 30% w/v.

9. Thus, the results that were obtained from experiments conducted under rigorously controlled conditions and with an expanded range of compositions, as shown in Attachment C, confirm the ultimate conclusions drawn from the results shown in Table 3 of the original application disclosure, namely that the addition of 10% to 25% w/v benzyl

benzoate to compositions having total alcohol concentrations in castor oil of between 10% to 30% w/v unexpectedly provides a positive effect on fulvestrant solubility, significantly increasing the solubility of fulvestrant in the compositions despite fulvestrant having a lower solubility in benzyl benzoate than in either alcohol or castor oil.

10. During the course of my study of the Evans Application and the underlying source materials it was drawn to my attention that some of the composition data given for Delestrogen and Delalutin somehow had been shifted one column to the right. Thus, for Delestrogen, the 78% and 58% figures shown under the BzBz column should have been under the OIL column; the 20% and 40% figures shown under the BzOH column should have been under the BzBz column; and the 2% figures shown under EtOH should have been under the BzOH column. Similarly for Delalutin, the "up to 2%" shown under the EtOH column should have been under the BzOH column. This table reports that the source of this data was J.Pharm.Sci (1964) 53(8) 891, which is Riffkin (1964) elsewhere referred to in this Declaration, and I have also verified the corrected data from the entries for Delalutin and Delestrogen in PDR (1973). A copy of Table 1 from the Evans Application is reproduced as Attachment D, on which these corrections have been made in handwriting, and I have additionally more correctly noted that Delalutin is 17-hydroxy progesterone *caproate*, and that the "COMP" designation for Delalutin should be "BMS" (Bristol-Myers Squibb). Attachment D also includes a one page explanation of the corrections to this Table 1.

11. In about early 2000, a person responsible for developing a sustained release injectable formulation suitable for administration to humans for a new steroidal compound such as fulvestrant, would have had specialized training and experience in developing pharmaceutical formulations and methods for their administration. In developing such a formulation for fulvestrant, the objective would have been to formulate an intramuscular (IM) injection that would provide for the satisfactory sustained release of fulvestrant over a period of at least two weeks and preferably over a period of at least four weeks to reduce the frequency of administration, and would have a target fulvestrant content of at

least 45 mg/mL so as to provide a fulvestrant dose of at least 250 mg in a single 5-6 mL injection. From my personal experience and knowledge of the literature at about that time, I believe that such an experienced formulator would likely have approached the task of developing a formulation for fulvestrant in about the following manner.

12. Given the foregoing objective, the experienced formulator would have appreciated that the traditional administration options to explore were intramuscular (IM) injection of a sustained release aqueous or oil suspension or an oil-based solution (depot) containing at least 250 mg of fulvestrant in a volume of vehicle that is tolerable for injection, *i.e.*, no more than 5 or 6 mL.

13. Because of the extremely low solubility of fulvestrant in water, a reasonable starting point would have been to investigate intramuscular injection of an aqueous or oil suspension of fulvestrant. However, the formulator would have found that injection of an aqueous suspension of fulvestrant resulted in extensive local tissue irritation at the injection site as well as a poor release profile, such as reported in paragraph [0042] of the Evans Application. Since suspensions thus were not an acceptable option for fulvestrant, the experienced formulator would have moved on to further explore whether 250 mg of fulvestrant could be solubilised in no more than 5-6 mL of an oil-based vehicle, *i.e.*, to achieve the target fulvestrant concentration of at least 45 mg/mL.

14. In the preformulation phase, the experienced formulator would have conducted a literature review or otherwise would have become familiar with commercially marketed injectable formulations, particularly injectable sustained release formulations of steroids or other relatively insoluble compounds such as those listed in Table 1 of the Evans Application, with the objective of identifying potential oil vehicles, co-solvents and other excipients that already had been found to be tolerated and/or to have passed through regulatory review, and which might be candidates for further consideration and testing for the fulvestrant formulation. This review also would have provided guidance with respect to concentration levels of such co-solvents and other excipients that generally had been found acceptable in sustained release oil-based intramuscular injections administered to

humans. This objective is confirmed, for example, in Nema (1997) at page 166:

> Generally, a knowledge of which excipients have been deemed safe by the FDA or are already present in a marketed product provides increased assurance to the formulator that these excipients will probably be safe for their new drug product. ... Regulatory bodies may view an excipient previously approved in an injectable dosage form favorably, and will frequently require less safety data.

The purpose of this Nema paper was thus "to present the various excipients that have been included in the formulation of injectable products marketed in the USA."[1] Similar objectives were intended to be served by the compilations of commercial formulations in Strickley I (1999), Strickley II (2000) and Strickley III (2000):

> This compilation will also be useful for those interested in knowing what additives are currently used in injectable products and at what concentrations they are administered in practice. This compilation only focuses on marketed formulations and does not delve into the subject of preclinical or drug discovery formulations associated with early-stages pharmacokinetics or proof-of-concept pharmacodynamics, where the formulation scientist is not bound by regulatory constraints.

(Strickley I (1999) at 324).

Powell (1998) similarly states at page 238 with respect to its compilation of commercially used excipients:

> Thus, the formulation scientist is often faced with a dilemma -- which excipients are truly available for use (based on what has been used previously), and which are not? ... And at what concentrations, and by what route? ...
>
> Herein are listed the excipients found in most of the approved and marketed parenteral formulations, given systematically by excipient name. In this format it is easy to determine what concentrations were used, the route of administration, the main rationale for addition of that excipient, the drug that was formulated, the manufacturer, brand name, etc.

15. From the literature review, the formulator would have noted reference to a number of intramuscular injectable sustained release oil-based steroidal formulations that had been

[1] Nema (1997) does caution, however, that there is no guarantee that the new drug product will be safe as excipients are combined with other additives and/or with a new drug, creating unforeseen potentiation or synergistic toxic effects.

commercially marketed:

- Strickley I (1999), Table VII:

  Haloperidol Decanoate/Haldol decanoate (50-100 mg/mL in sesame oil, benzyl alcohol 1.2%);

  Testosterone Enanthate/Delatestryl (200 mg/mL in sesame oil, chlorobutanol 5 mg/mL);

- PDR (1973) at pages 1277-1278

  Proluton/progesterone (50 mg/mL in sesame oil, 150 mg/ml benzyl benzoate, 5 mg/ml benzyl alcohol, 1 mg/ml propylparaben);

- PDR (1973) at pages 1349-1354

  Deladumone/Testosterone Enanthate & Estradiol Valerate (90 & 4 mg/mL in sesame oil, 0.5% chlorobutanol);

  Deladumone OB/Testosterone Enanthate & Estradiol Valerate (180 & 8 mg/mL in sesame oil, 2% benzyl alcohol);

  Delalutin/hydroxyprogesterone caproate (250 mg/mL in 52% castor oil, 46% benzyl benzoate, 2% benzyl alcohol);

  Delestrogen/estradiol valerate (20 mg/mL in 78% castor oil, 20% benzyl benzoate, 2% benzyl alcohol and 40 mg/mL in 58% castor oil, 40% benzyl benzoate, 2% benzyl alcohol);

  Delatestryl/Testosterone Enanthate (200 mg/mL in sesame oil, 0.5% chlorobutanol);

  Delaluteval 2X/hydroxyprogesterone caproate & estradiol valerate (250 mg/mL & 5 mg/mL in castor oil, 45% benzyl benzoate, 1.6% benzyl alcohol);

- PDR (1973) at pages 1391-1392

  Prolixin Enanthate/FluphenazineEnanthate (25 mg/mL in sesame oil, 1.5% benzyl alcohol);

- Wang (1980):

  Depo-Testosterone/testosterone cypionate (100 mg/mL in 87.4% cottonseed oil, 0.1 mL benzyl benzoate, 9.45 mg benzyl alcohol as a preservative);

- Mackey (1995):

  Testoviron Depot/testosterone enanthate (250 mg/mL in castor oil and benzyl

benzoate);

as well as a number of other commercialized oil based long-acting IM injectable formulations reported on Table 1 of the Evans Application.

16. As a further part of the preformulation phase, the experienced formulator would have conducted a preformulation solubility screen, separately measuring the solubility of fulvestrant in a range of pure solvents, including the potential oil and co-solvent candidates that had been identified in the above literature review as being suitable for inclusion in intramuscular injection formulations. See, for example, Gupta (1999), Chapter 17 at page 402, under the heading "Formulation Development":

> The activities necessary to develop a parenteral product can be placed into the following three broad areas: preformulation, formulation, and scale-up. While there are alternative development perspectives, all development ultimately needs to accomplish the same activities. Preformulation includes the characteristics of the bulk drug plus initial screening for excipient compatibility with the drug.

"Preformulation studies" are said to "provide fundamental data and experience necessary to develop formulations for a specific compound" including, as item 8.1 in the outline of areas of specific interest, a determination of "solubility" in "selected solvents" (at 403). "Significant formulation activities begin with initial preformulation data and knowledge of the specific route of administration" (at 405), which "formulation activities include the identification and selection of a suitable vehicle (aqueous, nonaqueous or co-solvent system) ..." (at 404). It is further noted that "injection volume is one of the most important considerations in the formulation development of a commercial product" (at 405). When carrying out such a preformulation solubility screen with fulvestrant, the formulator would have found that fulvestrant had extremely low solubility in water, low solubility in most oils (but highest in castor oil), low solubility in benzyl benzoate, and the highest solubility in ethanol and benzyl alcohol, such as reported in Table 2 of the Evans Application.

17. With the information on prior commercialized formulations and the fulvestrant solubility data from the preformulation screen (such as reported in Table 2 of the Evans

Application), the experienced formulator would have selected castor oil as the oil vehicle because of the higher solubility of fulvestrant in castor oil relative to the other oils tested. Nevertheless, he would have appreciated that the target fulvestrant concentration of at least 45 mg/mL could not be achieved with castor oil alone, and that a co-solvent would be required.

18. A number of the commercialized formulations that would have been identified in the literature review (including the castor oil-based formulations) have a substantial benzyl benzoate component, which may be present as a co-solvent. See, for example, Delalutin noted in paragraph 15 above, which is reported in PDR (1973) and noted in Table I of the Evans Application, and is one of the formulations discussed in Riffkin (1964), "Castor Oil as a Vehicle for Parenteral Administration of Steroid Hormones" (see Riffkin n. 6). Delalutin is 250 mg/mL 17-hydroxyprogesterone caproate dissolved in 52% castor oil, 46% benzyl benzoate and 2% benzyl alcohol. However, Riffkin Table II reports that the solubility of 17-hydoxyprogesterone caproate in castor oil alone is only 55.6 mg/mL, but the solutility of 17-hydroxyprogesterone caproate in benzyl benzoate is substantially higher, being at least 250 mg/mL (see example 4 of Huber (US '520) and Attachment E discussed below). Even if not needed as a cosolvent, Riffkin (1964) notes that "the addition of benzyl alcohol or benzyl benzoate to castor oil resulted in a lower and more favorable viscosity, making it easier to inject" (paragraph bridging pages 893-894).

19. However, the skilled formulator would have appreciated from the fulvestrant solubility data generated in the preformulation screen that fulvestrant had very different solubility characteristics relative to the steroids of previous commercial formulations. Attachment E is a compilation showing the chemical structures and relative solubilities in castor oil and sesame oil of the compounds named in Riffkin (1964) Table II compared to the structure and the solubility of fulvestrant in these oils. It can be seen that the solubility of fulvestrant in castor oil and in sesame oil (20 mg/mL and 0.58 mg/mL, respectively, from Table 2 of the Evans Application) is appreciably lower than the solubility of the other steroids in these oils (taken from Table II of Riffkin (1964)). The second page of Attachment E tabulates the concentration in benzyl benzoate of five named steroids, taken

from Examples 1-5 of Huber (US '520), ranging from 200 to 400 mg/ml.[2] By comparison, the solubility of fulvestrant in benzyl benzoate is reported in Table 2 of the Evans Application as being only 6.15 mg/mL, and only 3.8 mg/mL as determined in the recently conducted tests reported in Attachment C.

20. The experienced formulator thus would have expected that benzyl benzoate would *not* act as a co-solvent for fulvestrant in castor oil because the solubility of fulvestrant in benzyl benzoate was significantly lower than its solubility in castor oil. The addition of benzyl benzoate to castor oil, for whatever reason, would have been expected to *decrease, rather than increase*, the solubility of fulvestrant in the resulting castor oil/benzyl benzoate mixture. This is confirmed in Table 4 of the Evans Application, which reports a fulvestrant solubility of only 12.6 mg/mL in the castor oil vehicle containing only 15% benzyl benzoate, compared to the 20 mg/mL solubility of fulvestrant in castor oil alone as reported in Table 2.[3]

21. Based on the solubility data determined in the preformulation screen (such as reported in Table 2 of the Evans Application), ethanol and/or benzyl alcohol would have been seen as the best co-solvent candidates for raising the fulvestrant solubility to the 45 mg/mL target in the castor oil vehicle, and would also function to lower the viscosity of the resulting formulation and make it easier to inject. Consistent with this solubility data, Dukes (US '814) added 40% w/v benzyl *alcohol* in order to dissolve 50 mg/mL fulvestrant in the castor oil-based formulation used in the experimental rat studies of his Example 3. It thus would have been apparent that 40% w/v benzyl alcohol could function as a co-solvent in castor oil to achieve the target fulvestrant concentration. Nevertheless, the skilled formulator would have been concerned with using such a high alcohol content in intramuscular injectable formulations for administration to a human.

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[2] Data taken from the Examples of Huber (US '520); these are concentrations used in the examples and not necessarily the actual maximum solubility of each steroid in benzyl benzoate, which may be higher. Huber was a co-author on Riffkin (1964).

[3] It should be noted that in the further tests that were recently conducted under my guidance (paragraphs 7-9 above and Attachments B and C hereto), the solubility of fulvestrant in castor oil alone was again tested and found to be 21.4 mg/mL, and the solubility of fulvestrant in benzyl benzoate alone was again tested and found to be only 3.8 mg/mL, which further confirms that benzyl benzoate would not be expected to act as a cosolvent for fulvestrant in castor oil.

22. First of all, the experienced formulator would want to minimize the amount of co-solvents and excipients in any injectable formulation. For example, as stated in Gupta (1999), Chapter 17, "Formulation and Administration Techniques to Minimize Injection Pain and Tissue Damage Associated with Parental Products" at page 414:

> Cosolvents are commonly used to enhance drug solubility and stability. Cosolvents may include ethanol, propylene glycol, polyethylene glycols, and glycerine. These components have intrinsic effects on biologic tissue and can alter the properties of other excipients, thus influencing the tissue damage or pain caused by a product. There is a dearth of literature on the pain caused by cosolvents, but there is also a growing body of knowledge on the tissue damage that they can cause. It is not certain that tissue damage is always directly correlated with the injection pain, but minimization of both pain on injection and potential for tissue damage should be included in the product development plan.

See also Gupta (1999), Chapter 11, titled Cosolvent Use in Injectable Formulations, page 217:

> Ideally, it is best to select and use solvents that would maximize the solubility of the compound. Maximizing the solubility of a compound in a particular cosolvent system would result in lower total levels of the non-aqueous solvent(s) being administered to the patient, thereby lowering the chance for potential side effects.

This objective would have applied to aqueous and oil-based systems alike, in that the precedent of commercialized formulations identified in the literature review would have confirmed that fixed oils, such as castor oil, have long been commercially used and accepted as the major component of oil-based sustained release intramuscular injectable steroidal formulations. On the other hand, co-solvents such as ethanol or benzyl alcohol have generally been used only in far lesser concentrations, as discussed in the following paragraph.

23. Thus, use of such a high content of either benzyl alcohol or ethanol would have been contrary to precedent as shown from the review of commercialized oil-based intramuscular injectable sustained release formulations. The literature review as of early 2000 would have shown that any benzyl alcohol in such formulations was almost always

present as a preservative in a concentration of about 2% or less, occasionally at a concentration of up to 5%, but only rarely at higher concentrations. With respect to benzyl alcohol see, for example:

- Gupta (1999), Chapter 11 at page 229 stating that benzyl alcohol "is typically used in concentrations of up to 2 percent as a preservative and up to 5 present as a solvent," and then discussing reported toxicities.
- Nema (1997), Table V at page 168, reporting that benzyl alcohol was present as an antimicrobial preservative in 74 injectable formulations (not limited to oil-based IM formulations) at concentrations of from 0.75-5% (note that benzyl alcohol is not included at all in Nema Table I, "Solvents and Co-solvents";
- Powell (1998), the benzyl alcohol listing at pages 244-246, particularly those indicated as being used in IM formulations;
- Strickley I (1999) at page 329 notes the inclusion of 2% benzyl alcohol in an IM lorazapam formulation in a propylene glycol vehicle, but does not include benzyl alcohol at all in Table VI listing "Cosolvents Used in Parenteral Formulations;"
- Lopatin (1972) noting in Table 3 at page 727 opposite Benzyl alcohol, "Toxic. Used in concentration of not over 3%. Has irritant action in concentration of 5%;"
- Cornelius (US '863), col. 1, lines 30-35 stating, "It is known that the solubility of steroids in vegetable or animal oils can be increased by the addition of excipients such as benzyl alcohol and benzyl benzoate. An objection to the use of such excipients, and specifically benzyl alcohol in somewhat higher concentrations, is that these agents may irritate the tissues."

The literature review as of early 2000 also would have shown that, with few exceptions, ethanol was not included in such formulations in excess of about 10%. See, for example:

- Gupta (1999), Chapter 11 at page 225 noting that ethanol has been used at levels up to 50 percent, but these levels typically are associated with pain on injection;
- Strickley I (1999), Table VI, "List of Cosolvents Used in Parenteral Formulations" more specifically lists the ethanol content in IM formulations for specifically identified drugs, which concentrations range only from 2.5 to 10%; an IM/IV lorazapam formulation in a propylene glycol vehicle is noted at page 329 as having 18% alcohol, but is not included with the IM formulations in Table VI;

- Nema (1997), Table I, "Solvents and Co-solvents" at page 167, lists ethanol as being in 24 formulations with a concentration range of 0.6-80% (for Prograf); note that this is misleading, however, since Prograf is a *concentrate* for intravenous infusion only, and is to be diluted 250 to 1000 times before administration;
- Powell (1998), lists "alcohol" at page 242 and "ethyl alcohol" at page 255, wherein the ethanol concentration for IM formulations ranges from 0.61-10%.

24. Thus, even though Dukes (US '814) had demonstrated that the target 45 mg/mL fulvestrant concentration could be achieved by adding 40% benzyl alcohol to the castor oil vehicle, the precedent of commercialized IM oil-based systems would have motivated the experienced formulator to substantially reduce the benzyl alcohol content of the formulation intended for human use, and this commercial precedent would have made him very reluctant to replace benzyl alcohol with the substantial amount of ethanol that would be needed to maintain the target fulvestrant concentration. Benzyl benzoate clearly would not be considered to solve this dilemma, but rather would be expected to have a negative effect on fulvestrant solubility since fulvestrant was even less soluble in benzyl benzoate than in castor oil, that is, one would have expected that adding benzyl benzoate would require still *more* alcohol to maintain the target fulvestrant concentration.[4]

25. Under these circumstances, the discovery by Evans *et al.*, that the addition of benzyl benzoate to the castor oil/alcohol mixture actually increases the solubility of fulvestrant such that more fulvestrant could be dissolved in a given volume of formulation, was unexpected and truly surprising. This positive benzyl benzoate effect on fulvestrant solubility in the resulting formulation is shown in Table 3 of the specification (and is not changed by the above-noted corrections), and is confirmed and demonstrated over a broader range of formulation composition by the additional set of experiments conducted under my guidance and discussed in paragraphs 7-9 above, the results of which are reported in Attachments C.

[4] It should be noted that even apart from this solubility issue, there would have been no motivation to add benzyl benzoate for viscosity reduction since the significant quantity of alcohol would serve the dual function of acting as a co-solvent as well as reducing the injection viscosity and making it easier to inject, whereas the benzyl benzoate would be expected to have a negative effect on the fulvestrant solubility.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punished by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Signature: P. R. Mulick.

Date: 8th August 2008.

TABLE OF REFERENCES

Tab	Author/Inventor	Reference Citation/Patent
1	Cornelius (US '863)	US Patent 4,212,863
2	Dukes (EP '014)	EP 0 346 014 A1 (corresponds to US Patent 5,183,814)
3	Dukes (US '814)	US Patent 5,183,814 (corresponds to EP 0 346 013 A1)
4	Gupta (1999)	P.K. Gupta and G.A. Brazeau (eds). *Injectable Drug Development: Techniques to Reduce Pain and Irritation.* Chapters 11 & 17 Interpharm Press, Denver, Colorado (1999)
5	Huber (US '520)	US Patent 3,164,520
6	Lopatin (1972)	P.V. Lopatin, V. P. Safonov, T. P. Litvinova and L. M. Yakimenko. Use of nonaqueous solvents to prepare injection solutions. *Pharm. Chem. J.* **6**:724-733 (1972)
7	Mackey (1995)	M.A. Mackey, A.J. Conway and D.J. Handelsman. Tolerability of intramuscular injections of testosterone ester in oil vehicle. *Hum. Reprod.* **10**: 862-865 (1995)
8	Nema (1997)	S. Nema, R.J. Washkuhn, and R.J. Brendel. Excipients and their use in injectable products. *PDA J. Pharm. Sci. Technol.* **51**:166-71 (1997)
9	PDR (1973)	*Physicians' Desk Reference (27th edition).* 1277-1278, 1350-1354, 1391-1392 Medical Economics Company, Oradell, NJ (1973)
10	Powell (1998)	M. F. Powell, T. Nguyen, and L. Baloian. Compendium of excipients for parenteral formulations. *PDA J. Pharm. Sci. Technol.* **52**:238–311 (1998)
11	Riffkin (1964)	C. Riffkin, R. Huber and C.H. Keysser. Castor oil as a vehicle for parenteral adminstation of steroid hormones. *J.Pharm.Sci.* **53**: 891-5 (1964)
12	Strickley I (1999)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) -Part I. *PDA J. Pharm. Sci. Technol.* **53**:324–349 (1999)
13	Strickley II (2000)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part II *PDA J. Pharm. Sci. Technol.* **54**:69–96 (2000)
14	Strickley III (2000)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part III. *PDA J. Pharm. Sci. Technol.* **54**:152–169 (2000)
15	Wang (1980)	Y.C. J. Wang and R. R. Kowal. Review of excipients and pH's for parenteral products used in the United States. *J. Parenteral Drug Assoc.* **34**:452–462 (1980).

ATTACHMENT A

TABLE 3

EFFECT OF BENZYL BENZOATE ON FULVESTRANT SOLUBILITY IN CASTOR OIL AT 25°C / 4°C

	% w/v							
Ethanol (96%)	5	5	10	10	10	10	15	15
Benzyl Alcohol	5	5	5	5	10	10	15	15
Benzyl Benzoate		15		15		15		15
Castor Oil	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100
Mean Fulvestrant Solubility [mgml^{-1}]	27	36	46	54	45	68 / 64	78 / 74	102 / 103
Individual values [mgml^{-1}]	27.8 25.8	35.5 36.1	54.0 38.0	64.1 49.3 58.0 50.3	49.4 41.3	68.9 66.8 63.2 92.9	85.8 96.9 90.0 73.4	80.6 101.9 121.6 107.4

ATTACHMENT B:

Experimental Test Procedure for measuring the solubility of fulvestrant in different solvent vehicles at 25°C

1. Solvent vehicles for the solubility experiments were prepared by weighing the required amount of benzyl benzoate, benzyl alcohol and ethanol into a 20 ml volumetric flask and then diluting to volume with castor oil.
2. For each solvent vehicle in which the solubility of fulvestrant was to be determined, 1.0-1.5g of fulvestrant was weighed into each of 3 separate vials (2 dram size) and 5mls of the solvent vehicle was added to each vial, except for the pure castor oil vehicle, where 80mg of fulvestrant were weighed into each of the 3 separate vials and 2mls of the castor oil added to each vial. The reduced amount of fulvestrant and lower volume of solvent vehicle was needed to maintain stirring and achieve adequate mixing with the pure castor oil vehicle due to the combination of its higher viscosity and lower fulvestrant solubility/higher undissolved fulvestrant levels compared to the other solvent vehicles.
3. A magnetic stirrer bar was placed into each vial and the vials were capped and then placed on a magnetic stirrer block maintained at 25 ± 0.5°C.
4. After 5 days of stirring at 25 ± 0.5°C, an aliquot of each fulvestrant/solvent vehicle mixture was removed from each vial and placed into an Eppendorf tube which was then centrifuged at 12000 rpm for 5 minutes at ambient temperature.
5. For all but the fulvestrant/castor oil mixture, 1 ml of the supernatant was then removed from the Eppendorf tube and pipetted into a 10ml or 20ml volumetric flask and then diluted to volume with methanol and mixed to give a sample for analysis. The choice of whether to use a 10ml or 20ml volumetric flask for a particular sample was dependent on the likely concentration of fulvestrant in the sample and the quantifiable concentration range of the HPLC assay method used. For the fulvestrant/castor oil mixture, 100µl of the supernatant was removed from the Eppendorf tube and pipetted into a 1ml volumetric flask and then diluted to volume with methanol and mixed to give a sample for analysis.
6. Step 5 was repeated to give a duplicate sample for analysis. Thus, this gave 2 samples for each of the 3 vials, giving a total of 6 samples for analysis for each solvent vehicle tested.
7. The resultant samples were analysed for fulvestrant content by reverse phase High

Performance Liquid Chromatography (HPLC). The HPLC method that was used is described below at point 9. The fulvestrant content obtained for each sample was used to calculate a value for the concentration of fulvestrant dissolved in the corresponding solvent vehicle after stirring for 5 days at 25°C.

8. The mean solubility of fulvestrant for each different solvent vehicle tested was calculated as the arithmetic mean of the 6 individual values for the concentration of fulvestrant dissolved in the corresponding solvent vehicle.
9. HPLC Method details:

Gradient HPLC Method

Eluent A : 27% Methanol / 32% Acetonitrile / 41% Water

Eluent B : 41% Methanol / 49% Acetonitrile / 10% Water

Column : 15cm 3.5um Symmetry C8 4.6mm i.d.

Detection wavelength : 225 nm

Flow rate : 2 mL min-1

Temperature : 40°C

Injection volume : 10 µL

Gradient programme :

Time (min)	Eluent A (%)	Eluent B (%)
0	100	0
25	100	0
55	0	100
65	0	100
66	100	0
70	100	0

Retention time of fulvestrant: 21minutes approximately

ATTACHMENT C: EFFECT OF BENZYL BENZOATE ON FULVESTRANT SOLUBILITY IN CASTOR OIL AT 25°C

	% w/v																					
Ethanol (96%)	0	0	5	5	5	5	10	10	10	10	10	10	10	10	12.5	12.5	12.5	12.5	15	15	15	15
Benzyl Alcohol	0	0	5	5	5	5	5	5	5	5	10	10	10	10	12.5	12.5	12.5	12.5	15	15	15	15
Benzyl Benzoate	0	100		10	15	25		10	15	25		10	15	25		10	15	25		10	15	25
Castor oil	100	0	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100
Mean Fulvestrant solubility [mgml^{-1}]	**21.4**	**3.8**	**27.6**	**29.2**	**43.3**	**47.5**	**64.6**	**71.6**	**84.2**	**94.0**	**68.1**	**87.2**	**93.4**	**118.9**	**96.6**	**107.7**	**116.1**	**139.6**	**121.3**	**144.6**	**143.8**	**166.2**
Individual values [mgml^{-1}]	23.2	3.9	29.5	31.2	43.9	48.3	64.2	76.2	83.8	95.2	68.6	90.0	92.5	122.1	104.1	106.1	115.5	138.9	110.0	129.8	148.2	163.3
	17.8	4.0	28.3	26.3	45.1	50.7	66.8	72.1	81.9	97.8	68.9	84.9	92.1	120.3	74.0	86.6	117.9	141.0	120.0	133.5	147.1	164.8
	21.5	3.9	24.5	31.5	44.3	45.4	61.2	66.2	93.2	95.6	71.6	87.6	93.9	120.4	102.0	112.6	118.8	139.4	124.4	150.1	144.4	168.5
	21.8	3.8	26.6	29.3	45.4	45.2	66.0	65.7	84.6	96.1	67.6	88.1	93.0	118.3	98.6	117.9	116.1	142.1	125.6	151.7	144.4	169.7
	22.2	4.0	27.0	29.1	36.9	47.6	65.8	75.4	82.4	88.2	67.0	90.7	93.8	116.8	102.1	107.9	117.0	138.7	123.3	151.2	139.5	165.5
	22.0	3.2	29.6	27.8	44.3	47.6	63.6	73.9	79.1	91.0	64.8	82.1	95.3	115.7	98.4	115.1	111.5	137.9	124.6	151.1	139.1	165.5

ATTACHMENT D

TABLE 1

OIL BASED LONG-ACTING INTRAMUSCULAR INJECTIONS

PRODUCT NAME	STEROID	DOSE	TYPE	COMP.	SOURCE	OIL	BzBz	BzOH	EtOH	[illegible]	[illegible]
SUSTANON 250	Testosterone propionate	30 mg	Androgen	Organon	ABPI Data Sheet Comp. 1998	Arachis		0.1 ml		1 ml	3 weeks
	Testosterone phenyl-propionate	60 mg									
	Testosterone isocaproate	60 mg									
	Testosterone decanoate	100 mg									
PROLUTON DEPOT	Hydroxy progesterone hexanoate	250 mgml⁻¹	Progestogen	Schering HC	ABPI Data Sheet Comp. 1998	Castor	up to 46%			1 or 2 ml	1 week
TOCOGESTAN	Hydroxy progesterone enantate	200 mg	Progestogen	Theramex	Dict. Vidal 1990	Ethyl oleate	*40%			2 ml	<1 week
	Progesterone or Tocopherol	50 mg 250 mg									
TROPHOBOLENE	Estrapronicate	1.5 mg	Mixed	Theramex	Dict. Vidal 1997	Olive	40%			1 ml	15 to 30 days
	Nandrolone undecanoate	50 mg									
	Hydroxy-progesterone heptanoate	80 mg									
NORISTERAT	Norethisterone enanthate	200 mg	Contra-ceptive	Schering HC	ABPI Data Sheet Comp. 1998	Castor	YES			1 ml	8 weeks
BENZO-GYNOESTRYL	Estradiol hexahydro-benzoate	5 mg	Estradiol	Roussel	Dict. Vidal 1998	Arachis				1 ml	1 week
PROGESTERONE-RETARD	Hydroxy progesterone caproate	250 mgml⁻¹	Progestogen	Pharlon	Dict. Vidal 1999	Castor	YES			1 or 2 ml	1 week
GRAVIBINAN	Estradiol 17-β-valerate	5 mgml⁻¹	Mixed	Schering	Dict. Vidal	Castor	YES			1 or	1-2
	Hydroxy-progesterone caproate	250 mgml⁻¹		HC	1998					2 ml	weeks
PARABOLAN	Trenbolone	76 mg	Androgen	Negma	Dict. Vidal 1997	Arachis		79 mg	45 mg	1.5 ml	2 weeks
DELESTROGEN	Estradiol valerate	20 mgml⁻¹ 40 mgml⁻¹	Estradiol	BMS	J Pharm. Sci (1964) 53(8) 891	Castor 78% 58%	~~20%~~ ~~40%~~ 20% 40%	~~20%~~ ~~40%~~ 2% 2%	~~2%~~ ~~2%~~		
DELALUTIN	17-Hydroxy progesterone caproate	250 mgml⁻¹	Progestogen	~~BMS~~ BMS	J Pharm. Sci (1964) 53(8) 891	Castor	YES	YES up to 2%	~~up to 2%~~		

BzBz = benzylbenzoate
BzOH = benzylalcohol
EtOH = ethanol Diss.
Vidal = Dictionnaire Vidal % are w/v and
*approximate as measured directly from a single sample

Corrections to Table 1

In Table 1, the given values for the benzyl benzoate, benzyl alcohol and ethanol levels for the Delestrogen and Delalutin products have been incorrectly entered into the wrong columns. The entries are shown in their correct form in the attached corrected version of Table 1. The error is apparent from a review of the reference J.Pharm Sci (1964) 53 (8) 891 (Riffkin) which is stated in Table 1 as being the Source of the information for the Delestrogen and Delalutin products:

- In the Summary on page 895 of Riffkin, Delestrogen and Delalutin are identified as castor oil based commercially available products containing estradiol valerate at 20 & 40 mg/ml and 17-hydroxy-progesterone caproate at 250 mg/ml respectively.
- Furthermore, details of particular vehicle compositions for estradiol valerate and 17-hydroxy-progesterone caproate are given in Tables V and VI
    - In Table VI, the only 20 mg/ml formulation of estradiol valerate, also referred to as commercially available, has the composition castor oil 78%, benzyl benzoate 20% and benzyl alcohol 2%.
    - In Table VI, the only 40 mg/ml castor oil based formulation of estradiol valerate, has the composition castor oil 58%, benzyl benzoate 40% and benzyl alcohol 2%.
    - In Table V, there are three 250/mg/ml castor oil based formulations of 17-hydroxy-progesterone caproate that all contain benzyl benzoate. Two of these formulations also contain 2% benzyl alcohol and the other formulation does not contain benzyl alcohol ie they all contain up to 2% benzyl alcohol.

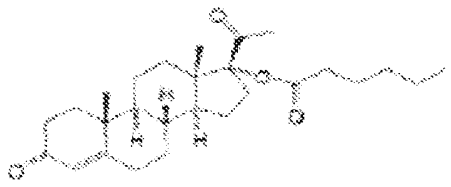
- None of the vehicle compositions disclosed in Tables V and VI in Riffkin contain ethanol. Therefore the entries in the Ethanol column of Table 1 for the Delestrogen and Delalutin products must have been incorrectly entered in the wrong column and should have been entered into the Benzyl Alcohol column.
- It is also apparent from Table VI that the 78% and 58% entries in the Benzyl Benzoate column of Table 1 for the Delestrogen products should have been entered into the Oil column and the 20% and 40% entries in the Benzyl Alcohol column should have been entered into the Benzyl Benzoate column
- The exact compositions for the Delestrogen and Delalutin products are confirmed in the Physicians Desk Reference (Edition 27, 1973) on page 1352.

In addition, the name of the steroid given in Table 1 for the Delalutin product should have been 17-hydroxy-progesterone caproate and not just 17-hydroxy-progesterone. Also the entry under the Company column for the same product should read BMS rather than DMS.

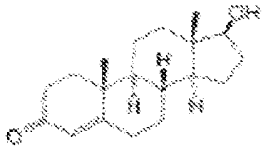
ATTACHMENT E

Structure of compounds disclosed in Riffkin et al.

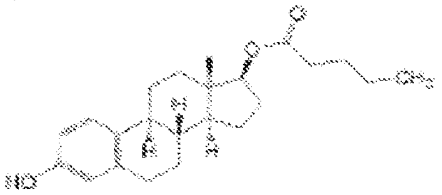
17-Hydroxyprogesterone caproate:



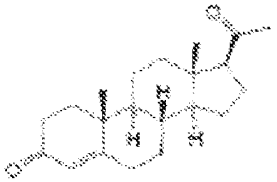
Testosterone:



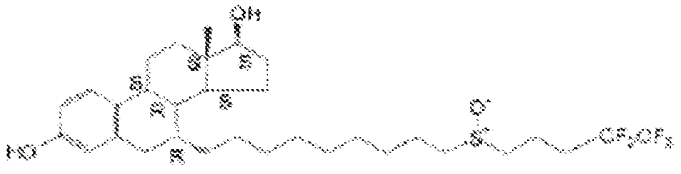
Estradiol valerate:



Progesterone:



On the other hand, fulvestrant has the following structure:



From Riffkin et al. Table II:

Steroid	Solubility [mg/ml] at 25°C	
	Castor oil	Sesame oil
Fulvestrant	20	0.58
17-Hydroxyprogesterone caproate	55.5	23.4
Testosterone	38.6	5.4
Estradiol valerate	60.6	16.1
Progesterone	52.0	22.9

Tabulation of data from Examples of Huber, 3,164,520:

Example	Steroid	Steroid concentration in benzyl benzoate (mg/ml)
1	16,17-dihydroxyprogesterone	200
2	testosterone palmitate	200
3	progesterone	250
4	Progesterone + 17-hydroxyprogesterone caproate	250 + 250
5	Testosterone enanthate	400

TABLE OF REFERENCES

Tab	Author/Inventor	Reference Citation/Patent
1	Cornelius (US '863)	US Patent 4,212,863
2	Dukes (EP '014)	EP 0 346 014 A1 (corresponds to US Patent 5,183,814)
3	Dukes (US '814)	US Patent 5,183,814 (corresponds to EP 0 346 013 A1)
4	Gupta (1999)	P.K. Gupta and G.A. Brazeau (eds). *Injectable Drug Development:Techniques to Reduce Pain and Irritation.* Chapters 11 & 17 Interpharm Press, Denver, Colorado (1999)
5	Huber (US '520)	US Patent 3,164,520
6	Lopatin (1972)	P.V. Lopatin, V. P. Safonov, T. P. Litvinova and L. M. Yakimenko. Use of nonaqueous solvents to prepare injection solutions. *Pharm. Chem. J.* ***6***:724-733 (1972)
7	Mackey (1995)	M.A. Mackey, A.J. Conway and D.J. Handelsman. Tolerability of intramuscular injections of testosterone ester in oil vehicle. *Hum. Reprod.***10**: 862-865 (1995)
8	Nema (1997)	S. Nema, R.J. Washkuhn, and R.J. Brendel. Excipients and their use in injectable products. *PDA J. Pharm. Sci. Technol.* **51**:166-71 (1997)
9	PDR (1973)	*Physicians' Desk Reference (27th edition).* 1277-1278, 1350-1354, 1391-1392 Medical Economics Company, Oradell, NJ (1973)
10	Powell (1998)	M. F. Powell, T. Nguyen, and L. Baloian. Compendium of excipients for parenteral formulations. *PDA J. Pharm. Sci. Technol.* **52**:238–311 [pages 238-255 provided] (1998)
11	Riffkin (1964)	C. Riffkin, R. Huber and C.H. Keysser. Castor oil as a vehicle for parenteral adminstation of steroid hormones. *J.Pharm.Sci.* **53**: 891-5 (1964)
12	Strickley I (1999)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) -Part I. *PDA J. Pharm. Sci. Technol.* **53**:324–349 (1999)
13	Strickley II (2000)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part II *PDA J. Pharm. Sci. Technol.* **54**:69–96 (2000)
14	Strickley III (2000)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part III. *PDA J. Pharm. Sci. Technol.* **54**:152–169 (2000)
15	Wang (1980)	Y.C. J. Wang and R. R. Kowal. Review of excipients and pH's for parenteral products used in the United States. *J. Parenteral Drug Assoc.* **34**:452–462 (1980).

Electronic Patent Application Fee Transmittal

Application Number:	12285887
Filing Date:	15-Oct-2008
Title of Invention:	Formulation
First Named Inventor/Applicant Name:	John R. Evans
Filer:	Carlos M. Tellez
Attorney Docket Number:	11285.0056-00000

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

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:				
Extension - 1 month with $0 paid	1251	1	150	150

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for continued examination	1801	1	930	930
Statutory or terminal disclaimer	1814	1	160	160
		Total in USD ($)		**1240**

Electronic Acknowledgement Receipt

EFS ID:	11825286
Application Number:	12285887
International Application Number:	
Confirmation Number:	1199
Title of Invention:	Formulation
First Named Inventor/Applicant Name:	John R. Evans
Customer Number:	22852
Filer:	Carlos M. Tellez
Filer Authorized By:	
Attorney Docket Number:	11285.0056-00000
Receipt Date:	17-JAN-2012
Filing Date:	15-OCT-2008
Time Stamp:	14:22:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1240
RAM confirmation Number	710
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Rule 130, 131 or 132 Affidavits	Exhibit_1--SawchukCV.pdf	573897 0db3336ec7b725b7384203972fb4278a02935cb7	no	33
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Information:					
2	Rule 130, 131 or 132 Affidavits	Exhibit_2--Office_Action_16-Sep-2011.PDF	731211 4f15e37d81f3921bc18cf2849f9c70a49b6f50c3	no	11
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Information:					
3	Rule 130, 131 or 132 Affidavits	Exhibit_5--Osborne.PDF	272030 4e32db3fb5737723f50c8e449ea0179da1e80b8d	no	5
Warnings:					
Information:					
4	Rule 130, 131 or 132 Affidavits	Exhibit_8--Faslox_claims_with_proposed_amendments--09-Dec-2011.pdf	124576 45495791c328a7d8966a65508cfa87928a54cd90	no	5
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Information:					
5	Rule 130, 131 or 132 Affidavits	Exhibit_9--US3164520.pdf	255520 89671a0136d2aec2d42cfce35a327341d1599cb6	no	2
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Information:					
6	Rule 130, 131 or 132 Affidavits	Exhibit_12--Merck_Index.PDF	283056 733b2ad6666064111e4d0f6e5f95aaf07a1191a7	no	8
Warnings:					
Information:					
7	Rule 130, 131 or 132 Affidavits	Exhibit_14--Lavy.pdf	145860 822312df838d475bdeca4823dde494952d18adbb	no	5
Warnings:					
Information:					
8	Rule 130, 131 or 132 Affidavits	Exhibit_15--Ismail.pdf	196801 d6457a120eb4a440923c0055fb92ab89a7365e4f	no	10
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Information:					
9	Non Patent Literature	Pages_from_File_History--23-Nov-2010_to_19-Dec-2011--EP_2_266_573--10180667.pdf	1273117 c2ebafe4125da30ba99dcc4b9b69e2b07d394ec6	no	46
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Information:					

10	Non Patent Literature	Pages_from_File_History--19-Jan-2011_to_19-Dec-2011--EP2286818--10180661.pdf	1199990 1c4606766d904accf7efedc7cafc03fa094e1e31	no	42
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11	Rule 130, 131 or 132 Affidavits	Exhibit_3--McLeskey-.pdf	2883384 679455e1c82a25a4cbd04f88ef0c5bbea905f6ad	no	15
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Information:					
12	Rule 130, 131 or 132 Affidavits	Exhibit_4--EP0346014-B1--Dukes-.pdf	1621917 a4de1af888d43ce8e2f49293549f04868eb2e018	no	14
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Information:					
13	Rule 130, 131 or 132 Affidavits	Exhibit_6--Wakeling-.pdf	138081 bde6a21a2cdce0a6dc4f595e036bd85a31ba7708	no	2
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Information:					
14	Rule 130, 131 or 132 Affidavits	Exhibit_7--US_2010-0152149A1-.pdf	711671 018c92234b63aecdffc82b816f1e4bf8fb65bb19	no	10
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Information:					
15	Rule 130, 131 or 132 Affidavits	Exhibit_10--Riffkin-.pdf	421271 6358b8b493e6d354b7c8e75be125f8ff2f9626ec	no	5
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Information:					
16	Rule 130, 131 or 132 Affidavits	Exhibit_11--Nema-.pdf	656507 6b87d551e40378b970cc2500b66aae11414fe376	no	6
Warnings:					
Information:					
17	Rule 130, 131 or 132 Affidavits	Exhibit_13--Guerrini-.pdf	388452 3aaebbcc6b379de33233ff81d4626ad73f7f5458	no	8
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Information:					
18	Rule 130, 131 or 132 Affidavits	Exhibit_17--WO_03-006064-.pdf	2208801 2ccc7cedbb97c919135349a171b4629417d96fa3	no	60
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Information:					

19	Non Patent Literature	Pages_from_file_history--07-Sep-2009_to_15-Dec-2011--EP_1_250_138--01900186.pdf	273713 62d9d727f5df88f5912b60f6bb78d071d86871d9	no	14
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20	Non Patent Literature	11285-0056--Ismail.pdf	196708 0764a889d53ad854199c5f607db58bc5c4f04c94	no	10
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Information:					
21	Non Patent Literature	11285-0056--Lavy.pdf	145800 5f924db76257b91b39716191b33ed33fc78d3853	no	5
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Information:					
22	Non Patent Literature	11285-0056--MerckIndex.pdf	286744 0f74063ace9c86c0b93120415c7982fb69d1e6db	no	8
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23	Non Patent Literature	11285--0056--Guerrini-.pdf	388489 02126ff82853baa264be55a03882e90192463c1c	no	8
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24	Rule 130, 131 or 132 Affidavits	Exhibit_16--Gellert_Declaration.pdf	26188243 e9d11fb259f0f64f886e275013489e678af70eeb	no	25
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25	Terminal Disclaimer Filed	11285-0056--Terminal_Disclaimer.pdf	117851 cf75f4f4f6c88c4b4e6d0640323124e0274331b7	no	4
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26		Response_to_16-Sep-2011_OA--Final_version_17-Jan-2012.pdf	156764 1f0800313b0a0d6ca584d647ee4efd2b0c29e3ef	yes	28

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	Claims	2	6

	Applicant Arguments/Remarks Made in an Amendment		7	28	
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Information:					
27	Rule 130, 131 or 132 Affidavits	Sawchuk_Declaration--Final_Executed_Version--13-Jan-2012-.pdf	227943 c28d18fc2d36703e478af54c1bdf52e48b72f77e	no	27
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Information:					
28	Request for Continued Examination (RCE)	11285-0056-RCE.pdf	58137 a4fcc2e01f43253b596af8013e379e9018c3de6f	no	1
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Information:					
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Information:					
30	Information Disclosure Statement (IDS) Form (SB08)	11285-0056--FormSB-08.pdf	60677 b0dab4c887c0778c5ee913f3a393d430d34c34bc	no	1
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32	Fee Worksheet (SB06)	fee-info.pdf	33259 8e6b3c56a1c465297b62998668e3c51581ca3b37	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	68474136		

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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.

RONALD J. SAWCHUK

PERSONAL DATA

Present Address:	Department of Pharmaceutics College of Pharmacy Room 9-149A Weaver-Densford Hall University of Minnesota 308 Harvard Street S.E. Minneapolis, MN 55455 Telephone: (612) 624-0646 E-mail: sawch001@umn.edu
Home Address:	14934 Pixie Point Circle SE Prior Lake, MN 55372 Telephone: (952) 226-6507
Born:	May 29, 1940, Toronto, Ontario, Canada
Marital Status:	Married, three children
Citizenship:	Dual: U.S. and Canadian

EDUCATION

1959	(High School)	Oakwood Collegiate Institute, Toronto Secondary School Grade XIII)
1963	B.Sc. Phm.	University of Toronto, Toronto Ontario College of Pharmacy Licentiate No. 10748
1966	M. Sc. Phm.	University of Toronto, Toronto
1972	Ph.D.	University of California, San Francisco Pharmaceutical Chemistry (Pharmacokinetics)

PROFESSIONAL AND ACADEMIC EXPERIENCE

1963 - 1965	Teaching Assistant, University of Toronto
1966	Community Pharmacist (part-time), Toronto
1966 - 1968	Teaching Assistant, University of California
1971 - 1972	Instructor in Pharmaceutics, University of Minnesota
1972 - 1977	Assistant Professor of Pharmaceutics, University of Minnesota
1977 -1983	Associate Professor of Pharmaceutics, University of Minnesota
1974 - 1982	Associate Director, Clinical Pharmacokinetics Laboratory, U of Minnesota
1982 - 1995	Director, Clinical Pharmacokinetics Laboratory, College of Pharmacy, U of Minnesota
1983 - 2010	Professor of Pharmaceutics, University of Minnesota
1983 - 1989	Director of Graduate Studies in Pharmaceutics, University of Minnesota
1983 - 1986	Acting Head, Department of Pharmaceutics, University of Minnesota
1984 (summer)	Quarter Leave, Sandoz Pharma, Pharmacokinetics and Drug Metabolism Dept., Basel, Switzerland (M. Lemaire)
1991 - 1994	Director of Graduate Studies in Pharmaceutics, University of Minnesota
1992 (Summer)	Quarter Leave, Sandoz Pharma, Drug Safety, Basel, Switzerland (W. Niederberger)
1996 - 1999	Member, Board of Directors, Century Mortar Club
1997 (Spring)	Semi-Quarter Leave, Toyama Medical and Pharmaceutical University, Japan (H. Sato)
1997 (Summer)	Semi-Quarter Leave, Novartis AG, PKDM, Basel, Switzerland (J. Vonderscher)
1998 - 1999	Head, Department of Pharmaceutics, University of Minnesota
2001 (Summer)	Faculty Development Leave, Novartis AG, PKDM, Basel, Switzerland (M. Lemaire)
2010 - present	Professor Emeritus of Pharmaceutics, University of Minnesota

APPOINTMENTS AND PROFESSIONAL RESPONSIBILITIES

1972 - present	Member, Graduate Program in Pharmaceutics, University of Minnesota
1982 - present	Consultant to the pharmaceutical industry
1995 - present	Director, Bioanalytic and Pharmacokinetic Services, University of Minnesota
1995 - present	Editorial Board, *Saudi Pharmaceutical Journal*
1996 - 2007	Editorial Board, *Journal of Pharmaceutical Sciences*
1996 - present	Member, Graduate Program in Neurosciences, University of Minnesota
2001 - present	Member, Graduate Program in Experimental and Clinical Pharmacology, U of M
2002 - present	Member, Graduate Program in Social, Administrative and Clinical Pharmacy, U of M
2008 - present	Editorial Advisory Board, *AAPS Journal*
2009 - present	Editorial Board, *Xenobiotica*

OTHER PROFESSIONAL ACTIVITIES

Prepared two videotapes on "Pharmacokinetics" for undergraduate instruction, 1974
Co-editor of a book with James Blanchard, Ph.D. and B.B. Brodie, Ph.D., entitled "Principles and Perspectives in Drug Bioavailability." S. Karger, Publisher, 1979
Assistant Director, Clinical Pharmacokinetics Laboratory, 1974-82
Consultant in the Establishment and Implementation of the Drug Quality Assurance Program, United Hospitals, St. Luke's Division, St. Paul, 1975
Participant in Critical Incidents Workshop, PDI - College of Pharmacy, 1977
Assessor in the Pharmacy Assessment Exercises, 1978
Coordinator for Continuing Education in Pharmacy, TV Series 1978, 1980
Expert, Bureau of Drugs and Biologics, Food and Drug Administration, 1982-84
Screening Committee, Abstracts, Basic Pharmaceutics Section, APS, APhA, 1981
Review of Grants, Medical Research Council (Canada) 1980-86
Review of Grants, British Columbia Health Care Foundation, 1981-84
Advisory Consultant, Site Visit Team NIH (NINCDS) Yale University School of Medicine, October 1979
Member, Site Visit Team NIH (NINCDS) University of Utah School of Medicine, January 1983
Member, Special Pharmacology Study Section NIH, April-June 1988
Review of Grants, Idaho State Board of Education, 1989-91
Review of Grants, Greater Minnesota Corporation, 1990-91
Organizer and Symposium Co-Chair, "Microdialysis in Drug Metabolism and Disposition Studies", for the Annual AAPS Meeting, San Antonio TX, 1992
Symposium Co-Chair, "Kinetic and Dynamic Challenges of the 90's", for the Annual AAPS Meeting, San Diego, CA, 1994
Organizing Committee Member for the NATO Advanced Study Institute, "Pharmacokinetics: From Theory to Practice", Erice, Italy, April 5-16, 1994
Co-organizer and Participating Instructor, "Pharmacokinetics for the Pharmacist and Pharmaceutical Scientist" University of Milan, Varese, September 10 -15, 1995.
Member, Board of Directors, Century Mortar Club, 1996-present.
National Advisory Committee, FAMU RCMI Program, Tallahasse, FL 1996-present
Co-organizer and Participating Instructor, "Pharmacokinetics for the Biomedical and Pharmaceutical Scientist", University of Milan, Varese, September 7 -12, 1997.
Scientific Advisory Committee, 1st Symposium on Microdialysis and Pharmacokinetics, Leiden, The Netherlands April 1998
Organizer and Participating Instructor, "Pharmacokinetics for the Biomedical and Pharmaceutical Scientist", University of Malta, Msida, September 6 -15, 1998.
Founder, Microdialysis Focus Group, American Association of Pharmaceutical Scientists, 1998.
Scientific Advisory Committee, 2nd International Symposium on Microdialysis in Drug Research and Development, Stockholm, Sweden, June 2000
Chair, Microdialysis Focus Group, American Association of Pharmaceutical Scientists, 1998-2000.
Co-Chair, Organizing Committee, 3rd International Symposium on Microdialysis in Drug Research and Development, Minneapolis, MN, USA, June 2002
Visiting Professor, Guilin Medical College, Guilin PRC (2002-2007)
Scientific Advisory Committee, 4th International Symposium on Microdialysis in Drug Research and Development, Vienna, Austria, June 2004

Scientific Advisory Committee, Abbott Laboratories, for the FDA Critical Path Initiative, September 2004
Scientific Advisory Committee, 5th International Symposium on Microdialysis in Drug Research and Development, Leiden, The Netherlands, June 2006
GLP-1 Scientific Advisory Panel, Medtronic, Minneapolis, MN, April 2009-present

CURRENT AND PAST MEMBERSHIP IN PROFESSIONAL AND SCIENTIFIC SOCIETIES

American Association of Pharmaceutical Scientists (Fellow)
American Association for the Advancement of Sciences (Fellow)
American Pharmacists Association (APhA)
American Society for Pharmacology and Experimental Therapeutics
International Society of Anti-Infective Pharmacology
International Society for the Study of Xenobiotics
Technology Park, Heidelberg, Germany
Century Mortar Club (Board of Directors, 1996-98)
Rho Chi Honor Society

SCHOLARSHIPS, HONORS AND AWARDS

1964	Scholarship, Canadian Foundation for the Advancement of Pharmacy
1965-66	National Research Council of Canada
1965	Warner-Lambert Research Fellowship
1968-70	National Institute of Health (NIH) Training Grant
1981-82	Teacher of the Year, College of Pharmacy, University of Minnesota
1986	Recipient of Horace T. Morse-Amoco Foundation Award
1988	Fellow, American Association of Pharmaceutical Scientists
1990	Fellow, American Association for the Advancement of Sciences
1996	Hallie Bruce Memorial Lecture Award
1997	Fellowship, Japanese Society for the Promotion of Science
1999	Meritorious Manuscript Award, American Association of Pharmaceutical Scientists
2001	Weaver Medal of Honor
2004	Distinguished Lecture, Creighton University School of Pharmacy and Health Professions
2005	Academy of Distinguished Teachers, University of Minnesota
2006	Distinguished Lecture, Temple University School of Pharmacy
2007	APhA Research Achievement Award in the Basic Pharmaceutical Sciences

COMMITTEE APPOINTMENTS

COLLEGE OF PHARMACY

1972-73, 1973-74	Student American Pharmaceutical Association Minnesota Chapter (Faculty Advisor)
1972-75	Student Admissions and Academic Standing Committee, College of Pharmacy
1972-73	Task Force on College of Pharmacy Organization
1973-74	Continuing Education Committee
1972-78	Admissions Committee for Pharm.D. Program, College of Pharmacy (Chair 1973-74; 1977-78)
1974-75	University of Minnesota Health Sciences B/C Implementation Committee
1974-77	Constitution and By-laws Committee
1974-75	Unit K Committee, Graduate School
1975-76	Task Force on Pharm.D. Admissions
1976-78	Professional Education Committee
1977-78	Task Force on Travel
1977-78	Anatomy, Physiology, Pathology Study Group
1977-78	Drug Product Design and Evaluation Study Group
1976-78	Search Committee for Biopharmaceutics Faculty Member
1977-78	Search Committee for Assistant Director HCMC
1977-78	Search Committee for Research Associate, CEP Project D-1 (Chairman)
1978-79	Pharm.D. Program Planning Committee (Chairman)
1978-79, 1979-80	Computer Systems Committee (Chairman)

1979-80	Professional Education Committee (Chairman)
1980-81	Educational Policy Committee (Chairman)
1980-82	Externship Committee
1981-82	Academic Standing Committee
1981-83	Health Sciences Policy and Review Council
1981-82	Graduate Faculty Nominations and Course Proposals Committee
1982-83	Academic Standing Committee (Chairman)
1982-83	Advisory Committee on Animal Care Facilities
1983-85	Council of Directors of Graduate Studies
1982-83	Task Force on Computers
1983	Search Committee for Department Chairman (Chairman)
1983	Search Committee for Clinical Faculty at HCMC
1984	Ad Hoc Committee on External Pharm.D. Program
1984	Executive Committee (Chairman)
1984	Search Committee for Dean of College of Pharmacy
1984	Search Committee for Psychiatry Position, St. Paul-Ramsey Medical Center
1984	Search Committee for Clinical Faculty at Hennepin County Medical Center
1985	Endowed Chair in Pharmaceutics Search Committee (Chair)
1985	Assistant Professor in Pharmaceutics Search Committee (Chair)
1985	Appointments, Promotion and Tenure Committee
1985	Space Committee
1985	Clinical Assistant Professor (MMC) Search Committee
1985-89	Executive Committee
1986-87	Appointments, Promotion and Tenure Committee (Chair)
1986-90	Educational Policy Committee
1986-87	Subcommittee of Educational Policy Committee
1986	Search Committee for Endowed Chair (Chair)
1986-87	College of Pharmacy Strategic Planning Committee
1986-87	Subcommittee of Strategic Planning Committee to Develop College Goals and Objectives
1987-90	Continuing Pharmacy Education Advisory Committee (Chair)
1987-88	Admissions Committee
1988-89	Admissions Committee (Chair)
1989-91	Promotion and Tenure Committee
1991-92	Promotion and Tenure Committee (Chair-Elect)
1991-92	General Research Support Committee
1992-93	Promotion and Tenure Committee (Chair)
1992-93	General Research Support Committee
1993-94	Academic Standing Committee (Chair-Elect)
1994-95	Academic Standing Committee (Chair)
1994-98	College Computer Committee
1995-96	Promotion and Tenure Committee
1995-96	Internal Organization and Leadership Task Force
1996-97	Nontraditional Pharm.D. Task Force
1997-98	Search Committee for Endowed Chair in Geriatric Pharmacotherapy
1997-98	Admissions Committee
1997-98	Search Committee for Immunotherapy Faculty Position (Chair)
1998-2000	Search Committee for Pharmaceutics Faculty Position
2000-2001	Educational Policy Committee
2001-2002	Search Committee for ECP Faculty Position
2001-2002	Educational Policy Committee (Chair)
2001-2002	Search Committee for Pharmaceutics Faculty Position
2001-2002	College of Pharmacy Phar. Sci. 2020 Committee, Capital Campaign (Co-Chair)
2001-2004	College of Pharmacy Faculty Consultative Committee
2002-2003	Educational Policy Committee (Past Chair)
2002-2003	College of Pharmacy Collegiate Review Committee (Chair)
2002-2003	College of Pharmacy Central Council (Faculty Representative)
2002-2003	College of Pharmacy Instructional Development Working Group for the Duluth Expansion
2003-2005	Search Committee for Pharmaceutics Faculty Position at UMD (Chair)
2004-2007	College of Pharmacy Assessment Committee

2005-2006	Search Committee for Endowed Chair in Geriatric Pharmacotherapy
2006-2007	Search Committee for Pharmaceutics Faculty Position

UNIVERSITY COMMITTEE APPOINTMENTS

1974-78	Subcommittee on Academic-Industrial Interface, Academic Relations Committee, 3M Technical Forum
1975-76	Health Sciences Primary Health Care Program Committee (Alternate), Solicitor for the University of Minnesota Consolidated Fund Drive
1977-78	Alternate Senator (U. of Minnesota)
1978-81	Senator (U. of Minnesota)
1984-85	Health Sciences Learning Resources Committee
1986	College Delegate to All-University Single Quarter Leave Working Group, Academic Affairs
1989	Health Sciences Policy and Review Council, Graduate School
1989-91; 1991-93	Biological Sciences (formerly Plant and Animal Sciences) Policy and Review Council, Graduate School
1991-93	Graduate Faculty Nominations Subcommittee, Biological Sciences Policy and ReviewCouncil, Graduate School
1992-93	Graduate Faculty Nominations Subcommittee (Chair), Biological Sciences Policy and Review Council, Graduate School
1995-1998	Biological Sciences Policy and Review Council, Graduate School
1997-98	Faculty Research Development Proposal Review Committee for the Academic Health Center
2001-2004	Academic Health Center Faculty Consultative Committee
2001-2002	SCFP Subcommittee on Twin Cities Facilities and Support Services (STCFSS)
2003	AHC Seed Grant Review Committee
2003	AHC FCC Internal Screening Committee for Academy of Excellence Nominees
2004-2007	All-University Honors Committee, University of Minnesota

STATE, NATIONAL, AND INTERNATIONAL COMMITTEE APPOINTMENTS

1974-76	Representative to AACP Council of Faculties
1977-78	AACP Task Force on Guidelines for Pharm.D. Accreditation
1980-82	Academic Advisory Committee, Kellogg Pharmaceutical Scientist Program
1981	Screening Committee for Academy of Pharmaceutical Sciences, Basic Pharmaceutics Section
1989-present	Member, Scientific Committee, International Pharmaceutical Technology Symposium (FIP)
1990	Academic Affairs Committee, AACP (Member)
1990	Program Committee, Controlled Release Society Annual Meeting (Member)
1989-91	Continuing Education Committee, State Board of Pharmacy (Member)
1990-95	USP Committee of Revision (Member)
1991-93	NIH/NINDS Antiepileptic Drug Development Program (Consultant)
1995	Fellows Nominations Committee for AAPS, PPDM Section
1995	Screening Committee for AAPS PPDM Section Abstracts
1997-2000	Fellows Nominations Committee for AAPS, PPDM Section
1999-2000	Committee on AAPS Section Structure and Procedure Guideline
2000-2001	PPDM Vice Chair, American Association of Pharmaceutical Scientists
2000-2002	Co-Chair, Organizing Committee, 3rd International Symposium on Microdialysis in Drug Research and Development
2001-2002	PPDM Chair Elect, American Association of Pharmaceutical Scientists
2001-2002	Annual Program Planning Committee, American Association of Pharmaceutical Scientists
2001-2002	Program Coordinating Committee, American Association of Pharmaceutical Scientists
2002-2003	PPDM Section Chair, American Association of Pharmaceutical Scientists
2002-2003	PPDM Committee for Graduate Student Symposium Awardees, American Association of Pharmaceutical Scientists
2002-2003	Short Course Program Review Team, American Association of Pharmaceutical Scientists
2003-2004	PPDM Section Past-Chair, American Association of Pharmaceutical Scientists
2004-2007	Member-at-Large, American Association of Pharmaceutical Scientists Executive Council
2004-2006	Clinical and Operational Working Group (CORWG), NASA
2004-2005	AAPS Executive Council Liaison to the Clinical Sciences section of AAPS
2005-2006	AAPS Executive Council Liaison to the DDD section of AAPS
2005-2006	AAPS Executive Council Liaison to the PDD section of AAPS
2005-2006	AAPS Executive Council Liaison to the 2006 Annual Meeting Program Committee

2005-2006	AAPS Executive Council Liaison to the 2006 Annual Meeting Screeners
2005-2006	AAPS Executive Council Liaison to the 2006 Program Coordination Committee
2006	AAPS Reference Resources Task Force
2006-2007	AAPS Executive Council Liaison to the APQ section of AAPS
2006-2007	AAPS Executive Council Liaison to the PT section of AAPS
2006-2007	AAPS Executive Council Liaison to the International Affairs Committee
2009-2011	Epilepsy NINDS Steering Committee
2009-2011	NINDS Consortium to Study Bioequivalence of AED Products

INVITED PRESENTATIONS

Continuing Education Program (6 hours) Minneapolis, MN, 1973.
Upper Midwest Hospital Conference, 1974.
Continuing Education Program (6 hours) Rochester, MN, 1974.
University of Illinois, Chicago, IL, 1974.
Department of Clinical Pharmacology, University of Minnesota, 1974.
AACP Annual Meeting and Teachers' Seminar (Workshop Leader), Lake Kiamesha, NY, 1975.
Debate Symposium, "Drug Product Selection," St. Paul, MN, 1977.
Continuing Education for Minneapolis Veteran Pharmacists (2 hours), Minneapolis, MN, 1978.
Continuing Education in Pharmacy (2 hours), Mankato, MN, 1978
Continuing Education in Pharmacy "Seminar at Sea" (4 hours of instruction), 1978.
HPLC Workshop, Invited Lecturer, Bloomington, MN, 1978.
University of Kentucky, Lexington, KY, 1979.
American Association of Clinical Chemists, Midwest Section, Minneapolis, MN, 1979.
University of Illinois, Chicago, IL, 1979.
Smith Kline Corp., Philadelphia, PA, 1979.
Department of Pathology, St. Cloud Hospital, St. Cloud, MN, 1979.
Comprehensive Epilepsy Program, Minneapolis, MN, 1979.
University of North Carolina, Chapel Hill, NC, 1979.
Burroughs Wellcome Co., Research Triangle Park, NC, 1979.
St. Paul-Ramsey Medical Center, St. Paul, MN, 1989.
Continuing Education in Pharmacy (4 hours) Minneapolis, MN, September-October, 1981.
Medical Research Council of Canada, Visiting Professor, University of British Columbia, Vancouver, 1982.
Invited Lecturer, National Institutes of Health, Epilepsy Branch, Bethesda, MD, 1982.
Geriatric Research, Education and Clinical Center, Bloomington, MN, September, 1982.
Continuing Education in Pharmacy (6 hours), Duluth, MN, September, 1982.
Ciba-Geigy, Pharmaceuticals Division, Ardsley, December 2, 1982.
Swiss Federal Institute of Technology, Zurich, Switzerland, June 19, 1984.
Biopharmacy Division, Sandoz AG, Basel, Switzerland, June 22, 1984.
Biopharmacy Division, Sandoz AG, Basel, Switzerland, July 24, 1984.
"Cyclosporine Pharmacokinetics in the Rabbit: In Vivo Disposition and In Situ Absorption Studies," Rhone-Poulenc Visiting Professor, University of Toronto, Ontario, February 5, 1985.
"Pharmacokinetics and Pharmacodynamics," Drug Therapy Symposium VI, St. Paul, MN, February 27, 1985.
"Absorption and Disposition Studies with Cyclosporine," Sandoz, AG, Basel, Switzerland, July 15, 1985.
"Absorption of Cyclosporine from Rabbit Small Intestine Using an In Situ Perfusion Model," Vorstand des Instituts fur Pharmazie U. Lebensmittlechemie der Ludwig-Maximilians-Universitat, Munich, West Germany, July 17, 1985.
"Analytic considerations in the Investigation of the Pharmacokinetics of Cyclosporine," Medizinischen Hochschule, Hanover, West Germany, September 11, 1985.
"Mixed-Order Absorption of a Sustained Release Carbamazepine Tablet in Humans," Institut fur Pharmazeutische Technologie der Johann Wolfgang Goethe-Universitat, Frankfurt am Main, West Germany, May 15, 1986.
"Simultaneous First- and Zero-order Absorption of Commercial Carbamazepine Tablets," 5th Symposium on Biopharmaceutics and Pharmacokinetics, Piestany, Czechoslovakia, May 22, 1986.
"Simultaneous First- and Zero-order Absorption of Tegretol in Human Volunteers," National Institutes of Health, Epilepsy Branch, NINCDS, Bethesda, MD, November 6, 1986.
"Comparison of Plasma AUCs using the Traditional Point-by-Point and Pooled Sample Methods: Application in the Analysis of Human Pharmacokinetics of Carbamazepine and its metabolites," Food and Drug Administration, Rockville, MD, July 20, 1987.

"Pharmacokinetics in Contemporary Pharmacy Practice," Minneapolis Veteran Pharmacists Association, Richfield, MN, September 15, 1987.

"The Absorption and Disposition Kinetics of Carbamazepine and its Metabolites in Humans," Ciba-Geigy, Summit, NJ, July 23, 1987.

The following four lectures were given in Beijing, Chengdu, and Guilin, China during a visit sponsored by the Chinese Academy of Medical Sciences in late October/early November 1987:

1. "Theory and Application of a Pharmacokinetic Model in Individualizing Dosing Regimens for the Aminoglycosides."
2. "First- and Zero-order Absorption of Carbamazepine from Commercial Tablets in Epileptic Patients and Normal Volunteers."
3. "Significance of Nonlinear Disposition Kinetics in the Adjustment of Dosing Regimens."
4. "Relative Bioavailability of Phenytoin Formulations: Problems in Assessment Due to Michaelis-Menten Elimination Kinetics."

"Does Tegretol need to be Dosed TID?" Comprehensive Epilepsy Program, Minneapolis, MN, March 21, 1988.

"The Kinetics of Absorption of Carbamazepine (Tegretol) and its Metabolism in Humans," Vorstand des Instituts der Pharmazie, Ludwig-Maximilians Universitat, Munich FRG, June 8, 1988.

"Pharmacokinetic and Physiologic Considerations in Oral Controlled Drug Delivery," Novel Drug Delivery Symposium, Minneapolis, MN, September 20, 1988.

"Clinical Applications of the Two-Compartment Open Model," Regional Kidney Disease Program, Hennepin County Medical Center, Minneapolis, MN, November 16, 1988.

The following five lectures were presented in a Continuing Education in Pharmacy Program: "Concepts and Applications in Pharmacokinetics, Parts I and II"; "Therapeutic Response and Toxicity"; "Monitoring Drug Therapy"; and "Bioavailability and Bioequivalence", St. Thomas, Virgin Islands, March 8-13, 1989.

"The Pharmacokinetics of Zidovudine (AZT) with Some Observations on the Interaction with Probenecid," Queen's University of Belfast, Belfast, North Ireland, June 15, 1989.

"Pharmacokinetic and Analytical Considerations in Monitoring Zidovudine (AZT) Levels in Children with Aids," Fourth International Congress on Pediatric Laboratory Medicine, Washington, DC, August 23, 1989.

"Inhibition of Zidovudine Metabolism and Excretory Transport," Department of Pharmacodynamics, Semmelweis University of Medicine, Budapest, Hungary, September 13, 1989.

"Evaluating Bioequivalence," Western Michigan Society of Hospital Pharmacists, Grand Rapids, MI, March 2, 1990.

"Effect of Temperature and Medium of Analysis on Cyclosporine Concentration," Canadian Consensus Meeting on Cyclosporine Monitoring, Minaki Lodge, Canada, May 11, 1990.

"Studies of the Interaction between Zidovudine (AZT) and Probenecid in Animals and Humans." Pharmaceutics and Process R & D, Ayerst Laboratories Inc., Rouse's Point, NY, August 17, 1990.

"Mechanistic Studies to Examine the Effect of Probenecid on the Brain Uptake of Zidovudine," Shanghai Medical University, Shanghai, P.R.C., October 13, 1990.

A lecture series (16 hrs) on the topic of "Clinical Pharmacokinetics and Therapeutic Drug Monitoring" was given to staff members of the Chinese Academy of Medical Sciences and Hospital Pharmacists, Beijing, P.R.C., October 15-20, 1990.

"Comparative Intestinal Absorption of Compounds of Varying Lipophilicity, and the Effect of Absorptive Water Flux." Lederle Laboratories, Pearl River, NY, September 12, 1991.

"Analysis of Zidovudine Distribution into Specific Brain Regions Utilizing Microdialysis," Bristol Myers-Squibb Research Institute, Princeton, NJ, September 17, 1991.

"Distribution of AZT Into Specific Brain Regions in the Rabbit Utilizing Microdialysis," University of Illinois College of Medicine, Peoria, IL, October 9, 1991.

"Studies on the Transport of Nucleosides into Specific Brain Regions Using Microdialysis with *In Vivo* Calibration." University of Florida, College of Pharmacy, Gainesville, FL, December 6, 1991.

"Analysis of Zidovudine Distribution into Specific Brain Regions Utilizing Microdialysis," University of Arizona College of Pharmacy, Tucson, AZ, February 17, 1992.

"Regional Considerations in the In Situ Intestinal Absorption of Glycylcycline and Minocycline, and the Effect of Solvent Drag," Lederle Laboratories, Pearl River, NY, May 11, 1992.

"Comparative Absorption of Fluorothymidine and Related Nucleosides in Different Anatomic Intestinal Regions," Lederle Laboratories, Pearl River, NY, May 11, 1992.

"Microdialysis Techniques for the Study of Drug Distribution, and the Problem of Recovery *In Vivo*," Europhor Toulouse, France, June 19, 1992.

"The Use of Microdialysis in Studying the Distribution of Exogenous Substances in Biological Tissues," Sandoz Pharma, Basel Switzerland, June 24, 1992.

"Inhibition of Brain Distribution and Systemic Clearance of AZT by Probenecid," Sandoz Pharma, Basel Switzerland, June 30, 1992.

"Uptake of Zidovudine (AZT) into Rabbit Brain Using Microdialysis with *In Vivo* Calibration," Knoll AG, Ludwigshafen, Germany, July 1, 1992.

"Microdialysis in the Study of the Distribution and Metabolism of Exogenous Substances," Pharmaceutical Chemical Institute, University of Heidelberg, Heidelberg, Germany, July 2, 1992.

"The Relationship Between Urine and Plasma Concentrations of Lipophilic Drugs: Implications for Therapeutic Drug Monitoring," Sandoz Pharma, Basel Switzerland, July 8, 1992.

"Estimation of the Elimination Rate Constant for Metabolites which Exhibit Formation-Rate Limited Disappearance," Sandoz Pharma, Basel Switzerland, July 23, 1992.

"Experimental Determination of Free Tissue Levels Using Microdialysis," 4th Biennial Conference on Chemotherapy of Infectious Diseases and Malignancies, Prague, Czechoslovakia, August 31, 1992.

"*In Situ* Intestinal Absorption of Tetracycline Derivatives and the Effect of Absorptive Water Flux," Lederle Laboratories, Pearl River, NY, November 13, 1992.

"Reversibility of Carbamazepine Autoinduction upon Dose Termination in Normal Volunteers," Abbott Laboratories, Abbott Park, IL, December 2, 1992.

"Barriers to the Oral Delivery of Drugs," Wyeth-Ayerst Research, Radnor, PA, February 23, 1993.

"Preliminary Results of Studies which Examine the Distribution of the NMDA Antagonist, EAB 515, to Rat Brain," Sandoz Pharma, Basel Switzerland, April 26, 1993.

"Microdialysis Calibration Using the Zero-Net Flux Method and Retrodialysis in Studying the Distribution of Exogenous Substances to Rat Brain," Sandoz Pharma, Basel Switzerland, April 26, 1993.

"Investigation of the Pharmacodynamics of the NMDA Antagonist, EAB 515, in the Rat During Intravenous and Intracerebroventricular Administration." Sandoz Research Institute, Berne, Switzerland, April 28, 1993.

"Comparative Distribution of AZT to Brain Tissue Extracellular Fluid During Intravenous and Intracerebroventricular Infusion." Food and Drug Administration, Rockville, MD, May 21, 1993.

"Interspecies Scaling of Pharmacokinetics in the Evaluation and Development of New Antiepileptic Drugs." Natural Resources Research Institute, University of Minnesota—Duluth, Duluth, MN, August 11, 1993.

"Application of Pharmacokinetic Principles in Practice." Minneapolis Veteran Pharmacists Association, St. Louis Park, MN, September 21, 1993.

"Microdialysis as a Tool to Study Drug Delivery to the Brain." North Jersey American Chemical Society Drug Metabolism Discussion Group, Somerset, NJ, October 7, 1993.

"Graduate Studies and Research Careers in Pharmaceutics." University of Minnesota—Duluth Department of Chemistry, Duluth, MN, December 3, 1993.

"Microdialysis in Pharmacokinetic and Drug Metabolism Studies." 95th Annual Meeting, American Society for Clinical Pharmacology and Therapeutics, New Orleans, LA, April 1, 1994.

"Modeling and Simulation of Complex Pharmacokinetic Systems." NATO Advanced Study Institute, Erice, Italy, April 12, 1994.

"Microdialysis in the Study of Drug Distribution." NATO Advanced Study Institute, Erice, Italy, April 13, 1994.

"Pharmacokinetic Studies Utilizing Microdialysis." Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, May 2, 1994.

"Pharmacokinetic Studies Utilizing Microdialysis and On-Line HPLC." 4th International Workshop in Bioanalysis, Lawrence, KS, July 12, 1994.

"Application of Microdialysis in Pharmacokinetic Studies." Gordon Research Conference in Drug Metabolism, Holderness School, Plymouth, NH, July 20, 1994.

"Microdialysis and its Application in Pharmacokinetic Studies." Ciba-Geigy, Pharmacokinetics and Bioanalytics Division, Ardsley, NY, July 25, 1994.

"Assessing Drug Transport in the Brain with Microdialysis." 9th Annual Meeting, American Association of Pharmaceutical Scientists, San Diego, CA, November 6-10, 1994.

"Applications of Microdialysis in Preclinical Pharmacokinetic Studies." 3M Pharmaceuticals, 3M Center, St. Paul, MN, November 29, 1994.

"Problems in Assessing the Absorption of Carbamazepine from Sustained Release Dosage Forms in Epileptic Patients." Pharmavene, Inc., Gaithersburg, MD, February 23, 1995.

"Selected Preclinical Pharmacokinetic Studies with Tacrine." Parke-Davis Pharmaceuticals, Ann Arbor, MI, May 5, 1995.

"Brain Distribution and Metabolism Studies with Tacrine and Two Hydroxylated Metabolites." Department of Pharmaceutics and Pharmacodynamics, University of Illinois, Chicago, IL, July 28, 1995.

"Microdialysis and its Application in Preclinical Drug Distribution and Absorption Studies." Chiron Corporation, Emeryville, CA, August 18, 1995.

"The Principle of Quantitative Microdialysis and its Application in Preclinical Drug Distribution Studies." Genentech, Inc., South San Francisco, CA, October 9, 1995.

"Graduate Programs and Research Opportunities in Pharmaceutics." 13th Annual Symposium on Pharmaceutical Sciences Graduate Programs, Merrillville, IN, October 21, 1995.

"Principles of Microdialysis and Applications in Preclinical Drug Distribution and Absorption Studies," Wyeth-Ayerst, Pearl River, NY, December 6, 1995.
"Microdialysis in Preclinical Drug Distribution Studies." Dupont Merck, Newark ,DE, December 8, 1995.
"Microdialysis and its Application to the Study of Drug/Metabolite Distribution in the Central Nervous System," University of Pittsburgh, Pittsburgh, PA, January 25, 1996.
"Therapeutic Drug Monitoring: A Fodor's Guide." Hallie Bruce Memorial Lecture Award, Minnesota Society of Health-Services Pharmacists, Minneapolis, MN, April 13, 1996"
"Preclinical Studies of Drug Distribution to the Brain using Microdialysis." Pharmaceutical Peptides Inc, Cambridge, MA, May 2, 1996.
"Microdialysis and its Application in Nonclinical Studies of Drug Distribution and Absorption." Bristol-Myers Squibb, Pinceton, NJ, June 24, 1996.
"Continuous Monitoring by Microdialysis in Neuropharmacokinetic Investigations." Faculty of Pharmacy, University of Tanta, Tanta, Egypt, March 5, 1997.
"Preclinical Studies of Drug Distribution to the Brain using Microdialysis," Toyama Medical and Pharmaceutical University, Toyama, Japan, April 11, 1997.
"Application of Pharmacokinetic Principles in Individualizing Aminoglycoside Dosing," Toyama Medical and Pharmaceutical University, Toyama, Japan, April 11, 1997.
"Preclinical Studies of Drug Distribution to the Brain using Microdialysis," Meiji College of Pharmacy, Japan, April 18, 1997.
"Individualizing Aminoglycoside Dosing and Once-a-Day Aminoglyocosides," Meiji College of Pharmacy, Japan, April 18, 1997.
"Education of Pharmacists and Pharmaceutical Scientists at the University of Minnesota," 260th Meeting on Continuing Education of Pharmacists, Okuda-Shinmachi, Toyama, Japan, April 26, 1997.
"Pharmacokinetic Basis of Drug-drug Interactions," Novartis Workshop on Metabolic Drug-Drug Interactions, Schluchsee, Germany, October 14, 1997.
"Microdialysis and its Application in Preclinical Pharmacokinetic Studies," Merck Research Laboratories, West Point PA, December 16, 1997.
"Microdialysis and its Application in Preclinical Pharmacokinetic Studies," Merck and Co, Inc. Rahway NJ, December 17, 1997.
"Brain Distribution Studies employing Microdialysis and Crossover Designs," *1st International Symposium in Drug Research and Development*, Noorwijkerhout, Netherlands, April 3, 1998.
"Application of Sample Pooling in the Time Domain to Estimate CL, Vss and MRT in the Search for Lead Compounds." Chiron Corporation, Emeryville, CA, May 5, 1998.
"Microdialysis as a Sampling Technique in Preclinical Pharmacokinetic Studies." Pfizer Inc, Groton CT, June 18,1998
"Assessing Drug Delivery to the CNS Using Microdialysis Sampling." School of Medicine, University of Minnesota, Duluth, October 19,1998.
"Pharmacokinetic Studies Using Microdialysis Sampling." American Association of Pharmaceutical Scientists Annual Meeting, San Francisco CA, November 18, 1998.
"Applications of Microdialysis in Pharmacokinetics: Brain, Blood, and Middle Ear Fluid." Bristol-Myers Squibb, Wallingford CT, May 14, 1999.
"Applications of Microdialysis in Preclinical Pharmacokinetics: Brain, Blood and Middle Ear Fluid." Parke-Davis, Ann Arbor, MI, May 21, 1999.
"Blood Sample Pooling and the Determination of Mean Residence Times in High-Throughput Pharmacokinetic Screening"." Parke-Davis, Ann Arbor, MI, May 21, 1999.
"Role of controlled release formulations in the steady-state pharmacokinetics and pharmacodynamics of anticonvulsants" Impax Pharmaceuticals, Inc, Hayward CA, June 9, 1999.
"Investigating Neuropharmacokinetics and Drug Delivery to the CNS using Microdialysis" *8th International Conference on In Vivo Methods: Monitoring Molecules in Neuroscience.* Stony Brook NY, June 19-23, 1999.
"Use of Microdialysis in Pharmacokinetics" at the 8th BMSR Workshop on *Advanced Methods of Pharmacokinetic and Pharmacodynamic System Analysis,* Marina del Rey, CA June 25-26, 1999.
"Applications of Microdialysis in Preclinical Pharmacokinetics." Amgen, Inc., Thousand Oaks, CA, June 28, 1999.
"Pharmacokinetic –Pharmacodynamic Principles in Drug Development." Chiron Corporation, Emeryville, CA, August 20, 1999.
"Microdialysis and its Application in Pharmacokinetics: Brain, Blood, and Middle Ear Fluid." Abbott Labs, Abbott Park IL Aug 27, 1999.
"Distribution kinetics of antibiotics to the chinchilla middle ear" Department of Biopharmaceutical Sciences, Uppsala University, Uppsala, Sweden, March 16, 2000.
"In Vivo Microdialysis as a Tool to Study Site Specific Drug Delivery" *Millennial World Congress of Pharmaceutical Sciences.* San Francisco CA, April 17, 2000.

"In Vivo Microdialysis as a Tool to Study Site Specific Drug Delivery" *Engebretson Symposium on Drug Discovery and Development.* Minneapolis, MN. May 18, 2000.

"In Vivo Microdialysis as a Tool to Study Drug Delivery". *19th Annual Robert S. Rozman Memorial Symposium,* Langhorne PA , May 25, 2000.

"Basic Principles of Microdialysis, Experimental Setup". *Course on Basic and Advanced Aspects of In Vivo Microdialysis*", Stockholm, Sweden, June14, 2000.

"Recovery: Basic Idea and Practical Methods". *Course on Basic and Advanced Aspects of In Vivo Microdialysis*", Stockholm, Sweden, June14, 2000.

"Studies of Distribution of Antibiotics to the Middle Ear by Microdialysis" *2nd International Symposium on Microdialysis in Drug Research and Development*, Stockholm, Sweden, June15, 2000.

"Basic Concepts in Clinical Pharmacokinetics" A 2-Day Course. Abbott Laboratories, Abbott Park IL and Victory Hospital, Waukegan, IL, July 18-19, 2000

"Microdialysis and its Application in Preclinical Pharmacokinetics: Brain, Blood, and Middle Ear Fluid." Dupont Pharmaceuticals, Wilmington, DE July12, 2000.

"Pharmacokinetic –Pharmacodynamic Principles in Drug Development." Abbott Labs, Abbott Park IL Jan 9, 2001

"Biopharmaceutical and Pharmacokinetic Considerations in Delivering Drug to the CNS" Medtronic Neuro Division, Minneapolis. January 25, 2001

"Clinical Pharmacokinetic Principles in Drug Development." Novartis Pharma, Tokyo, April 12, 2001

"In Vivo Microdialysis as a Tool to Study Site Specific Drug Delivery" Showa University, Tokyo, Japan, April 13, 2001

"In Vivo Microdialysis as a Tool to Study Drug Delivery in Preclinical Studies". Xi'an Medical College, Xi'an, PRC. April 25, 2001

"Principles of Pharmacokinetics and their Application in Drug Development" Novartis Pharma, Basel, Switzerland, July 3, 2001.

"Microdialysis and its Application in Preclinical Studies of Drug Delivery to Target Tissues" Boehringer-Ingelheim Pharma KG, Dept. of Pharmacokinetics & Drug Metabolism, Biberach, Germany, July 5, 2001.

"Estimation of Intrinsic Clearances and Organ Partition Coefficients in an Organ Perfusion Model" Novartis Pharma, Basel, Switzerland, July 26, 2001.

"Pharmacodynamic Modeling of the Sigmoid Emax Model"Novartis Pharma, Basel, Switzerland, July 31, 2001.

"Prediction of the Pharmacokinetics of Cefdinir in Children from the Results of Animal Studies. Omnicef® Clinical Advisory Meeting, Dallas, TX, February 9, 2002.

"Applications of Microdialysis in Studying Drug Delivery to Specific Targets". Guilin Medical School, Guilin PRC, March 28, 2002

"Microdialysis: A Tool to Study Brain Uptake?" Gordon Research Conference on the Barriers of the CNS, Tilton School, Tilton NH, June 25, 2002

"A Model for the Distribution of Drugs between Plasma, CSF and Parenchyma", Workshop on Microdialysis Techniques in the CNS, Gordon Research Conference on the Barriers of the CNS, Tilton School, Tilton NH, June 26, 2002

"Microdialysis in the Study of Drug Delivery to the Central Nervous System", Department of Pharmaceutics, Seoul National University, Seoul, South Korea, November 25, 2002.

"Investigating Antibiotic Delivery to the Middle Ear". Chong Kun Dang Pharma, Cheonan, South Korea, November 27, 2002.

"Microdialysis and its Application in Preclinical Pharmacokinetic and Drug Delivery Investigations", 32nd Annual Meeting of the Korean Pharmaceutical Society, Seoul, South Korea, November 28, 2002.

"Applications of Pharmacokinetic Principles in Drug Development". Schering-Plough Research Institute. Kenilworth, NJ. December 19, 2002

"A Course in Pharmacokinetics in Pharmaceutical Development". Abbott Laboratories. Harrison Conference Center, Lake Bluff, IL. May 15-16, 2003

"Characterizing Antibiotic Delivery to the Middle Ear for the Treatment of Otitis Media. Biomedical Simulations Resource Workshop: Advanced Methods of PK/PD Systems Analysis. Marina del Rey, CA. June 20-21, 2003.

"Cerebrospinal Fluid Distribution of Intrathecally Administered Antiviral Nucleosides". Monitoring Molecules in Neuroscience. 10th International Conference on In Vivo Methods. Department of Neuroscience, Karolinska Institutet Stockholm, Sweden. June 24-27, 2003

"Microdialysis Sampling in Drug Development: Applications in Preclinical Research." Sunrise School, American Association of Pharmaceutical Scientists Annual Meeting, Salt Lake City, UT, October 26, 2003.

"Clinical Pharmacokinetics in Pharmaceutical Development." Abbott Laboratories. Harrison Conference Center, Lake Bluff, IL. July 23-24, 2003.

"Microdialysis Sampling in Drug Development: Applications in Preclinical Research." Sunrise School, American Association of Pharmaceutical Scientists Annual Meeting, Salt Lake City, UT. October 26, 2003.

"The Role of Pharmacokinetics in Drug Discovery." Abbott Laboratories. Harrison Conference Center, Lake Bluff, IL. March 18, 2004.

"Microdialysis and its Application in Preclinical Pharmacokinetic and Drug Delivery Investigations." CDER, Food and Drug Administration, Rockville, MD. March 29, 2004.
"Interspecies Scaling, PB-PK modeling and Microdialysis in Antibiotic Drug Development." Novartis Institute for Biomedical Research, Cambridge, MA. April 9, 2004.
"Does it get to the Target Site? Microdialysis as a Tool to Study Preclinical Drug Distribution and Delivery" Amgen Inc., Thousand Oaks, CA. April 30, 2004.
"Microdialysis of Antibiotics." 4th International Symposium on Microdialysis in Drug Research and Development, Vienna, Austria, June 19, 2004.
"The Chinchilla Microdialysis AOM Model" Pfizer Global Pharmaceuticals, New York, NY. June 25, 2004.
"Advantages of the Chinchilla Microdialysis Model" Scientific Basis for Tissue-Directed Antimicrobial Therapy Symposium, Boston MA, July 21-22, 2004.
"Evaluating Drug Distribution to the Target Site and Predicting Tissue Exposure in Humans from Animal Data" Scientific Advisory Committee, Abbott Laboratories. The FDA Critical Path Initiative and the Role of Modeling/Simulation in Improving the Efficiency of Drug Development. Lake Forest, IL. September 89, 2004.
"Assessing Drug Delivery to the Target Site: The Role of Microdialysis in Measuring Tissue Exposure in Animals and Humans." Distinguished Lecture, Creighton University School of Pharmacy and Health Professions, Omaha NE, November 30, 2004.
"Microdialysis—Introduction to Basic Principles and Applications". AAPS Workshop on Microdialysis Principles, Application, and Regulatory Perspectives, Nashville TN, November 4, 2005.
"A Phase I Open-Label, Dose-Ranging Study to Investigate the Safety and Tolerability of Gabapentin Injection Administered Intrathecally in Individuals with Chronic, Intractable Pain: A Pharmacokinetic Report". Medtronic WHQ, Fridley, MN, February 16, 2006.
"Public Outreach and AAPS: Students are the Future of Our Association". Temple University School of Pharmacy, Philadelphia, PA. February 20, 2006.
"Assessing Drug Delivery: Using Microdialysis to Measure Target Site Exposure in Animals and Humans". Wyeth Distinguished Lecture Series, Temple University School of Pharmacy, Philadelphia, PA. February 20, 2006.
"Pharmacokinetics for Scientists Engaged in Drug Discovery". Lundbeck Research,USA. Paramus NJ. February 24, 2006.
"Pharmacokinetic Issues related to Intrathecal Drug Dosing". Medtronic WHQ, Fridley, MN, March 15, 2006.
"TTM Technology: Antibiotic Distribution to Middle Ear Fluid" Abbott Laboratories, Abbott Park, IL. May 16, 2006
"Trans-tympanic Membrane (TTM) Drug Delivery to the Middle Ear" Alcon Laboratories, Fort Worth TX. Feb 2, 2007.
"Bugs and Drugs: Does the Anti-infective Agent get to the Target Site?". Science Luncheon Presentation. APhA Annual Meeting. Atlanta, GA. March 18, 2007
"Future Perspectives on the Contributions of Microdialysis in Drug Research and Development" Keynote Address. Fifth International Symposium on Microdialysis in Drug Research and Development. Leiden, NE. April 25, 2007.
"Drug Delivery to the Middle Ear across the Tympanic Membrane for Therapy of Acute Otitis Media". Global Gators 6th Symposium on Clinical Pharmacy and Clinical Pharmacology. Munich, Germany. June 9, 2007.
"The Pharmacokinetics of Hydrophilic Drugs during Intrathecal Infusion: the Concept of a Targeted Delivery Advantage". Novartis Pharma AG, Basel, Switzerland. June 13, 2007.
"Trans-tympanic Membrane Delivery of an Antibiotic into Chinchilla Middle Ear" Alcon Laboratories, Fort Worth TX. October 15, 2008.
"A Phase I Study to Investigate the Safety and Pharmacokinetics of Intrathecal Gabapentin Injection in Individuals with Chronic Pain". University of Poitiers, Poitiers, France, April 29, 2009.
"Cerebrospinal fluid flow, and the convective/diffusive transport of drugs in the CSF" Abbott GmbH and Co., Ludwigshafen, Germany. Oct 15, 2010.
"The Neuropharmacokinetics of Hydrophilic Drugs during Intrathecal Infusion: the Concept of a Targeted Brain Delivery Advantage" Abbott GmbH and Co., Ludwigshafen, Germany. Oct 15,2010.
"A Brief Introduction to Pharmacokinetics" Upsher-Smith Laboratories, Inc., Maple Grove, MN. December 2, 2010.
"CSF flow, and convective/diffusive transport of drugs" Upsher-Smith Laboratories, Inc., Maple Grove, MN. December 2, 2010.
"Modeling the delivery of drugs to target sites in the CNS" Upsher-Smith Laboratories, Inc., Maple Grove, MN. December 2, 2010.

TEACHING AT THE UNIVERSITY OF MINNESOTA

Undergraduate

1971 - 1972	Co-instructor in Phar 5680 "Pharmacokinetics"
1971 - 1975	Discussant in Pharm.D. Conferences
1972 - 1973	Participating instructor in Phar 5670
1972 - 1978	Discussion leader in Pharm.D. I conferences
1972 - 1985	Course director, Phar 5680 "Pharmacokinetics"
1975 - 1995	Course director, Phar 5685 "Clinical Pharmacokinetics"
1991 - 1999	Course director, Phar 5681 "Basic Pharmacokinetic Modeling"
1996 - 1998	Course director and Participating instructor, Phmc 5460 "Pharmacokinetics"
1998 - 2003	Course director and instructor, Phar 6216 "Pharmacokinetic Simulation and Data Analysis using SAAM"
1999 - 2004	Course director and Participating instructor, Phar 6163 "Pharmacokinetics"
1998 - 2004	Participating instructor in Phar 6164 "Biopharmaceutics"
2004 - 2010	Participating instructor, Phar 6163 "Pharmacokinetics"

Graduate

1972 - 1999	Course director in Phm 8420 "Modeling Approaches in Pharmacokinetics" participating instructor in Phm 8421, Phm 8425
1972 - 2005	Participating instructor in Phm 8100 (Seminar) and Phm 8101 (Pharmaceutics Readings)
1984 - 1999	Participating instructor in Phm 8425 "Advanced Topics in Pharmacokinetics"
1986 - 1999	Course co-director in Phm 8105 "Pharmacokinetics Research Seminar"
2000 - 2006	Course co-director in Phm 8150 "Pharmacokinetics Research Seminar"
2000 - 2006	Course Co-director and Participating instructor in Phm 8421 "Advanced Pharmacokinetics"
2004 - 2010	Participating instructor in Phm 8481 "Advanced Neuropharmaceutics"
2006 - 2010	Participating instructor in Phm 8421 "Advanced Pharmacokinetics"

TEACHING AT OTHER SITES

"An Introduction to Clinical Pharmacokinetics" Abbott Laboratories. Abbott Park, IL. January 9-10, 2001.
"An Introduction to Clinical Pharmacokinetics" Abbott Laboratories. Abbott Park, IL. March 29-30, 2001.
"An Introduction to Clinical Pharmacokinetics" Abbott Laboratories. Abbott Park, IL. May 17-18, 2001.
"An Introduction to Clinical Pharmacokinetics" Abbott Laboratories. Abbott Park, IL. May 17-18, 2001.
"An Introduction to Clinical Pharmacokinetics" Abbott Laboratories. Lake Bluff, IL. November 8-9, 2001.
"An Introduction to Pharmacokinetics" Abbott Laboratories. Lake Bluff, IL. March 14-15, 2002.
"An Introduction to Pharmacokinetics" Abbott Laboratories. Abbott Park, IL. July 22-23, 2002.
"An Introduction to Pharmacokinetics" Abbott Laboratories. Parsippany, NJ. Aug 26-27, 2002.
"An Introduction to Pharmacokinetics" Bristol-Myers Squibb. Wilmington, DE. September 19-20, 2002.
"An Introduction to Pharmacokinetics" Schering Plough Corp. Kenilworth, NJ. December 19-20, 2002.
"An Introduction to Pharmacokinetics" Abbott Laboratories. Lake Bluff, IL. July 23-24, 2003.
"Short Course in Pharmacokinetics for Drug Discovery" Abbott Laboratories. Lake Bluff, IL. March 17, 2004.
"Preclinical Pharmacokinetics in Pharmaceutical Discovery." Bristol-Myers Squibb, Princeton, NJ. May 6-7, 2004.
"Introduction to Pharmacokinetics" Abbott Laboratories. Abbott Park, IL, July 27-28, 2004.
"Introduction to Clinical Pharmacokinetics" Millennium Pharmaceuticals, Inc. Cambridge, MA, December 23, 2004.
"Introduction to Clinical Pharmacokinetics" Gilead Sciences, Foster City, CA. December 8-9, 2005.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist" Co-instructor. Boehringer-Ingelheim Pharmaceuticals, Inc., USA. Ridgefield, CT, April 13-14, 2006
"An Introduction to Pharmacokinetics" Lundbeck Research, USA, Inc. Paramus, NJ, February 24, 2006.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist" Co-instructor. Abbott Laboratories. Abbott Park, IL. June 4-5, 2007.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist" Co-instructor. Theravance, Inc. South San Francisco, CA. August 20-21, 2007.
"Basic Pharmacokinetic Concepts" Co-instructor. US Patent and Trademark Office. Alexandria, VA. October 4, 2007.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist" Co-instructor. Allergan, Inc. Irvine, CA. July 24-25, 2008.

"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist". Co-instructor. Abbott Laboratories, Abbott Park, IL. August 19-20, 2008.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist". Co-instructor. Gilead Sciences, Foster City, CA. October 9-10, 2008.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist". Co-instructor. Genentech, South San Francisco, CA. July 23-24, 2009.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist". Co-instructor. Abbott Laboratories. Abbott Park, IL. July 30-31, 2009.
"Neuropharmacokinetic Concepts for CNS Drug Delivery". Co-instructor. Abbott Laboratories. Abbott Park, IL. January 8, 2010.
"Basic Pharmacokinetic Concepts for the Pharmaceutical Scientist". Co-instructor. Abbott Laboratories. Abbott Park, IL. August 4-5, 2010.
"Basic Pharmacokinetic Concepts for the Upsher-Smith Pharmaceutical Scientist". Co-instructor. Upsher-Smith Laboratories, Maple Grove, MN. September 21-23, 2011.
"Basic Pharmacokinetic Short Course for Pharmaceutical Scientists". Co-instructor. Novartis Pharma, Florham Park, NJ. November 17-18, 2011.

GRADUATE STUDENTS SUPERVISED

Graduate Students supervised as Primary Advisor:

1978	Wargin, W.A.	Ph.D.
1978	El-Yazigi, A.	Ph.D.
1980	Mugure Pyron	M.S.
1981	Sue-Chi Wu	M.S.
1983	Hsuehling Su	M.S.
1984	Dale Yu	Ph.D.
1984	Walid Awni	Ph.D.
1985	Lillian Riad	M.S.
1985	Rose Eggerth	Ph.D.
1987	Hisham Abou-Auda	Ph.D.
1989	Mohsen Hedaya	Ph.D.
1989	Ajit K. Shah	Ph.D.
1989	Lillian Riad	Ph.D.
1991	Helen Chan	Ph.D.
1992	William Elmquist	Ph.D.
1992	Shekman Wong	Ph.D.
1994	Yanfeng Wang	Ph.D.
1994	Bimal Malhotra	Ph.D.
1996	Richard Brundage	Ph.D.
1997	Zheng Yang	Ph.D
1998	Belinda Cheung	Ph.D.
2001	Yue Huang	Ph.D
2001	Guanfa Gan	Ph.D.
2002	Joanna Peng	Ph.D
2002	Tong Zhu	Ph.D.
2004	Ji Ping	Ph.D
2004	Wei Liu	Ph.D.
2005	Yan Song	Ph.D.
2007	Nael Mostafa	Ph.D.
2007	Zhihong Li	Ph.D.

GRANTS , CONTRACTS, and OTHER SUPPORT

1972-73	University of Minnesota Graduate School
1973-74	University of Minnesota Media Production Fund
1975-78; 1978-80	NIH/NINCDS Comprehensive Epilepsy Program Contract (Principal Investigator, Project D-1)
1976; 1977	Medical Education and Research Foundation Grant (Co-investigator with John W. McBride, M.D.)
1976-78	FDA Contract to Study the Pharmacokinetics and Toxicology of Phenytoin Sodium Products in Clinical Patients
1980-81; 1981-83	Grant to Support Research Involving the Analysis of Cyclosporin A in Biological Fluids (Sandoz, Inc.)
1982-83; 1983-84	"Pharmacokinetics and Biopharmaceutic Studies of Cyclosporin A in Selected Animal and*In Vitro* Systems" (NIH; Principal Investigator; Co-investigator, R.P. Enever)
1984	Comparative Bioavailability of Sodium Phenytoin in Normal Volunteers (ZenithLabs)
1984	Relative Bioavailability of Carbamazepine in Chewable and Conventional Tablets (Ciba-Geigy)
1984	Transdermal Delivery of Propranolol (Medtronics)
11/84 - 1/85	Absorption and Metabolism of Carbamazepine in Normal Volunteers (Ciba-Geigy)
1/85 - 4/85	Transdermal Absorption of □-Blockers (Medtronics, Inc.)
11/85 - 4/86	Relative Bioavailability of Sustained Release Oral Dosage Forms of Carbamazepine (Ciba-Geigy)
1/86 - 6/86	Analysis of Analgesics in Receptor Media (Medtronics)
8/86 - 12/86	Bioequivalence of Carbamazepine Oral Dosage Forms (Ciba-Geigy)
1/86 - 12/88	Pharmacokinetics of Diltiazem in the Rabbit (Marion)
2/87 - 9/87	Bioequivalence of Carbamazepine Dosage Forms Demonstrating Varying Dissolution Rates (Ciba-Geigy)
6/1/87 - 10/15/87	Effect of Urine Flow on the Renal Clearance of Carbamazepine and its Metabolites in Humans (Ciba-Geigy)
1/88 - 6/88	Effect of Fasting on the Absorption of Diclofenac Sodium in Normal Human Volunteers (Ciba-Geigy)
4/1/89 - 3/31/92	Enhancing Brain Uptake of AZT by Transport Inhibition, (NINCDS / NIH)
7/1/89 - 6/30/90	Induction of Carbamazepine Metabolism as a Function of Dosing Rate in Normal Volunteers (Ciba-Geigy)
9/91 - 6/92	Brain Distribution of EAB-515 in the Rabbit (Sandoz, Ltd.)
11/91 - 5/92	*In Situ* Absorption from Rabbit Intestine (Lederle Laboratories)
3/92 - 8/92	Clinical Studies of the Absorption of an Oral Immunosuppressant (Apotex Laboratories)
11/92 - 10/93	Brain Distribution of an NMDA-Receptor Antagonist in the Rat (Sandoz, Ltd).
10/92 - 5/93	Brain Uptake of a CNS-Active Agent (Warner-Lambert)
11/92 - 3/93	*In Situ* Absorption from Rabbit Intestine (Lederle Laboratories)
9/94 - 5/95	Population Pharmacokinetic Analysis of A General Anesthetic in Man (Abbott Laboratories)
7/94 - 5/95	Brain Uptake of a Cholinesterase Inhibitor and its Metabolites (Warner-Lambert)
10/94 - 9/95	Distribution of Antiviral Nucleosides into Rat Cortex (Bristol-Myers Squibb)
9/95 - 8/96	Bioanalytical Methods Development of Selected Drugs and Metabolites (MedTox)
1/96 - 9/96	Pharmacokinetic Analysis of IL-2 in the Pig (Chiron)
1/96 - 6/98	Analysis of Selected Macrolides by High-pressure Liquid Chromatography (TAP)
4/96 - 10/97	Brain Penetration of Fosphenytoin and Phenytoin in the Rabbit (Warner-Lambert)
4/96 - 9/96	Analysis and Brain Uptake of PPI-457 (Pharmaceutical Peptides, Inc)
7/97 - 3/98	Regional Intestinal Absorption of Anti-CMV agents (Bristol-Myers Squibb)
8/97 - 6/98	EM574 absorption in the rabbit in situ (TAP)
7/97 - 9/97	Pharmacokinetics of macrolides in Protocol EM-97-006 (TAP)
11/97 - 12/97	Drug Interaction Pharmacokinetic Analysis (McNeil)
8/97 - 3/98	Analysis and Pharmacokinetics of Macrolides in EM-97-008 (TAP)
10/97 - 12/97	Pharmacokinetics of Slow Release Agents in the CNS (Chiron)
1/98	LC/MS/MS Equipment Grant (TAP)
2/98 - 12/99	Analysis of Macrolides and Metabolites in EM-97-013 (TAP)
3/98 - 12/99	Chemical Stability of Selected Agents (Medtronic)
5/98	Validation of Analysis of Macrolides in Dog Plasma (TAP)
8/98	Validation of Analysis of Macrolides in Rabbit Plasma (TAP)
8/98	Stability of anticancer drugs in solution (Medtronic)
8/98	EM574 toxicokinetics in the dog (TAP)
12/98	EM574 toxicokinetics in the rabbit (TAP)
1/99	Pharmacodynamics of EM574 on LES Pressure (protocol 004) (TAP)
3/99	Effect of Time of Dosing on Absorption of EM574 (protocol 007(TAP)
4/99	Effect of gastric emptying on the pharmacokinetics of EM574 and its metabolites (protocol 002)(TAP)

2/9	Stability of FUDR and heparin in solution (Medtronic)
2/99	Pharmacodynamics and PKs of EM574 and its metabolites during chronic dosing (protocol 029)(TAP)
3/00 - 8/01	Pharmacokinetics of CDTR and distribution to middle ear fluid (TAP)
8/00 - 6-01	Distribution of ketolides to middle ear fluid (Abbott)
10/01 - 09/03	Pharmacokinetics of Ketolides (Abbott)
12/01 - 11/03	Pharmacokinetics and Distribution of cefdinir (Abbott)
12/02 – 12/03	Effect of a P-Glycoprotein Inhibitor on the Middle Ear Distribution of Clarithromycin (Abbott)
12/02 – 6/04	Distribution a Cephalosporin into Middle Ear Fluid in Children with Otitis Media (H LaRoche)
12/02 – 12/04	Development and Testing of Formulations for Delivery of Antibiotics to the Middle Ear (Abbott)
5/03 - 4/05	A New Approach for the Therapy of Otitis Media (Abbott)
8/04 - 7/05	Distribution of Macrolide antibiotics to tissue sites (Pfizer)
5/05 - 11/05	Testing the Distribution of Amoxicillin into Middle Ear Fluid in the Chinchilla following Pulsatile Dose Administration (Advancis)
1/06 – 9/06	Distribution of Macrolide antibiotics to Pulmonary Tissue and Skeletal Muscle (Pfizer)
9/07 – 10/08	Transtympanic Membrane Delivery of an Antibiotic to the Middle Ear (Alcon)
11/07 – 12/08	Development of an Acute Otitis Media Middle Ear Microdialysis Model in the Chinchilla with Implanted Tympanostomy Tube (Alcon)
1/10 – 12/10	Testing the Penetration of an Antibiotic into Chinchilla Middle Ear using Transtympanic Membrane Delivery Formulations – Phase II (Alcon)
1/11 – 12/11	Testing the Penetration of an Antibiotic into Chinchilla Middle Ear using Transtympanic Membrane Delivery Formulations – Phase II B (Alcon)
1/12 – 6/12	Testing the Penetration of Moxifloxacin into Chinchilla Middle Ear– Phase II, Supplement II (Alcon)

PATENTS

United States Patent. Number 7,220,431 "METHODS AND COMPOSITIONS FOR APPLYING PHARMACOLOGIC AGENTS TO THE EAR." UMN Docket # Z01159. RJ Sawchuk and BW Cheung. Issue Date: May 22, 2007. Filing Date: November 27, 2002: #06,306,517

PUBLICATIONS

BOOKS AND CHAPTERS

J. Blanchard, R.J. Sawchuk and B.B. Brodie, Editors. Principles and Perspectives in Drug Bioavailability. S. Karger, Basel, 1979.

J. Blanchard and R.J. Sawchuk, "Drug Bioavailability: An Overview" in Principles and Perspectives of Drug Bioavailability, J. Blanchard, R.J. Sawchuk and B.B. Brodie (Editors), S. Karger, Basel, 1979.

I.E. Leppik, J. Shope, R.J. Sawchuk, W.A. Hauser and B. Van Dyne, "Variability of Antiepileptic Drug Levels During Chronic Therapy" in Epileptology, M. Dam, L. Gram and K. Penry (Editors), Raven Press, NY, 1981.

R.J. Sawchuk, "Drug Absorption and Disposition in Burn Patients" in The Pharmacokinetic Basis of Drug Treatment, N. Massoud, L.Z. Benet, and J.G. Gambertoglio (Editors), Raven Press, NY, 1984.

"Use of Microdialysis in Drug Delivery Studies." Theme Issue. W.F. Elmquist and R.J. Sawchuk (Editors) *Advanced Drug Delivery Reviews* 45, Nos. 2-3 (2000).

R. J. Sawchuk and B.W.Y. Cheung, "Application of Microdialysis in Pharmacokinetic Studies" in Handbook of Microdialysis: Methods, Applications and Clinical Aspects, B.H.C. Westerink and T.I.F.H. Cremers (Editors), Academic Press, Amsterdam, 2007.

PROFESSIONAL PUBLICATIONS

R.J. Sawchuk, "The Plateau Principle in Drug Therapy. Part 1: The Principle and Its Application." *Minn. Pharmacist* 27(6): 19 (1973).

R.J. Sawchuk, "The Plateau Principle in Drug Therapy. Part 2: Factors Governing Plateau Levels During Chronic Drug Therapy." *Minn. Pharmacist* 27(7): 8 (1973).

M.C. Meyer, A.B. Straughn, L.J. Leeson, R.H. Levy and R.J. Sawchuk, "Meprobamate Bioavailability Monograph." *JAPhA* NS17, 173 (1977).

R.J. Sawchuk and T.S. Rector, "Burn-Induced Alterations in Drug Absorption and Disposition." *Minn. Pharmacist* 35: 6-9 (1981).

SCIENTIFIC PUBLICATIONS (NON-REFEREED)

I.E. Leppik, J. Cloyd and R.J. Sawchuk, "Coefficient of Variation as a Measure of Compliance" Letter. *Lancet*, October 14, 1978.

SCIENTIFIC PUBLICATIONS (REFEREED)

1. R.J. Sawchuk, J.M. Anderson and J.G. Nairn. "Stirring apparatus for the investigation of unstable strongly adsorbing chemicals." *J. Pharm. Sci.* 55: 1463 (1966).

2. R.J. Sawchuk and J.G. Nairn. "Rate studies on the binding of bilirubin by ion-exchange resins." *J. Pharm. Sci.* 57: 1896 (1968).

3. R.J. Sawchuk, J. Robayo and K.W. Miller. "The distribution of propranolol between blood and plasma in hypertensive patients." *Br. J. Clin. Pharmacol.* 1: 440 (1974).

4. R.J. Sawchuk and D.E. Zaske. "Pharmacokinetics of dosing regimens which utilize multiple intravenous infusions: gentamicin in burn patients." *J. Pharmacokin. Biopharm.* 4: 183 (1976).

5. D.E. Zaske, R.J. Sawchuk, D.N. Gerding and R.G. Strate. "Increased dosage requirements of gentamicin in burn patients." *J. Trauma* 16: (1976).

6. R.J. Sawchuk, D.E. Zaske, R.J. Cipolle, W.A. Wargin and R.G. Strate. "Kinetic model for gentamicin dosing with the use of individual patient parameters." *Clin. Pharmacol. Therap.* 21: 362 (1977).

7. J.C. Cloyd, D.E. Bosch and R.J. Sawchuk. "Concentration-time profile of phenytoin after admixture with small volumes of intravenous fluids." *Am. J. Hosp. Pharm.* 35: 45-48 (1978).

8. J.D. Wirtschafter, C.R. Volk and R.J. Sawchuk. "Trans-aqueous diffusion of acetylcholine to denervated iris sphincter muscle: A hypothetical mechanism for the tonic pupil syndrome (Adie's Syndrome). *Annals of Neurology* 4: 1-5 (1978).

9. D.E. Zaske, R.J. Sawchuk and R.G. Strate. "The necessity of increased doses of amikacin in burn patients." *Surgery* 84: 603-608 (1978).

10. S.M. Ehlers, D.E. Zaske and R.J. Sawchuk. "Massive theophylline overdose: rapid removal by charcoal memoperfusion. *JAMA* 240: 474 (1978).

11. I.E. Leppik, V. Ramani, R.J. Sawchuk and R.J. Gumnit. "Increased clearance of phenytoin during mononucleosis." *NEJM* 300: (1979).

12. H.G. McCoy, R.J. Cipolle, S.M. Ehlers, R.J. Sawchuk and D.E. Zaske. "Severe methanol poisoning: application of a pharmacokinetic model for ethanol therapy and hemodialysis." *Am. J. Med.* 67: 804-807 (1979).

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16. I.E. Leppik, J.C. Cloyd, R.J. Sawchuk and S.M. Pepin. "Compliance and variability of plasma phenytoin levels." *Therap. Drug Monitoring* 1: 475-483 (1979).

17. R.L. Kriel, J.C. Cloyd, K.H. Green, R.J. Sawchuk, L.A. Lockman and R. Eggerth. "The pharmacokinetics of valproic acid in children." *Ann. Neurology* 6: 179 (1979).

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22. R.J. Sawchuk* and T.S. Rector. "Drug kinetics in burn patients." *Clin. Pharmacokinetics* 5: 548-556 (1980).

23. G.R. Matzke, J.C. Cloyd and R.J. Sawchuk. "Acute phenytoin and primidone intoxication, a pharmacokinetic analysis." *J. Clin. Pharmacol.* 21: 92-99 (1981).

24. El-Yazigi and R.J. Sawchuk. "Theophylline absorption and disposition in the rabbit: oral, intravenous, and concentration-dependent kinetic studies." *J. Pharm. Sci.* 70: 452-456 (1981).

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26. R.J. Sawchuk and L.L. Cartier. "Liquid-chromatographic determination of cyclosporin A in blood and plasma." *Clinical Chemistry* 27: 1368-1371 (1981).

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29. R.J. Sawchuk, S.M. Pepin, I.E. Leppik and R.J. Gumnit. "Rapid and slow release phenytoin in epileptic patients at steady state: assessment of relative bioavailability utilizing Michaelis-Menten parameters." *J. Pharmacokin. Biopharm.* 10: 383-391 (1982).

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31. R.J. Sawchuk and L.L. Cartier. "Liquid-chromatographic method for simultaneous determination of carbamazepine and its epoxide metabolite in plasma." *Clin. Chem.* 28: 2127-2130 (1982).

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33. J.C. Cloyd, R.L. Kriel, J.H. Fischer, R.J. Sawchuk and R.M. Eggerth. "Pharmacokinetics of valproic acid in children. I. Multiple antiepileptic drug therapy." *Neurology* 33: 185-191 (1983).

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35. D.K. Yu and R.J. Sawchuk. "Gas-liquid chromatographic determination of propylene glycol and plasma and urine." *Clin. Chem.* 29: 2088-2090 (1983).

36. R.J. Sawchuk and G.R. Matzke. "Contribution of 5-(4-hydroxyphenyl)-5-phenylhydantoin to the discrepancy between phenytoin analyses by EMIT and high pressure liquid chromatography." *Ther. Drug Monit.* 6: 97-103, (1984).

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39. W. Awni and R.J. Sawchuk. "The pharmacokinetics of cyclosporine. II. Blood-plasma distribution and binding studies." *Drug Metab. Disposition* 13(2): 133-138 (1984).

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41. A. El-Yazigi and R.J. Sawchuk. "*In vitro - in vivo* correlation and dissolution studies with oral theophylline dosage forms." *J. Pharm. Sci.* 74(2): 161-164 (1985).

42. D.K. Yu, W.F. Elmquist and R.J. Sawchuk. "Pharmacokinetics of propylene glycol in humans during multiple dosing regimens." *J. Pharm. Sci.* 74(8): 876-878 (1985).

43. R.J. Sawchuk and W. Awni. "Absorption of cyclosporine from rabbit small intestine *in situ*." *J. Pharm. Sci.* 75(12): 1151-1156 (1986).

44. G.R. Matzke, R.C. Brundage and R.J. Sawchuk. "Protein binding of phenytoin, p-hydroxyphenytoin, and p-hydroxy phenytoin glucuronide." *J. Clin. Pharmacol.* 26: 677-679 (1986).

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46. C. Fletcher, R.J. Sawchuk, B. Chinnock, P. de Miranda and H.H. Balfour. "Human pharmacokinetics of the antiviral drug DHPG." *Clin. Pharmacol. Therap.* 40: 281-286 (1986).

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48. D.K. Yu and R.J. Sawchuk. "Pharmacokinetics of propylene glycol in the rabbit" *J. Pharmacok. Biopharm.* 15: 1-8 (1987).

49. A.K. Shah and R.J. Sawchuk. "Liquid chromatographic determination of cyclosporine and its metabolites in blood." *Clinical Chemistry* 34: 1467-1471 (1988).

50. M.A. Hedaya and R.J. Sawchuk. "A sensitive liquid chromatographic method for the determination of 3'-azido-3'-deoxythymidine (AZT) in plasma and urine." *Clinical Chemistry* 34:1565-1568 (1988).

51. L.E. Riad and R.J. Sawchuk. "A sensitive method for the simultaneous determination of carbamazepine, its epoxide and transdiol metabolites in plasma by microbore liquid chromatography." *Clinical Chemistry* 34: 1863-1866 (1988).

52. M.A. Hedaya and R.J. Sawchuk. "Effect of probenecid on the renal clearance of zidovudine (AZT) and its distribution into cerebrospinal fluid." *J. Pharm. Sci.* 78: 716-722 (1989).

53. R.J. Sawchuk and M.A. Hedaya. "Modeling the enhanced uptake of zidovudine (AZT) into cerebrospinal fluid: the effect of probenecid." *Pharmaceutical Research* 7: 332-338 (1990).

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58. L.E. Riad and R.J. Sawchuk. "Effect of polyethylene glycol 400 on the intestinal permeability of carbamazepine in the rabbit." *Pharmaceutical Research* 8: 491-497 (1991).

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75. R.J. Sawchuk. "Pharmacokinetic and analytical considerations in monitoring zidovudine (AZT) levels in children with AIDS." The Fourth International Congress on Pediatric Laboratory Medicine, Washington, DC, August 23, 1989.

76. K.D. Lake, A.K. Shah, R.W. Emery, D.R. Holder and R.J. Sawchuk. "The effect of urine flow rate on the renal clearance of cyclosporine in heart transplant recipients." International Society for Heart Transplantation, Tenth Annual Meeting, Fort Lauderdale, FL, April 4-6, 1990.

77. R.J. Sawchuk. "Effect of temperature and medium of analysis on CsA concentration." Canadian Consensus Meeting on Cyclosporine Monitoring, Minaki Lodge, Ontario, May 10-13, 1990.

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79. S.M. Wong and R.J. Sawchuk. "Interaction of zidovudine and probenecid at steady state." NATO Advanced Study Institute Symposium "New Trends in Pharmacokinetics." Erice, Sicily, September 4-15, 1990.

80. W.F. Elmquist and R.J. Sawchuk. "Tissue mean transit time determination from steady-state partition coefficients and tissue/plasma concentration ratios in the postdistributive phase." NATO Advanced Study Institute Symposium "New Trends in Pharmacokinetics," Erice, Sicily, September 4-15, 1990.

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83. W.F. Elmquist, L.E. Riad, I.E. Leppik and R.J. Sawchuk. "The relationship between carbamazepine urine and plasma concentrations: implications for therapeutic drug monitoring." AAPS Fifth Annual Meeting, Las Vegas, NV, November 4-8, 1990.

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86. L.E. Riad and R.J. Sawchuk. "Carbamazepine enzymatic induction: extent and effects of dosing rate in normal volunteers." AAPS Fifth Annual Meeting, Las Vegas, NV, November 4-8, 1990.

87. Y.-F. Wang, M.A. Hedaya and R.J. Sawchuk. "Comparative absorption and disposition pharmacokinetics of AZT and AZddU in rabbits." AAPS Fifth Annual Meeting, Las Vegas, NV, November 4-8, 1990.

88. S.L. Wong and R.J. Sawchuk. "Investigation of distribution of zidovudine into the brain by microdialysis." AAPS Fifth Annual Meeting, Las Vegas, NV, November 4-8, 1990.

89. S.L. Wong and R.J. Sawchuk. "Interaction of zidovudine and probenecid at steady state." AAPS Fifth Annual Meeting, Las Vegas, NV, November 4-8, 1990.

90. S.L. Wong, Y.-F. Wang and R.J. Sawchuk. "Brain/plasma distribution kinetics of zidovudine (AZT) in the rabbit using microdialysis." 1991 Symposium on Microdialysis and Allied Analytical Techniques, Indianapolis, IN, May 16-17, 1991.

91. K. Van Belle, S.L. Wong, Y.-F. Wang and R.J. Sawchuk. "Comparative delivery and distribution kinetics of zidovudine (AZT) and AZddU into the rabbit brain by microdialysis." 1991 Symposium on Microdialysis and Allied Analytical Techniques, Indianapolis, IN, May 16-17, 1991.

92. Y.-F. Wang, S.L. Wong and R.J. Sawchuk "*In vitro* and *in vivo* calibration of microdialysis probes using retrodialysis." 1991 Symposium on Microdialysis and Allied Analytical Techniques, Indianapolis, IN, May 16-17, 1991.

93. B.W. Cheung, L.E. Riad and R.J. Sawchuk. "Comparative uptake of selected anticonvulsant drugs into rabbit brain." 1991 Symposium on Microdialysis and Allied Analytical Techniques, Indianapolis, IN May 16-17, 1991.

94. L.E. Riad, K.K. Chan, J.A. Maloney and R.J. Sawchuk. "Reversibility of carbamazepine autoinduction upon dose termination in normal volunteers." AAPS Sixth Annual Meeting, Washington, DC, November 17-21, 1991.

95. S.L. Wong, M.A. Hedaya and R.J. Sawchuk. "Modeling the competitive inhibition of renal and nonrenal clearance of zidovudine by probenecid." AAPS Sixth Annual Meeting, Washington, DC, November 17-21, 1991.

96. K. Van Belle, S.L. Wong, Y.-F. Wang and R.J. Sawchuk. "Comparison of pharmacokinetics and CNS distribution of coadministered AZT and AZddU by microdialysis." AAPS Sixth Annual Meeting, Washington, DC, November 17-21, 1991.

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101. R.J. Sawchuk. "Study of zidovudine distribution into the CNS utilizing microdialysis." 25th Annual Higuchi Research Seminar, Lake of the Ozarks, MO, March 8-11, 1992.

102. Y.F. Wang and R.J. Sawchuk. "Microdialysis studies of brain/plasma distribution of AZT during intraventricular and intravenous infusion." AAPS Seventh Annual Meeting, San Antonio, TX, November 15-19, 1992.

103. S.L. Wong, K. Van Belle, and R.J. Sawchuk. "A microdialysis study of probenecid's effect on the transport kinetics of zidovudine in rabbit brain." AAPS Seventh Annual Meeting, San Antonio, TX, November15-19, 1992.

104. S.L. Wong, and R.J. Sawchuk. "Pharmacokinetic interaction of zidovudine and salicylic acid during continuous infusion." AAPS Seventh Annual Meeting, San Antonio, TX, November 15-19, 1992.

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108. W.F. Elmquist and R.J. Sawchuk. "Simulation of the effect that the time delay in sampling from the bladder has on urine concentrations: implications for therapeutic drug monitoring using urine specimens." AAPS Seventh Annual Meeting, San Antonio, TX, November 15-19, 1992.

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111. R.J. Sawchuk. "Comparative distribution of AZT into brain tissue extracellular fluid during intravenous and intraventricular infusion using microdialysis." 26th Annual Higuchi Research Seminar, Lake of the Ozarks, MO, March 14-17, 1993.

112. Y. Wang and R.J. Sawchuk, "Comparison of renal clearance of AZdU following IV bolus and constant-rate Infusion." 8th Annual Meeting, American Association of Pharmaceutical Scientists, Orlando, FL, November 14-18, 1993.

113. Y. Wang and R.J. Sawchuk. "Assessment of oral absorption of AZT and AZdU in the rabbit using deconvolution." 8th Annual Meeting, American Association of Pharmaceutical Scientists, Orlando, FL, November 1418, 1993.

114. Y. Wang, Y. Wei and R.J. Sawchuk. "Microdialysis studies of carrier-mediated transport of AZT from brain to plasma during intracerebroventricular infusion." 8th Annual Meeting, American Association of Pharmaceutical Scientists, Orlando, FL, November 14-18, 1993.

115. B.K. Malhotra, M. Lemaire, and R.J. Sawchuk. "Investigation of the CNS distribution of EAB 515 in freely moving rats by microdialysis." 8th Annual Meeting, American Association of Pharmaceutical Scientists, Orlando, FL, November 14-18, 1993.

116. A.K. Shah, R.C. Brundage, K.D. Lake and R.J. Sawchuk. "Estimation of plasma free fraction (fu) of cyclosporine (CYA) in the rabbit and heart transplant (HT) patients by NONMEM using a physiological model of renal clearance (CLr)." 8th Annual Meeting, American Association of Pharmaceutical Scientists, Orlando, FL, November 14-18, 1993.

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118. R.C. Brundage, B.K. Malhotra, J.A. Maloney and R.J. Sawchuk. "Brain distribution of tacrine and the 1-hydroxy and 2-hydroxy tacrine metabolites determined by microdialysis in freely-moving rats." 5th Symposium: Frontiers of Pharmacokinetics and Pharmacodynamics, Baltimore, MD, April 18-20, 1994.

119. Y. Wang and R.J. Sawchuk. "CNS Distribution of inulin-[^{14}C]-carboxylic acid in rabbits." 9th Annual Meeting, American Association of Pharmaceutical Scientists, San Diego, CA, November 6-10, 1994.

120. B.K. Malhotra, R.C. Brundage and R.J. Sawchuk. "Simultaneous microdialysis of portal and jugular blood following IV bolus and oral lavage in freely-moving rats." 9th Annual Meeting, American Association of Pharmaceutical Scientists, San Diego, CA, November 6-10, 1994.

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122. B.K. Malhotra, R.C. Brundage, Y. Wang and R.J. Sawchuk. "Dialysis membrane-limited and aqueous boundary layer-limited *in vitro* microdialysis recovery." 9th Annual Meeting, American Association of Pharmaceutical Scientists, San Diego, CA, November 6-10, 1994.

123. B.W.Y. Cheung, Y. Wang and R.J. Sawchuk. "Preliminary studies of effects of hydroxy-propyl-beta-cyclodextrin on carbamazepine distribution into rabbit brain." 9th Annual Meeting, American Association of Pharmaceutical Scientists, San Diego, CA, November 6-10, 1994.

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127. R.J. Sawchuk, R.C. Brundage, E.D. Kharasch and M.D. Karol. "Physiological-based pharmacokinetic (PBPK) modeling of sevoflurane, a volatile anesthetic." 10th Annual Meeting, American Association of Pharmaceutical Scientists, Miami Beach, FL, November 5-9, 1995.

128. R.C. Brundage, S. Thomas Forgue and R.J. Sawchuk. "Comparative distribution of a series of aminoacridines of varying polarities into rat cortex using microdialysis." 10th Annual Meeting, American Association of Pharmaceutical Scientists, Miami Beach, FL, November 5-9, 1995.

129. Z. Yang, R.C. Brundage, L.L. Cartier, J.A. Maloney and R.J. Sawchuk. "Development of a microdialysis method to study brain distribution of stavudine (d4t) in freely-moving rats." 10th Annual Meeting, American Association of Pharmaceutical Scientists, Miami Beach, FL, November 5-9, 1995.

130. B.K. Malhotra, R.C. Brundage and R.J. Sawchuk. "Estimation of presystemic disposition of drugs based upon combination of area ratios and deconvolution." 10th Annual Meeting, American Association of Pharmaceutical Scientists, Miami Beach, FL, November 5-9, 1995.

131. M.A. Osman, R. J. Sawchuk, and M.K. Youssef. "In Situ Absorption of the Antiviral drug, stavudine, from the rabbit intestine." European Symposium on Formulation of Poorly Available Drugs for Oral Administration, Paris, February 5- 6, 1996.

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142. Z Yuan, P. Ji, S. Giebink, and R.J. Sawchuk. "Antibiotic Middle Ear Pharmacokinetics by Microdialysis." American Association of Pharmaceutical Scientists Annual Meeting, San Francisco, CA, November 15-19, 1998.

143. R.J. Sawchuk, P. Ji, Y. Huang. "Distribution of Amoxicillin to Middle Ear Fluid Using Microdialysis Sampling." Higuchi Research Seminar, Lake of the Ozarks, MO, March 14-17, 1999.

144. Y. Huang, and R.J. Sawchuk. "Antibiotic Pharmacokinetics in Chinchilla Middle Ear using Microdialysis." Association of Pharmaceutical Scientists Midwest Regional Meeting, Chicago, IL, May 17, 1999

145. R.J. Sawchuk, P. Ji, Y. Huang, and S. Giebink. "Kinetics of Transport of Antibiotics to Middle Ear Fluid Using Microdialysis Sampling." Seventh International Symposium on Recent Advances in Otitis Media, Fort Lauderdale, FL, June 1-5, 1999.

146. G. Gan, and R.J. Sawchuk. "Intestinal Absorption and Pre-Systemic First Pass Elimination of Minocycline and Propranolol in Rabbits" *American Association of Pharmaceutical Scientists Annual Meeting*, New Orleans, LA, November 14-18, 1999.

147. B.W.Y. Cheung, L.L. Cartier, H. Q. Russlie, and R.J. Sawchuk. "Using Sample Pooling Methods in the Determination of AUC and AUMC in Pharmacokinetic Studies" *American Association of Pharmaceutical Scientists Annual Meeting*, New Orleans, LA, November 14-18, 1999.

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150. R. J. Sawchuk, B. W. Y. Cheung, L. L. Cartier, H. Q. Russlie, T. Zhu, Y. Huang, G. S. Giebink, D. Mulford and M. Mayer. "Kinetics of Cefditoren Distribution to Middle Ear Fluid in The Unanesthetized Chinchilla" *40th Interscience Conference on Antimicrobial Agents and Chemotherapy.* Toronto, ON, September 17-20, 2000

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152. Y. Huang, R. J. Sawchuk. "Studies of the Middle Ear Distribution Kinetics of Amoxicillin in the Awake Chinchilla Using Microdialysis". *American Association of Pharmaceutical Scientists Annual Meeting*, Indianapolis, IN, October 29- November 2, 2000.

153. J.Z. Peng, R.C. Brundage, and R.J. Sawchuk. "Study of Presystemic Elimination of 4-mono-methylamino-antipyrine (MAAP) after Consecutive Doses in Freely Moving Rats Using On-line Microdialysis". *American Association of Pharmaceutical Scientists Annual Meeting*, Indianapolis, IN, October 29- November 2, 2000.

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156. L. C. Musib, J. C. Cloyd, A.K. Birnbaum, T. J. Hietpas, R. J. Sawchuk, I. E. Leppik, T. R. Browne, S. F. Holloway, G. S. Holden, J. O. Rarig. "Preliminary Report on Phenytoin Bioavailability in the Elderly Using an Intravenous Stable-Labeled Isotope". *American Association of Pharmaceutical Scientists Annual Meeting*, Indianapolis, IN, October 29- November 2, 2000.

157. Z. Yang, L. M. Zadjura, C. J. D'Arienzo, D. B. Wang-Iverson, R. J. Sawchuk "Use of Sample Pooling in Drug Discovery to Screen for Pharmacokinetic Properties of Compounds in Rats" *American Association of Pharmaceutical Scientists Annual Meeting*, Indianapolis, IN, October 29- November 2, 2000.

158. R.J. Sawchuk, Y. Huang, P. Ji, M. Quartey, G.S. Giebink. "Influx/Eflux Penetration Clearance of Amoxicillin between Plasma and Middle Ear Fluid in Freely Moving Chinchillas using Microdialysis" *4th Extraordinary International Symposium on Recent Advances in Otitis Media,* Sendai, Japan, April 16-20, 2001.

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163. J.Peng, R.J.Sawchuk, and R.P.Remmel "Mechanism-based inactivation of CYP1A2 by tacrine" *11th North American Meeting of the International Society for the Study of Xenobiotics*, Orlando, FL. October 27-31, 2002.

164. Y.Song, L.L.Cartier, B.W Cheung, R J Sawchuk. "An Animal Model for Multi-site CSF Disposition Studies of Intrathecally Administered Agents". *American Association of Pharmaceutical Scientists Annual Meeting*, Toronto, Ontario, Canada. November 10-14, 2002.

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166. Y.Song, L.L.Cartier, B. Cheung, R.J. Sawchuk. "Multi-site CSF Disposition Studies of Intrathecally Administered Antiviral Nucleosides in a Novel Animal Model". *8th International Meeting of the International Society for the Study of Xenobiotics*, Dijon France. April 27-31, 2003

167. W. Liu, B. W. Y. Cheung, R. J. Sawchuk. "Distribution Kinetics of Cethromycin in the Chinchilla Middle Ear". *8th International Symposium on Recent Advances in Otitis Media.* Fort Lauderdale, FL. June 3 - 7, 2003

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170. Y. Song and R. J. Sawchuk. "Pharmacokinetics of Zidovudine and Stavudine in the Cerebrospinal Fluid after Intrathecal Administration in a Novel Rabbit Model". *American Association of Pharmaceutical Scientists Annual Meeting*, Baltimore, MD. November 8, 2004.

171. N. Mostafa and R. J. Sawchuk. "Determination of Ofloxacin Clearance from the Middle Ear Fluid using Microdialysis". *American Association of Pharmaceutical Scientists Annual Meeting*, Nashville, TN. November 7, 2005.

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173. R. J. Sawchuk, L. M. Page, and R. L. Rauck. "Pharmacokinetics of Gabapentin Injection in Cerebrospinal Fluid and Plasma with Intrathecal Administration". FDA Science Forum, Washington, DC. April 18, 2006.

174. R. J. Sawchuk. "Bugs and Drugs: Does the Anti-infective Agent get to the Target Site?" *American Pharmacists Association Annual Meeting*, Atlanta, GA. March 18, 2007.

175. R. J. Sawchuk. "Future Perspectives on the Contributions of Microdialysis in Drug Research and Development" Fifth International Symposium on Microdialysis in Drug Research and Development. Leiden, NE. April 25, 2007.

176. R.J. Sawchuk. "Drug Delivery to the Middle Ear across the Tympanic Membrane for Therapy of Acute Otitis Media". Global Gators 6th Symposium on Clinical Pharmacy and Clinical Pharmacology. Munich, Germany. June 9, 2007.

177. T. Wang, R. J. Sawchuk and W. F. Elmquist. "Modeling Distributional Kinetics: Comparison of Two Methods Based on a Partial-Areas Analysis". *American Association of Pharmaceutical Scientists Annual Meeting*, San Diego, CA, November 13, 2007.

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179. R. J. Sawchuk, G. M. Wall, B. W. Y. Cheung, L. L. Cartier, and D. B. Madhura. "Trans-tympanic Membrane Delivery of an Antibiotic into Chinchilla Middle Ear: a Model for the Treatment of Otitis Media". *European Society of Pediatric Infectious Diseases Meeting 2010*, Nice, FR, May 4-8, 2010.

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 June 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *24-53* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *24-53* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This is a continuation of US 10/872,784, filed 6/22/2004, now patent 7,456,160, which is a continuation of US 09/756,291, filed 1/9/2001, now patent 6,774,122. The instant application also claims the benefit of UNITED KINGDOM 0000313.7, filed 01/10/2000 and UNITED KINGDOM 0008837.7, filed 04/12/2000.

Applicant's amendments filed 6/20/2011 have been entered. Claims 24-53 are pending.

The outstanding rejection under 35 USC 103(a) is withdrawn due to the cancellation of the claims.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422

F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 24-53 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-9 of U.S. Patent No. 6,774,122 ('122). Although the conflicting claims are not identical, they are not patentably distinct from each other because '122 teaches the method of treating hormonal dependent benign or malignant disease of reproductive tract by employing the herein claimed composition. The ratio of the solvents and the excipients are within the range taught in '122. The optimization of result effect parameters (e.g., dosing regimen, weight ratio of the actives and the excipients) is obvious as being within the skill of the artisan. The optimization of known effective amounts of known active agents to be administered, is considered well in the competence level of an ordinary skilled artisan in pharmaceutical science, involving merely routine skill in the art. It has been held that it is within the skill in the art to select optimal parameters, such as amounts of ingredients, in a composition

in order to achieve a beneficial effect. See *In re Boesch*, 205 USPQ 215 (CCPA 1980). It is also noted that "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Claims 24-53 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 7,456,160 ('160). Although the conflicting claims are not identical, they are not patentably distinct from each other because '160 teaches the method of treating hormonal dependent benign or malignant disease of reproductive tract by employing the herein claimed composition. The ratio of the solvents and the excipients are within the range taught in '160. The optimization of result effect parameters (e.g., dosing regimen, weight ratio of the actives and the excipients) is obvious as being within the skill of the artisan. The optimization of known effective amounts of known active agents to be administered, is considered well in the competence level of an ordinary skilled artisan in pharmaceutical science, involving merely routine skill in the art. It has been held that it is within the skill in the art to select optimal parameters, such as amounts of ingredients, in a composition in order to achieve a beneficial effect. See *In re Boesch*, 205 USPQ 215 (CCPA 1980). It is also noted that "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, 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 at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 24-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over McKeskey et al., from IDS filed 6/20/2011 in view of Dukes (EP 0 346 014), Osborne et al., Journal of National Cancer Institute, 1995;87(20):746-750, and the abstract of Wakeling et al., The Journal of Steroid Biochemistry and Molecular Biology, 1992;43(1-3):173-177.

McKeskey et al. teaches a studies employing subcutaneous injection of fulvestrant to nude mice. The fulvestrant formulation contains 50mg/ml in a vehicle of 10% ethanol, 15% benzyl benzoate, 10% benzyl alcohol brought to volume with castor oil (see page 698, col. 2, Drugs section).

McKeskey et al. does not expressly teach the use of fulvestrant in treating hormonal dependent diseases of breast. It does not expressly teach the dosing regimen to be once a month, intramuscular administration, or the volume administered. McKeskey et al. does not expressly teach the herein claimed serum concentration of fulvestrant.

Dukes teaches antiestrogen agents, including fulvestrant, are useful in treating postmenopausal symptoms such as urogenital atrophy affecting the vagina (See page 3, lines 56-page 4, line 1; also page 7, line 28-29). Dukes teaches that antiestrogen

agent, including fulvestrant, via intramuscular route of administration may be used in a dosage of 50mg to 5g in vehicle comprising castor oil and benzyl alcohol (See page 7, line 20-24).

Osborne et al. teaches fulvestrant as useful in treating human breast cancer (See pages 747- 748, Result Section).

Wakeling et al. teaches the administration of fulvestrant (ICI 182780) demonstrating the antiestrogenic effect for over a 1 month period (see the abstract).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ fulvestrant in McKeskey's, in the herein claimed dosing regimen and dosage, for treating hormonal dependent diseases such as breast cancer and postmenopausal symptoms.

One of ordinary skill in the art would have been motivated to employ fulvestrant in McKeskey's, in the herein claimed dosing regimen and dosage, for treating hormonal dependent diseases such as breast cancer and postmenopausal symptoms. It is known in the art that fulvestrant as being useful in treating hormonal dependent disease. It is also known in the art that fulvestrant can be administered intramuscularly and its antitumor effect can last for more than 1 month. Employing McKeskey's formulation of fulvestrant for intramuscular administration would be seen as obvious since administering a relative large volume of fulvestrant (5ml) would not be appropriate for subcutaneous administration. The examiner notes that in McKeskey's study, only 0.1ml was injected via subcutaneous administration. Furthermore, the optimization of result effect parameters (e.g., dosing regimen, weight ratio of the actives and the excipients) is

obvious as being within the skill of the artisan. The optimization of known effective amounts of known active agents to be administered, is considered well in the competence level of an ordinary skilled artisan in pharmaceutical science, involving merely routine skill in the art. It has been held that it is within the skill in the art to select optimal parameters, such as amounts of ingredients, in a composition in order to achieve a beneficial effect. See *In re Boesch*, 205 USPQ 215 (CCPA 1980). It is also noted that "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

The examiner notes that the herein claimed serum concentration is considered to be an inherent effect of the formulation of fulvestrant.

Response to Arguments

Applicant's arguments with respect to claims 24-53 have been considered but are moot in view of the new ground(s) of rejection.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not

mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAN-MING HUI whose telephone number is (571)272-0626. The examiner can normally be reached on Mon - Fri from 9:00 to 5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brandon Fetterolf can be reached on (571) 272-2919. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

San-ming Hui

Primary Examiner
Art Unit 1628

/San-ming Hui/
Primary Examiner, Art Unit 1628

Notice of References Cited	Application/Control No. 12/285,887	Applicant(s)/Patent Under Reexamination EVANS ET AL.	
	Examiner SAN-MING HUI	Art Unit 1628	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	The abstract of Wakeling et al., The Journal of Steroid Biochemistry and Molecular Biology, 1992;43:1-3:173-177
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Comparison of the Effects of a Pure Steroidal Antiestrogen With Those of Tamoxifen in a Model of Human Breast Cancer

*C. Kent Osborne, Ester B. Coronado-Heinsohn, Susan G. Hilsenbeck, Bryant L. McCue, Alan E. Wakeling, Richard A. McClelland, David L. Manning, Robert I. Nicholson**

Background: **Tamoxifen, a nonsteroidal estrogen antagonist, is the most prescribed drug for the treatment of breast cancer. The use of tamoxifen is limited, however, by the development of resistance to this compound in most patients. Although tamoxifen behaves primarily as an estrogen antagonist, it has agonist (or growth-stimulatory) activity as well. ICI 182,780 is a 7α-alkylsulfinyl analogue of estradiol lacking agonist activity. The absence of agonist activity may make this steroidal antiestrogen superior to tamoxifen in suppressing tumor cell growth.** ***Purpose:*** **We compared the inhibitory effects of ICI 182,780, tamoxifen, and estrogen withdrawal on the growth of established tumors and on tumorigenesis in a model system that uses estrogen-dependent, human MCF-7 breast tumor cells growing in athymic nude mice. We also studied the hormonal responsiveness of tumors that became resistant to the two estrogen antagonists and the effects of these drugs on estrogen-regulated gene expression.** ***Methods:*** **MCF-7 cells were injected subcutaneously into the flanks of castrated, female nude mice. The effects of repeated doses of tamoxifen and ICI 182,780 (500 μg and 5 mg, respectively) on the growth of established tumors (8-10 mm in size) were determined after supplemental estrogen was removed. The effects of antiestrogen treatments on the process of tumorigenesis, in the absence of estrogen supplementation, were determined by initiating drug administration on the same day as tumor cell inoculation. To evaluate the hormonal responsiveness of tumors resistant to tamoxifen and ICI 182,780, 1-mm^3 segments of the tumors were transplanted onto the flanks of new recipient mice, which were then treated with estrogen or the antiestrogens—alone or in combination. Tumor growth was monitored by measuring tumor volumes twice a week. Expression of the estrogen-responsive genes, pLIV1 and pS2, in the tumors of treated animals was analyzed using blots of total cellular RNA and complementary DNA probes.** ***Results:*** **Treatment with ICI 182,780 suppressed the growth of established tumors twice as long as treatment with tamoxifen or estrogen withdrawal. Tumorigenesis, in the absence of supplemental estrogen, was delayed to a greater extent in ICI 182,780-treated mice than in tamoxifen-treated mice. ICI 182,780 was found to be more effective than tamoxifen in reducing the expression of estrogen-regulated genes. Most tumors eventually became resistant to ICI 182,780 and grew independently of estrogen.** ***Conclusions:*** **ICI 182,780 is a more effective estrogen antagonist than tamoxifen in the MCF-7 tumor cell/nude mouse model system.** **[J Natl Cancer Inst 87:746-750, 1995]**

Tamoxifen, a nonsteroidal antiestrogen, is the most prescribed drug for the treatment of breast cancer. When used in the adjuvant setting after surgery for primary breast cancer, about one fifth of the deaths at 10 years are avoided by 2 years or more of treatment (*1*). Tamoxifen is also effective in inducing remissions in women with estrogen receptor (ER)-positive metastatic breast cancer. Invariably, however, tumors become resistant to tamoxifen, and tumor progression and death ensue. The evolution to tamoxifen resistance in metastatic breast cancer occurs after an average treatment duration of only 10-12 months, severely limiting the usefulness of this approach.

The mechanisms by which tumors acquire resistance to tamoxifen are poorly understood. Loss of ER from the tumor can occur by selection of an ER-negative clone or by suppression of receptor expression, but this loss explains only a minority of cases (*2*). Growing experimental and clinical evidence suggests that resistance in some patients may be caused by the intrinsic estrogen agonist properties of tamoxifen. Although tamoxifen is predominantly an estrogen antagonist in breast cancer cells, acquisition of increasingly dominant agonist activity over time may result in clinical resistance because of the acquired ability of the drug to stimulate, rather than to inhibit, tumor growth (*3-7*). The mechanisms for tamoxifen-stimulated tumor growth are not clear, but these data suggest that antiestrogens with pure antagonist properties might have superior antitumor activity.

ICI 182,780 is a 7α-alkylsulfinyl analogue of estradiol that differs substantially from tamoxifen in terms of its chemical, pharmacologic, and biologic properties. This agent has no intrinsic estrogen-agonist activity and, thus, is considered a "pure" antiestrogen (*8,9*). It has potent antiestrogenic activity in preclinical in vitro and in vivo model systems (*10*). We recently reported (*7*) that treating nude mice with ICI 182,780 inhibits the growth of MCF-7 human breast tumor implants that had acquired tamoxifen resistance through the mechanism of tamoxifen-stimulated growth. Similar results were obtained with another analogue, ICI 164,384, studied earlier (*11*). These data suggest the possibility that pure steroidal antiestrogens may be effective in some tamoxifen-resistant patients.

In the present study, we have investigated the preclinical activity of ICI 182,780 in more detail. We compared the inhibitory effects of ICI 182,780, tamoxifen, and estrogen withdrawal on the

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See "Notes" section following "References."

growth of established tumors and on tumorigenesis in a model system that uses estrogen-dependent, human MCF-7 breast tumor cells growing in athymic nude mice. We also studied the hormonal responsiveness of tumors that had become resistant to the two estrogen antagonists and the effects of these drugs on estrogen-regulated gene expression.

Materials and Methods

Nude Mouse Model System

ER-positive MCF-7 human breast cancer cells (passage 100-200) were cultured as described previously (*12*). The athymic nude mice used in these experiments were 4- to 5-week-old female castrated BALB/c-nu$^+$/nu$^+$ mice purchased from Harlan Sprague-Dawley, Inc. (Madison, Wis.). The methods for maintenance and housing of the mice and for growing MCF-7 tumors from cell suspensions and from tumor transplants have been published in detail (*3,7*). Animal care was in accordance with institutional guidelines.

Approximately 5×10^6 MCF-7 cells were injected subcutaneously into the flanks, just under the forelimb, of female nude mice to initiate tumor formation. Estrogen supplementation was provided in the form of a 0.25-mg estradiol (E_2) pellet (Innovative Research, Rockville, Md.) placed subcutaneously in the interscapular region of the mice. The effects of tamoxifen and ICI 182,780 on the growth of established tumors were studied after the tumors had reached a size of 8-10 mm (3-5 weeks). At this time, the animals were randomly allocated into four treatment groups: 1) continued estrogen supplementation, 2) removal of the E_2 pellet, 3) removal of the E_2 pellet plus treatment with 500-µg tamoxifen citrate (Zeneca Pharmaceuticals, Wilmington, Del.) in peanut oil (injected subcutaneously each day, Monday through Friday), or 4) removal of the E_2 pellet and treatment with the indicated doses of ICI 182,780 (Zeneca Pharmaceuticals, Macclesfield, England) in castor oil (subcutaneous injections once a week). Initial dose–response studies with ICI 182,780 were performed in the presence of continued estrogen supplementation. Tumor growth was assessed, and tumor volumes were measured twice a week as described previously (*12*).

In tumorigenesis experiments, various treatments were begun on the same day tumor cells were injected. Inoculated mice were randomly allocated immediately into four treatment groups: 1) estrogen supplementation, 2) 500 µg tamoxifen once a day, Monday through Friday, 3) 5 mg ICI 182,780 once a week, or 4) drug vehicle (peanut oil and/or castor oil). Tumor volumes were measured twice a week.

To investigate the hormonal responsiveness of tumors that had become resistant to ICI 182,780, mice with resistant tumors were killed by cervical dislocation, and the tumors were resected and cut into 1-mm^3 fragments. The fragments were then transplanted subcutaneously on the flank just under the forelimb of new 4- to 5-week-old recipient mice that were then treated with estrogen, tamoxifen, ICI 182,780, or vehicle alone.

Estrogen and Progesterone Receptor Assays

ER content was determined in tumors homogenized in 0.4 *M* KCl–Tris buffer, using the ER antibody kit (ER-EIA; Abbott Laboratories, North Chicago, Ill.). Progesterone receptor (PgR) levels were measured by a ligand-binding, dextran-coated charcoal method (*3*).

Estrogen-Regulated Gene Expression

Expression of the estrogen-responsive genes, pLIV1 and pS2, was determined by northern blot analysis, using complementary DNA (cDNA) probes labeled with [^{32}P]deoxycytidine triphosphate (3000 Ci/mmol; Amersham Ltd., Amersham, England, U.K.) by the random-priming method as described previously (*13*). Briefly, total RNA was obtained from the tumors of treated mice by cell lysis in 4 *M* guanidinium thiocyanate and 1% 2-mercaptoethanol and centrifugation through 5.7 *M* caesium chloride (Beckman L-80 ultracentrifuge, SW50 rotor, 34 000 rpm at 20 °C for 17 hours). Purified samples were stored in RNase-free water at −70 °C before electrophoresis (10 µg/lane), blotting, and hybridization. Densitometric analysis of autoradiographs was performed using a model 620 video densitometer (Bio-Rad Laboratories, Richmond, Calif.), and values obtained were corrected for equivalence of RNA loading by comparison with the signals generated using a cDNA probe to human glyceraldeyhyde 3-phosphate dehydrogenase (G3PDH) (Clontech Laboratories, Inc., Palo Alto, Calif.).

Recorded densitometry values represent the area of peak values obtained, following background subtraction, from equivalently exposed autoradiographs (where x = band width in mm and y = optical density value). Hybridizations of each set of filters in the study were carried out simultaneously with the same labeled probes. The reported values represent means of groups, and at least two separate hybridizations of different filters were performed for each probe (stripping the previous probe with high-stringency washes and checking for clearance by autoradiography).

Statistical Analysis

Analyses were performed using either the Kruskal–Wallis one-way analysis of variance (when there were more than two groups) or the Wilcoxon signed rank test for two samples. All statistical tests were two-sided.

Results

ICI 182,780 Dose–Response

ICI 182,780 inhibited estrogen-induced growth of MCF-7 tumors in a dose-dependent manner. Estrogen-supplemented mice with established MCF-7 tumors were randomly allocated to receive either continued estrogen treatment or estrogen treatment plus injections of ICI 182,780 once a week in doses ranging from 0.5 mg to 10.0 mg. Inhibitory activity was modest with doses of 0.5 mg or 1.0 mg, while more dramatic—but approximately equivalent—inhibitory effects were observed with 5.0-mg and 10.0-mg doses (data not shown). For subsequent experiments, a dose of 5.0 mg per mouse, given once a week, was used.

Effect of Estrogen Withdrawal, Tamoxifen, and ICI 182,780 on MCF-7 Tumor Growth

Treatment of mice by removal of the E_2 pellet alone or with tamoxifen or ICI 182,780 significantly inhibited MCF-7 tumor growth (Fig. 1). In this experiment, tumor volumes remained stable for nearly 100 days after estrogen withdrawal before progression ensued. In contrast, tumor volumes decreased slightly with tamoxifen and ICI 182,780 treatment, and tumor size remained stable for variable periods of time. A consistent observation was the delayed time to progression that was evident in mice treated with ICI 182,780. With estrogen withdrawal alone or with tamoxifen, tumors developed resistance, and progression was evident in all mice after 3-4 months of treatment (median, 97 and 104 days, respectively). However, the median time to progression was nearly twice as long with ICI 182,780, and the growth of some tumors remained controlled for extended periods of time (median, 200 days). In fact, two of the 10 tumors from ICI 182,780-treated mice still had not progressed after 11 months and one small tumor (4 mm diameter) completely regressed and did not reappear during the course of the experiment (data not shown).

Effect of ICI 182,780 on Tumorigenesis

ICI 182,780 also had a greater impact on tumor formation in mice in which drug treatments were begun on the day of tumor cell inoculation (Fig. 2). Tumors grew rapidly in mice treated with estrogen. Tumor growth was substantially delayed in mice treated with tamoxifen, but after 2 months, the growth rate increased. Tumors grew very slowly, or not at all, in mice treated with ICI 182,780—similar to the growth pattern observed in estrogen-deprived mice (*12*). By day 70, barely measurable tumors were present in the majority of mice. In another experiment (data not shown), three of six mice

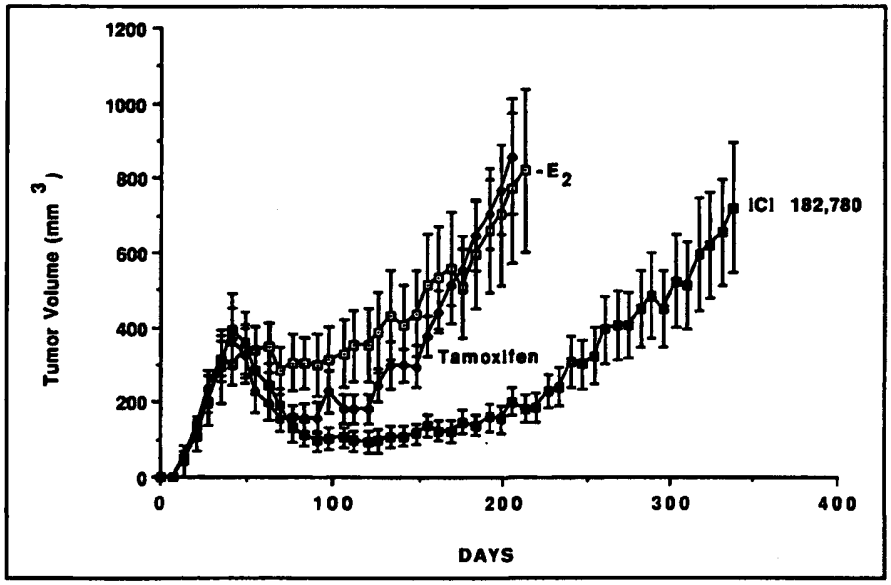

Fig. 1. Effects of estrogen (estradiol [E_2]) withdrawal, tamoxifen, and ICI 182,780 on MCF-7 tumor growth. Estrogen-supplemented mice were inoculated with MCF-7 cells. On day 36 when tumors had formed, mice were randomly allocated to treatment by withdrawal of estrogen (–E_2; -□-); withdrawal of estrogen and treatment with 500 µg tamoxifen given once a day, Monday through Friday (-◆-); or 5 mg ICI 182,780 given once a week (-■-). Tumor volumes were determined at the times shown. n = 10 mice per group; means ±SE.

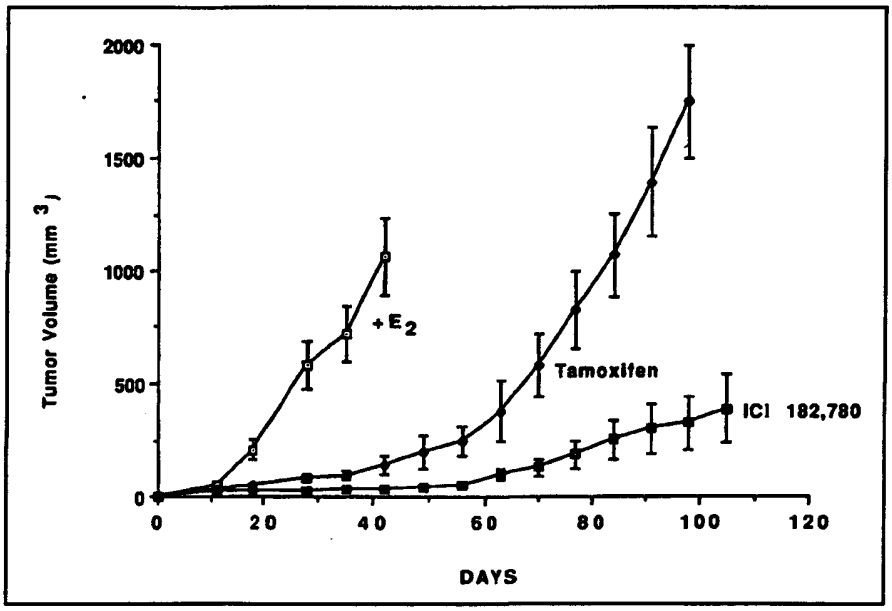

Fig. 2. Effect of estrogen, tamoxifen, and ICI 182,780 on MCF-7 tumorigenesis. Mice were inoculated with MCF-7 cells on day 0 and randomly allocated immediately to receive treatment with a 17β estradiol (E_2) pellet (+E_2; -□-); 500 µg tamoxifen given once a day, Monday through Friday (-◆-); or 5 mg ICI 182,780 given once a week (-■-). Tumor volumes were determined at the times shown. n = 8 mice per group; means ±SE.

treated with ICI 182,780 failed to grow measurable tumors even after 6 months of treatment.

ICI 182,780-Resistant Tumors

As indicated above, tumor resistance eventually occurred in most, but not all, mice treated with ICI 182,780. This resistance was manifested by regrowth of tumors, usually after many months of treatment. To investigate the hormonal sensitivity of these resistant tumors, fragments of a tumor that had progressed after months of treatment with ICI 182,780 were transplanted into new castrated, recipient mice that were then treated with estrogen, tamoxifen, ICI 182,780, tamoxifen plus ICI 182,780, or vehicle alone. This experiment was conducted five times with different tumor transplants, and a representative result is shown in Fig. 3. Transplanted tumor fragments grew well in all mice, even those treated with vehicle alone (–E_2), suggesting estrogen independence. However, in four of five experiments, tumor growth was slightly increased by estrogen treatment (+E_2), indicating continued sensitivity to the hormone. As expected, growth of these transplanted ICI 182,780-resistant tumors was also observed in recipient mice treated with ICI 182,780.

Interestingly, in four of the five experiments, treatment of recipient mice with tamoxifen alone or tamoxifen plus ICI 182,780 resulted in a slight retardation of tumor growth compared with treatment using vehicle alone or ICI 182,780 alone, although the observed differences in the individual experiments were modest and not statistically significant. A total of six of the 25 mice in these experiments showed slower tumor growth with tamoxifen treatment, indicating some heterogeneity among the transplanted fragments in response to tamoxifen. However, most mice resistant to ICI 182,780 showed cross-resistance to tamoxifen.

Resistance to ICI 182,780 was not due to a complete loss of tumor ER, although treatment with this drug reduced expression of both ER and PgR. Tumors harvested 4 weeks after initiating treatment with ICI 182,780 (ER = 37 ± 3 fmol/mg protein; PgR = 27 ± 7 fmol/mg protein) as well as those harvested at the time of resistance to ICI 182,780 (ER = 16 ± 4 fmol/mg protein; PgR = 17 ± 8 fmol/mg protein) expressed both ER and PgR at markedly reduced levels compared with estrogen-treated controls (ER = 208 ± 81 fmol/mg protein; PgR = 103 ± 20 fmol/mg protein) (P = .024).

Expression of two estrogen-responsive genes, pS2 and pLIV1, was also measured in these tumors (Table 1). pS2 and pLIV1 messenger RNA (mRNA) expression was reduced by 20%-74% in tumors from tamoxifen-treated mice (P = .013). It is interesting that pS2 and pLIV1 expression remained suppressed even after

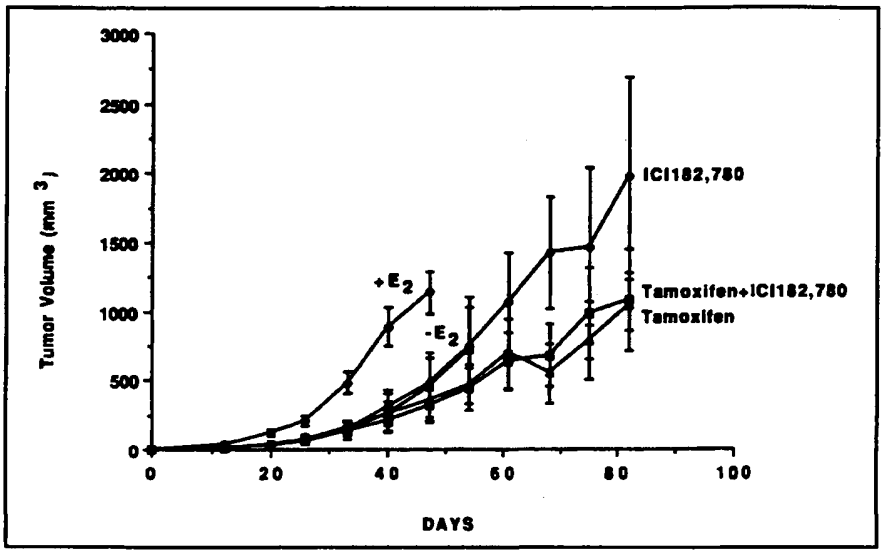

Fig. 3. Hormonal sensitivity of ICI 182,780-resistant tumors. Fragments of a tumor that had developed resistance in a mouse treated with ICI 182,780 were transplanted into new recipient female castrated nude mice. The recipient mice were then randomly allocated to receive vehicle alone (-E_2; -□-), an estradiol (E_2) pellet (+E_2; -◆-); tamoxifen alone (-▲-); ICI 182,780 alone (-◇-), or a combination of tamoxifen plus ICI 182,780 (-■-). Tumor volumes were calculated on the days shown. n = 6 mice per group; means ±SE.

evolution to tamoxifen resistance when the drug was stimulating tumor growth (*3*). In fact, pS2 was significantly lower in tamoxifen-resistant tumors than in tamoxifen-sensitive tumors (P = .012). This finding suggests that the agonist activity of tamoxifen, if responsible for tamoxifen-stimulated tumor growth, may be specific to genes associated with cell proliferation, while its antagonist activity continues to suppress the activity of genes less crucial for tumor survival. In contrast to the results obtained with tamoxifen, mRNA expression was nearly abrogated by treatment with ICI 182,780 (P<.004), and there was no difference between sensitive and resistant tumors. It is unlikely, therefore, that ICI 182,780 resistance is caused by metabolic conversion of the drug to E_2, since expression of these estrogen-regulated genes remained low.

Table 1. Expression of estrogen-sensitive genes*

Treatment group (No. of blots analyzed)	Gene, relative mRNA level	
	pS2	pLIV1
Estrogen (4)	12.2 ± 0.7	12.2 ± 0.6
Tamoxifen-sensitive (5)	9.8 ± 0.5	6.0 ± 1.5
Tamoxifen-resistant (5)	3.2 ± 0.4	7.5 ± 1.8
ICI-sensitive (5)	0.3 ± 0.05	0 ± 0
ICI-resistant (8)	0.6 ± 0.23	2.3 ± 1.3

*mRNA expression was measured by northern blot analysis of total RNA extracted from MCF-7 tumors taken from mice treated with estrogen (controls), tamoxifen for 3 weeks (tamoxifen-sensitive), tamoxifen until the time of tumor progression (tamoxifen-resistant), ICI 182, 780 for 4 weeks (ICI-sensitive), or ICI 182,780 until tumor progression (ICI-resistant). Values shown are the means ± SE of scanning densitometry units corrected for RNA loading.

Discussion

Clinical data demonstrate that, in some patients, the current endocrine therapies for breast cancer result in temporary tumor regression or growth stabilization, followed by tumor regrowth, usually within 6-18 months of treatment. We have developed an experimental in vivo model that mimics this clinical scenario. Our data suggest that, in this experimental model system, ICI 182,780 possesses a greater ability to suppress estrogen-sensitive gene expression and greater antitumor activity than the partial estrogen antagonist tamoxifen. In addition, MCF-7 tumorigenesis was significantly delayed by ICI 182,780 when compared with tamoxifen. Moreover, a proportion of treated mice failed to develop tumors even after prolonged follow-up, an event rarely encountered in our experience treating mice with tamoxifen. ICI 182,780 also suppressed growth of established tumors for a significantly longer duration than treatment by estrogen withdrawal alone or with tamoxifen. Finally, expression of the estrogen-regulated genes pS2 and pLIV1 was nearly abolished by treatment with ICI 182,780.

Previous reports by us and by other investigators (*7,11,14-16*) have also shown that the growth of tumors with acquired tamoxifen resistance can be inhibited or blocked by treatment with a pure antiestrogen such as ICI 182,780, suggesting that the pure antiestrogens work by a different mechanism of action than tamoxifen and other similar antiestrogens. Tamoxifen resistance in our model system is associated with drug-induced tumor growth stimulation that occurs after an initial period of growth suppression (*3*). The ability of tamoxifen alone to stimulate the growth of these tumors is less than that of estrogen. Interestingly, when combined with estrogen, tamoxifen can still inhibit estrogen-stimulated growth, indicating that it continues to possess both estrogen-agonist and antagonist properties (*7*). The increasingly dominant agonist properties of tamoxifen that develop after prolonged treatment can be blocked by the addition of pure antiestrogens (*7,11*). Evidence for tamoxifen-stimulated tumor growth as a mechanism for acquired tamoxifen resistance in patients has also been presented (*5,6,17*). On the basis of these preclinical studies, it has been suggested that treatment with ICI 182,780 might induce tumor regression in some patients who have developed tamoxifen resistance. One recent study (*18*) has shown that short-term ICI 182,780 treatment of patients who have ER-positive tumors causes statistically significant reductions in the Ki67 labeling index and reductions in the expression of estrogen-regulated genes such as PgR and pS2. In addition, remissions have now been reported in tamoxifen-resistant patients treated with this drug (*19*).

Although ICI 182,780 controls MCF-7 tumor growth for longer durations than tamoxifen, eventual resistance to this agent is common. MCF-7 tumors that progress after prolonged treatment are estrogen-independent (grow in the absence of estrogen supplementation) although they are still estrogen-sensitive (growth is enhanced by estrogen). The mechanisms by which resistance to

ICI 182,780 develops are not clear, but reduced levels of ER and reduced expression of estrogen-regulated genes (compared with tamoxifen-sensitive or with tamoxifen-resistant tumors) are evident. Reduced ER levels have also been seen in tumors from patients treated with ICI 182,780, in cultured breast cancer cells, and in mouse uterine tissue following the administration of the prototype pure antiestrogen ICI 164,384 (*18-20*). Other data suggest that the pure antiestrogen-ER complex may be more fragile and more susceptible to receptor degradative pathways (*16*). In contrast, ER levels are high in tamoxifen-resistant tumors obtained with our model system (*3*). On the basis of our data, we would predict that most patients with ICI 182,780-resistant tumors would not respond well to subsequent treatment with tamoxifen.

Even if pure antiestrogens are shown to have superior antitumor activity in women with breast cancer, they may not be the optimal antiestrogens for clinical use. The estrogenic properties of tamoxifen in bone and on blood lipids may help to reduce bone loss and prevent cardiovascular disease, which are added benefits when treating breast cancer patients for prolonged periods after surgery for primary tumors or for breast cancer prevention (*21,22*). The effect of ICI 182,780 on these parameters is not yet known, but it might be deleterious given its lack of estrogenic qualities. However, treatment with ICI 182,780 might not be associated with the increased risk of endometrial cancer recently attributed to tamoxifen (*23*). Further clinical study of pure antiestrogens in tamoxifen-resistant and in tamoxifen-naive patients is clearly indicated.

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Notes

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Pending Claims in U.S. Application No. 12/285,887 as of December 9, 2011, with proposed amendments

Claims 24, 32, 34, 36, 44, and 46 are proposed to be amended. New claims 54-57 are proposed to be added. Claims 25, 28, 31, 33, 37, 40, 43, 45, and 48-53 are proposed to be cancelled. Deletions appear in ~~strikethrough font~~ and/or [[inside double brackets]] and additions are underlined.

Claims 1-23 (Cancelled)

24. (Currently amended) A method for treating a hormonal dependent benign or malignant disease of the breast or reproductive tract comprising administering intramuscularly to a human in need of such treatment a formulation comprising:

~~at least 45 mgml^{-1} of fulvestrant;~~

~~a mixture of from 17 – 23% w/v of ethanol and benzyl alcohol;~~

~~12 - 18% w/v of benzyl benzoate; and~~

about 50 mgml^{-1} of fulvestrant;

about 10% w/v of ethanol;

about 10% w/v of benzyl alcohol;

about 15% w/v of benzyl benzoate; and

a sufficient amount of castor oil vehicle;

wherein the method achieves a therapeutically significant blood plasma fulvestrant concentration of at least 2.5 ngml^{-1} for at least ~~two~~ four weeks.

25. (Cancelled)

26. (Previously presented) The method of claim 24, wherein the therapeutically significant blood plasma fulvestrant concentration is at least 8.5 $ngml^{-1}$.

27. (Previously presented) The method of claim 24, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

28. (Cancelled)

29. (Previously presented) The method of claim 24, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

30. (Previously presented) The method of claim 24, wherein the method further comprises once monthly administration of the formulation.

31. (Cancelled)

32. (Currently amended) The method of ~~claim 31~~ claim 26, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

33. (Cancelled)

34. (Currently amended) The method of ~~claim 33~~ claim 32, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

35. (Previously presented) The method of claim 34, wherein the method further comprises once monthly administration of the formulation.

36. (Currently amended) A method for treating a hormonal dependent benign or malignant disease of the breast or reproductive tract comprising administering intramuscularly to a human in need of such treatment a formulation consisting essentially of:

~~at least 45 mgml^{-1} of fulvestrant;~~

~~a mixture of from 17 – 23% w/v of ethanol and benzyl alcohol;~~

~~12 - 18% w/v of benzyl benzoate; and~~

~~a sufficient amount of castor oil vehicle;~~

about 50 mgml^{-1} of fulvestrant;

about 10% w/v of ethanol;

about 10% w/v of benzyl alcohol;

about 15% w/v of benzyl benzoate; and

wherein the method achieves a therapeutically significant blood plasma fulvestrant concentration of at least 2.5 ngml^{-1} for at least ~~two~~ four weeks.

37. (Cancelled)

38. (Previously presented) The method of claim 36, wherein the therapeutically significant blood plasma fulvestrant concentration is at least 8.5 ngml^{-1}.

39. (Previously presented) The method of claim 36, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

40. (Cancelled)

41. (Previously presented) The method of claim 36, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

42. (Previously presented) The method of claim 36, wherein the method further comprises once monthly administration of the formulation.

43. (Cancelled)

44. (Currently amended) The method of ~~claim 43~~ claim 38, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

45. (Cancelled)

46. (Currently amended) The method of ~~claim 45~~ claim 44, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

47. (Previously presented) The method of claim 46, wherein the method further comprises once monthly administration of the formulation.

Claims 48-53 (Cancelled)

54. (New) The method according to claim 24, wherein the formulation is administered in a divided dose.

55. (New) The method according to claim 35, wherein the formulation is administered in a divided dose.

56. (New) The method according to claim 36, wherein the formulation is administered in a divided dose.

57. (New) The method according to claim 47, wherein the formulation is administered in a divided dose.

United States Patent Office

3,164,520

Patented Jan. 5, 1965

3,164,520

INJECTABLE STEROID COMPOSITIONS CONTAINING AT LEAST 75% BENZYL BENZOATE

Raymond Charles Huber, Martinsville, N.J., assignor to Olin Mathieson Chemical Corporation, New York, N.Y., a corporation of Virginia

No Drawing. Filed Oct. 29, 1962, Ser. No. 233,931
4 Claims. (Cl. 167—58)

This invention relates to compositions of matter and more particularly to new parenterally administrable pharmaceutical compositions comprising one or more active medicaments and a physiologically acceptable non-toxic pharmaceutical vehicle, comprised essentially of benzyl benzoate.

The active medicament which may be incorporated in the novel compositions of this invention may be any one which is administered for use in comparatively large unit dosages, for example, 10 mg./ml. to 500 mg./ml. and which is soluble in benzyl benzoate. Examples of the medicaments which may be employed in this invention include inter alia, steroid hormones, especially those steroid hormones which exhibit anabolic, estrogenic, androgenic and progestational activity, for example, 17-hydroxyprogesterone and the esters thereof, testosterone, estradiol and the acid esters thereof, progesterone and its derivatives and Δ^1-testololactone and its derivatives. In the most preferable embodiment of this invention the active medicament is a steroid hormone although other pharmaceutically active compounds may also be employed, with satisfactory results.

Heretofore it has been well recognized in the preparation of parenterally administrable pharmaceutical compositions that a suitable solvent must be employed to render the composition injectable. However, as the science of medicine has progressed it has been found that increasingly higher dosages of certain medicaments must be employed in the treatment of certain ailments in order to achieve several advantages. Among these advantages can be numbered the prolongation of activity of the medicaments involved and the lessening of the total number of individual injections which are needed to obtain the same results.

Additionally, it has been found that new chemical modifications of medicaments are continually being discovered and the solubility of these modified medicaments in the solvents commonly employed, appears to be more and more limited and it has therefore become increasingly difficult to dissolve these new modified medicaments in parenterally acceptable vehicles. It is well-known that certain pharmaceutical vehicles yield satisfactory results at low level medicament concentrations when employed in compositions for parenteral administration. Such vehicles are the vegetable oils such as cotton seed oil, peanut oil, sesame oil, or corn oil, in combination with small amounts of benzyl benzoate. However, when an increased dosage level of the medicaments is employed, along with a correspondingly necessary increased amount of pharmaceutical vehicle it has been found that certain undesirable disadvantages exist.

The undesirable disadvantages which are present when the prior art vehicles are employed with a high dosage level of medicaments, are many. In addition to the prior art vehicles being incapable of solubilizing any great quantities of the medicaments, it has been found that the compositions heretofore employed produce an undue amount of irritation at the site of injection, when parenterally administered into the animal being treated.

It has now been found that the disadvantages encountered in the parenteral administration of high dosage levels of the medicaments of this invention can be avoided by employing the novel pharmaceutical compositions of

this invention. It has been found that these disadvantages can be overcome by employing benzyl benzoate as the essential component of the pharmaceutical vehicle of parenterally administrable compositions. The benzyl benzoate has been found to be capable of dissolving great quantities of the medicaments of this invention and the resulting parenterally administrable composition employing this vehicle does not produce undue irritation when injected into the animals being treated.

The amount of benzyl benzoate which may be employed in the compositions of this invention while still yielding satisfactory results has been found to range from about 75% to 100% by volume of the pharmaceutical vehicle employed. Thus the ratio of benzyl benzoate present in the pharmaceutical vehicle as compared to any other ingredients therein must be at least 3 to 1. In the most preferable embodiment of this invention it has been found that a pharmaceutical vehicle consisting essentially of pure benzyl benzoate yields the best results although at lower levels satisfactory results are also obtained.

As is common in the art of preparing parenterally administrable pharmaceutical compositions other additives such as preservatives, antioxidants or anesthetics, such as benzyl alcohol and the other like well known additives may also be included in the pharmaceutical compositions of this invention. However, their use herein is permissive and not mandatory as their incorporation or omission in the final product of this invention does not substantially affect the results herein obtained.

The compositions of this invention are easily prepared by merely taking the desired amount of medicament and dissolving it in the pharmaceutical vehicle of this invention by any means known in the art, for example, by mere stirring.

The final compositions of this invention are parenterally administrable to the animal being treated. The administration of the composition may be accomplished intramuscularly, subcutaneously or in any other manner known to the art as may be determined in the individual cases wherein this invention is employed. It has been generally found that the most preferable results are obtained when an intramuscular route of administration is employed, although other methods of administration will also give satisfactory results.

The invention is more particularly illustrated by the following examples:

Example 1

Two g. of the acetophenone derivative of 16,17-dihydroxyprogesterone are dissolved in 10 ml. of benzyl benzoate with stirring and warming. The resultant solution is then filled in vials of 5 ml. each and sterilized by autoclaving at 121° C. for two hours.

0.25 ml. of the resulting solution is then injected into the vastus lateralis muscle of a rabbit producing a lesion at the site of the injection having the size of about 640 cubic millimeters after two days.

When 2 g. of the acetophenone derivative of 16,17-dihydroxyprogesterone are dissolved in 4.5 ml. of benzyl benzoate and 5.5 ml. of castor oil in accordance with the procedure of Example 1 and 0.25 ml. of the resultant solution is injected intramuscularly into the rabbit a lesion at the site of injection having a size of 967 cubic millimeters after two days.

Example 2

The procedure of Example 1 is followed except that 2 g. of testosterone palmitate are substituted for the acetophenone derivative of 16,17-dihydroxyprogesterone of Example 1.

0.25 ml. of the resultant solution is injected intramus-

cularly into a rabbit producing a lesion at the site of injection having the size of about 420 cubic millimeters after two days. When 2 g. of testosterone palmitate are dissolved in a vehicle consisting of 40% castor oil and 60% benzyl benzoate and the resultant solution is injected intramuscularly into the rabbit, a lesion at the site of injection having a size of 610 cubic millimeters is produced after two days.

Example 3

A 25% solution of progesterone is prepared by dissolving 2.5 g. of progesterone in benzyl benzoate to make 10 ml. Sterilization is obtained by autoclaving the solution at 121° C. for 2 hours. When 0.25 mg. of this solution is injected into the vastus lateralis muscle of the rabbit, a lesion is produced which, after 2 days, measures 672 cubic millimeters.

When 2.5 g. of progesterone are dissolved to make 10 ml. in a mixture of 50% benzyl benzoate and 50% castor oil as the vehicle, and 0.25 ml. of this solution is injected into the rabbit muscle, a lesion size of 898 cubic millimeters is produced after two days.

Example 4

A 50% solution of hormones is prepared by dissolving 2.5 g. of progesterone and 2.5 g. of 17-hydroxyprogesterone caproate in benzyl benzoate to make 10 ml. of final product. After autoclaving at 121° C. for 2 hours to sterilize, 0.25 ml. of the solution is injected into a rabbit muscle and the lesion size is measured after 2 days. A lesion consisting of 572 cubic millimeters was produced. When this same hormone combination in the same proportions was dissolved in a vehicle consisting of 46% benzyl benzoate and 54% castor oil, a rabbit muscle lesion size of 1047 cubic millimeters is produced 2 days after injection of 0.25 ml. of test material.

Example 5

A 40% solution of testosterone enanthate is prepared by dissolving 4.0 g. in benzyl benzoate to make 10 ml. of final volume. After autoclaving at 121° C. for 2 hours to sterilize, 0.25 ml. of the solution is injected into the vastus lateralis muscle of the rabbit and the lesion size is measured after 2 days. A lesion consisting of 847 cubic millimeters is produced.

When this same quantity of hormone is dissolved in a vehicle consisting of 20% benzyl benzoate and 80% sesame oil and 0.25 ml. is injected a lesion size of 1441 cubic millimeters is produced.

Example 6

A 5% solution of Δ^1-testololactone is prepared by dissolving 50 mg./ml. in benzyl benzoate and after autoclaving to sterilize, 0.25 ml. of the solution is injected into a rabbit muscle. After 2 days a lesion size of only 483 cubic millimeters is produced.

Example 7

15 mg. of Δ^1-testololactone is dissolved in a solution comprised of 7.5 ml. of benzyl benzoate and 2.5 ml. of castor oil. The resultant solution is sterilized, then filled in vials of 5 ml. each and sterilized by autoclaving at 121° C. for 2 hours. The injectable solution may then be administered to the patient being treated.

This invention may be variously otherwise embodied within the scope of the appended claims.

What is claimed is:

1. A parenterally administrable pharmaceutical composition comprising the acetophenonide of 16,17-dihydroxyprogesterone and a physiologically acceptable non-toxic pharmaceutical vehicle wherein at least 75% by volume of said vehicle is benzyl benzoate.

2. A parenterally administrable pharmaceutical composition comprising testosterone palmitate and a physiologically acceptable non-toxis pharmaceutical vehicle wherein at least 75% by volume of said vehicle is benzyl benzoate.

3. A parenterally administrable pharmaceutical composition comprising testosterone enanthate and a physiologically acceptable non-toxic pharmaceutical vehicle wherein at least 75% by volume of said vehicle is benzyl benzoate.

4. A method of administering a large single dosage of a steroid which comprises parenterally administering to the patient being treated a composition comprising a steroid selected from the group consisting of 17-hydroxyprogesterone, the caproate ester of 17-hydroxyprogesterone, testosterone, the enanthate ester of testosterone, the palmitate ester of testosterone, estradiol, progesterone, and Δ^1-testololactone, and a pharmaceutical carrier, said carrier being at least 75% by volume of benzyl benzoate.

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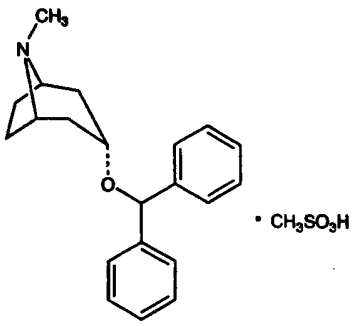
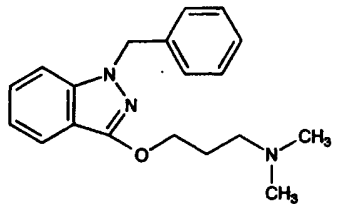
CM-cellulose	*O*-(carboxymethyl) cellulose
CMI	cell-mediated immunity
CML	cell-mediated lymphocytotoxicity
CMP	cytidine 5′-monophosphate (cytidylic acid)
CNS	central nervous system
CoA or CoASH	coenzyme A
coll. vol.	collective volume
compd	compound
compn	composition
Con A	concanavalin A
conc(d)	concentrated
concn	concentration
config	configuration
constit	constituent
contd	continued
contg	containing
CoQ	coenzyme Q (ubiquinone)
cor(r)	corrected
corresp	corresponding, corresponds
cp	centipoise
C.P.	chemically pure
cpd	compound
crit press	critical pressure
crit temp	critical temperature
cryst	crystalline, crystals
crystn	crystallization
CSF	colony stimulating factor; cerebral spinal fluid
CTFA	Cosmetic, Toiletry and Fragrance Assoc.
CTP	cytidine triphosphate
Cys	cysteine
Cyt	cytosine
d	density; specific gravity (d_4^{19} specific gravity at 19° referred to water at 4°)
d-	*dextro*(rotatory), the opposite of *l*
D-	*dextro* (in configurational sense only), the opposite of L
Da	daltons
DEAE cellulose	*O*-(diethylaminoethyl)cellulose
dec, decomp	decompose(s), decomposition
decompn	decomposition
deg	degree
deliquesc	deliquescent
Δ (delta)	indicates the locant of the double bond
deriv	derivative
determn	determination
DFP, DIFP, DIPF	diisopropyl fluorophosphate or diisopropyl phosphofluoridate
Dha	dihydroalanine
Dhb	dehydrobutyrine, β-methyldehydroalanine
diff	difference
dil(d), (n)	dilute, diluted, dilution
distln	distillation
dl-	racemic
DL-	optically inactive by external compensation as contrasted with *meso-*
dm	decimeter(s)
DMA	dimethylacetamide
DMARD	disease modifying antirheumatic drug
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
cDNA	complementary DNA
mtDNA	mitochondrial DNA
DNAase	deoxyribonuclease
DNFB	2,4-dinitro-1-fluorobenzene
DNP	2,4-dinitrophenyl or 2,4-dinitrophenol
Dopa	dihydroxyphenylalanine
dp, DP	degree of polymerization (number of monomeric units in the polymer)
D.R.P.	*(Deutsches Reichs-Patent)*–German patent
ds	double stranded
DTT	dithiothreitol
dyn	dynes
(*E*)-	*entgegen*, stereodescriptor, *see Glossary*
$E^{1\%}_{1cm}$	the absorbance of a solution containing one gram per 100 ml contained in a cell having an absorption path of one cm
E_M	molar extinction coefficient (concn in g-moles/l)
EAA	excitatory amino acid
EAC	erythrocyte coated by antibody and complement
EAE	experimental allergic encephalomyelitis
EC	electron capture
ECF-A	eosinophil chemotactic factor of anaphylaxis
ECG	electrocardiogram
E.C. No.	Enzyme Commission Number
ed.	edition
ED	effective dose
Ed(s).	editor(s)
EDTA	ethylenediaminetetraacetic acid
EEG	electroencephalogram
e.g.	(*exempli gratia*) for example
EGF	epidermal growth factor
eidem	the same (authors), plural of *idem*
EINECS	European Inventory of Existing Chemical Substances
EKG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
emf	electromotive force
en	ethylenediamine (in formulas)
endo-	stereochemical descriptor, *see Glossary*
EPA	Environmental Protection Agency
EPO	erythropoietin; European Patent Office
ε (epsilon)	molar extinction coefficient (concn in g-moles/l); dielectric constant
eq	equation
equilib	equilibrium
equiv	equivalent
esp	especially
esu	electrostatic units of electrical charge
Et	ethyl C_2H_5—
η (eta)	viscosity
et al.	(*et alii*) and others
etc.	*et cetera*
Et_2O	ether
EtOH	ethyl alcohol
Eur. pat. Appl.	European patent application
ev	electron volt
evac	evacuated
evapn	evaporation
exo-	stereochemical descriptor, *see Glossary*
expt(ly)	experimental(ly)
ext(d)	extract, extracted
extern	externally
°F	Fahrenheit degrees; also Fourneau
F-1-P	fructose 1-phosphate
F-6-P	fructose 6-phosphate
FA	fatty acid
FAB	fast atom bombardment
FAD ($FADH_2$)	flavin adenine dinucleotide (reduced form)
FCA	Freund's complete adjuvant (same as CFA)
Fd	ferredoxin
F.D.A.	Food and Drug Administration (U.S.A.)
FD & C	Food, Drug and Cosmetic (U.S.A).



Crystals from acetone + ether, mp 143°. uv max: 259 nm (E_M = 437). Soluble in water. pH about 6.

THERAP CAT: Anticholinergic.

1157. Benzydamine. *N,N-Dimethyl-3-[[1-(phenylmethyl)-1H-indazol-3-yl]oxy]-1-propanamine; 1-benzyl-3-[3-(dimethylamino)propoxy]-1H-indazole;* 1-benzyl-1*H*-indazol-3-yl 3-(dimethylamino)propyl ether; benzindamine. $C_{19}H_{23}N_3O$; mol wt 309.41. C 73.76%, H 7.49%, N 13.58%, O 5.17%. Prepn: Fr. pat. 1,382,855; Palazzo, U.S. pat. 3,318,905 (1964, 1967 both to Angelini Francesco); Palazzo *et al., J. Med. Chem.* 9, 38 (1966). Pharmacology: Lisciani *et al., Eur. J. Pharmacol.* 3, 157 (1968). Metabolism: Catanese *et al., Arzneimittel-Forsch.* 16, 1354 (1966); Kataoka *et al., Chem. Pharm. Bull.* 19, 1511 (1971). Toxicology: B. Silvestrini *et al., Toxicol. Appl. Pharmacol.* 10, 148 (1967). Series of articles on pharmacology: *Arzneimittel-Forsch.* 37, 587-646 (1987).



$bp_{0.05}$ 160°.

Hydrochloride, $C_{19}H_{23}N_3O.HCl$, *Afloben, Andolex, Benalgin, Benzyrin, Difflam, Dorinamin, Enzamin, Imotryl, Ririlim, Riripen, Salyzoron, Saniflor, Tamas, Tantum, Verax.* Crystals, mp 160°. uv max: 306 nm ($E^{1\%}_{1cm}$ 160). Very sol in water; rather sol in ethanol, chloroform, *n*-butanol. LD_{50} in mice, rats (mg/kg): 110, 100 i.p.; 515, 1050 orally (Silvestrini).

THERAP CAT: Analgesic; anti-inflammatory; antipyretic.

THERAP CAT (VET): Anti-inflammatory.

1158. Benzyl Acetate. *Acetic acid phenylmethyl ester; acetic acid benzyl ester.* $C_9H_{10}O_2$; mol wt 150.18. C 71.98%, H 6.71%, O 21.31%. $C_6H_5CH_2OOCCH_3$. Occurs in a number of plants, particularly jasmine: S. Arctander, *Perfume and Flavor Materials of Natural Origin* (Elizabeth, N.J., 1960) pp 313-314. Prepd from benzyl chloride, acetic acid or sodium acetate and triethylamine: Merker, Scott, *J. Org. Chem.* 26, 5180 (1961); Hennis *et al., Ind. Eng. Chem., Prod. Res. Develop.* 6, 193 (1967). Toxicity study: P. M. Jenner *et al., Food Cosmet. Toxicol.* 2, 327 (1964).

Liquid; pear-like odor. bp 213°, bp_{102} 134°. mp −51°. d^{25}_4 1.050. n^{20}_D 1.5232, n^{25}_D 1.4998. Flash pt, closed cup: 216° F (102° C). Practically insol in water. Misc with alcohol, ether. LD_{50} orally in rats: 2490 mg/kg (Jenner).

Caution: If ingested can cause G.I. irritation with vomiting and diarrhea. Also irritating to skin, eyes, respiratory tract.

USE: In perfumery, solvent for cellulose acetate and nitrate.

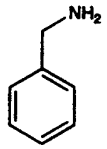
1159. Benzyl Alcohol. *Benzenemethanol;* phenylcarbinol; phenylmethanol; α-hydroxytoluene. C_7H_8O; mol wt 108.14. C 77.75%, H 7.46%, O 14.80%. $C_6H_5CH_2OH$. Constituent of jasmine, hyacinth, ylang-ylang oils, Peru and Tolu balsams, storax, where it occurs in ester form also. Originally prepd by the Cannizzaro reaction from benzaldehyde + KOH: Cannizzaro, *Ann.* 88, 129 (1853); *cf.* Hickinbottom, *Reactions of Organic Compds.* (Longmans, London, 3rd ed., 1957) p 251; A. I. Vogel, *Practical Organic Chemistry* (Longmans, London, 3rd ed., 1959) p 711; Gattermann-Wieland, *Praxis des organischen Chemikers* (de Gruyter, Berlin, 40th ed., 1961) p 193. Produced on a large scale by the action of sodium or potassium carbonate on benzyl chloride: Ger. pat. 484,662; *Chem. Zentr.* 1930, I, 1052; *Frdl.* 16, 426; *cf.* Kirk-Othmer *Encyclopedia of Chemical Technology* vol. 3 (Interscience, New York, 1964) pp 442-449. Toxicity: Smyth *et al., Arch. Ind. Hyg. Occup. Med.* 4, 119 (1951).

Liquid. Faint aromatic odor. Sharp burning taste. d^{20}_4 1.04535; d^{25}_4 1.04156. mp −15.19°. bp_{760} 204.7°; bp_{400} 183.0°; bp_{200} 160.0°; bp_{100} 141.7°; bp_{60} 129.3°; bp_{40} 119.8°; bp_{20} 105.8°; bp_{10} 92.6°; bp_5 80.8°; $bp_{1.0}$ 58.0°. n^{20}_D 1.54035; n^{25}_D 1.53837: Dreisbach, Martin, *Ind. Eng. Chem.* 41, 2875 (1941). Absorption spectrum: Brode, *J. Phys. Chem.* 30, 61 (1926). Vapor density 3.72 (air = 1.00). Flash pt, closed cup 213°F, open cup 220°F. Autoignition temp 817°F. One gram dissolves in about 25 ml water. One volume dissolves in 1.5 vols of 50% ethyl alcohol. Misc with abs and 94% alcohol, ether, chloroform. LD_{50} orally in rats: 3.1 g/kg (Smyth).

USE: Manuf other benzyl compds. Pharmaceutic aid (antimicrobial). Solvent for gelatin, casein (when hot), solvent for cellulose acetate, shellac. Used in perfumery and in flavoring (mostly in form of its aliphatic esters). In microscopy as embedding material.

THERAP CAT (VET): Has been used for relief from pruritis.

1160. Benzylamine. *Benzenemethanamine;* aminotoluene; phenylmethylamine; moringine. C_7H_9N; mol wt 107.16. C 78.46%, H 8.47%, N 13.07%. Prepn from benzylchloride and ammonia: Mason, *J. Chem. Soc.* 63, 1311 (1893); by redn of benzonitrile: Carothers, Jones, *J. Am. Chem. Soc.* 47, 3051 (1925); from benzyl bromide + acetamide: Erikson, *Ber.* 59, 2665 (1926); from *N*-benzylphthalimide + hydrazine hydrate: Ing, Manske, *J. Chem. Soc.* 129, 2348 (1926). Identity with moringine: Chakravarti, *Bull. Calcutta School Trop. Med.* 3, 162 (1955); *C.A.* 50, 16891e (1956).



Liquid; strongly alkaline reaction. bp 185°; bp_{12} 90°. d^{19}_4 0.983. n^{20}_D 1.5401. Miscible with water, alcohol, ether.

Hydrochloride, $C_7H_9N.HCl$, crystals, mp 253°.

Hydroiodide, $C_7H_9N.HI$, leaflets, mp 162°.

Caution: Highly irritating to skin, mucous membranes.

USE: In organic synthesis.

1161. Benzylaniline. *N-Phenylbenzenemethanamine; N-phenylbenzylamine;* benzylphenylamine. $C_{13}H_{13}N$; mol wt 183.25. C 85.21%, H 7.15%, N 7.64%. $C_6H_5CH_2NHC_6H_5$. Prepn from benzyl alc and aniline in the presence of KOH: Sprinzak, *J. Am. Chem. Soc.* 78, 3207 (1956); from benzaldehyde and aniline in the presence of $NaBH_4$: Schellenberg, *J. Org. Chem.* 28, 3259 (1963).

Prisms, mp 37-38°. bp 306-307°. Practically insol in water; sol in alcohol, chloroform, ether.

1162. Benzyl Benzoate. *Benzoic acid phenylmethyl ester;* benzoic acid benzyl ester; benzylbenzenecarboxylate; Ascabin; Venzonate; Ascabiol. $C_{14}H_{12}O_2$; mol wt 212.25. C 79.23%, H 5.70%, O 15.08%. $C_6H_5COOCH_2C_6H_5$. Contained in Peru and Tolu balsams. Prepd by the action of sodium benzylate on benzaldehyde: Kamm, Kamm, *Org. Syn.* coll. vol. 1, 104 (2nd ed., 1941); by the dry esterification of sodium benzoate and benzyl chloride in the presence of triethylamine: Thorp, Nottorf, *Ind. Eng. Chem.* 39, 1300 (1947). Toxicity studies: Graham, Kuizenga, *J. Pharmacol. Exp. Ther.* 84, 358 (1945); Draize *et al., J. Pharmacol. Exp. Ther.* 93, 26 (1948). Comprehensive description: M. M. A.

Hassan, J. S. Mossa, *Anal. Profiles Drug Subs.* **10**, 55-74 (1981).

Leaflets or oily liq; faint, pleasant, aromatic odor; sharp burning taste. mp 21°. d_4^{25} 1.118. bp 323-324°. bp_{16} 189-191°. $bp_{4.5}$ 156°. Sparingly volatile with steam. n_D^{21} 1.5681. Insol in water or glycerol. Miscible with alc, chloroform, ether, oils. LD_{50} in rats, mice, rabbits, guinea pigs (g/kg): 1.7, 1.4, 1.8, 1.0 orally (Draize).

Caution: In exptl animals, ingestion causes progressive incoordination, excitation, convulsions, death. May cause skin irritation in humans. Avoid contact with eyes, *Clinical Toxicology of Commercial Products*, R. E. Gosselin *et al.*, Eds. (Williams & Wilkins, Baltimore, 4th ed., 1976) Section II, p 137.

USE: As solvent of cellulose acetate, nitrocellulose and artificial musk; substitute for camphor in celluloid and plastic pyroxylin compds; perfume fixative; in confectionery and chewing gum flavors.

THERAP CAT: Scabicide, pediculicide.

THERAP CAT (VET): Acaricide, pediculicide. *Contraindicated* in cats.

1163. Benzyl Bromide. *(Bromomethyl)benzene; α-bromotoluene; ω-bromotoluene.* C_7H_7Br; mol wt 171.04. C 49.16%, H 4.13%, Br 46.72%. $C_6H_5CH_2Br$. Prepd by the action of bromine on toluene in ultraviolet light: v. Konek, Loczka, *Ber.* **57**, 679 (1924); Zelinsky, Ger. pat. 478,084; *Chem. Zentr.* **1929 II**, 1216; *Frdl.* **16**, 335; by the action of bromine on dibenzyl ether: Lachman, *J. Am. Chem. Soc.* **45**, 2359 (1923).

Lacrimatory liquid. mp −3.9°. bp 198-199°. bp_{80} 127°. d_0^{22} 1.4380; d^{17} 1.443; d_4^{44} 1.3886. Slowly decomp by water.

Caution: Intensely irritating to skin, eyes, mucous membranes. Large doses cause CNS depression.

1164. Benzyl Chloride. *(Chloromethyl)benzene; α-chlorotoluene.* C_7H_7Cl; mol wt 126.59. C 66.42%, H 5.57%, Cl 28.01%. $C_6H_5CH_2Cl$. Made by cautious chlorination of toluene: A. I. Vogel, *Practical Organic Chemistry* (Longmans, London, 3rd ed., 1959) p 538; Gattermann-Wieland, *Praxis des organischen Chemikers* (de Gruyter, Berlin, 40th ed., 1961) p 92. Manuf: Faith, Keyes & Clark's *Industrial Chemicals*, F. A. Lowenheim, M. K. Moran, Eds. (Wiley-Interscience, New York, 4th ed., 1975) pp 145-148.

Very refractive liquid; rather unpleasant, irritating odor. d_{20}^{20} 1.100. bp 179°. mp −48° to −43°. n_D^{15} 1.5415. Insol in water. Miscible with alcohol, chloroform, ether. Rapidly dec when heated in the presence of iron.

Caution: Potential symptoms of overexposure are irritation of eyes and nose; weakness; irritability; headache; skin eruption; pulmonary edema. *See NIOSH Pocket Guide to Chemical Hazards* (DHHS/NIOSH 90-117, 1990) p 46.

USE: Manuf benzyl compds, perfumes, pharmaceutical products, dyes, synthetic tannins, artificial resins.

1165. Benzyl Cinnamate. *3-Phenyl-2-propenoic acid phenylmethyl ester; trans-cinnamic acid benzyl ester;* cinnamein. $C_{16}H_{14}O_2$; mol wt 238.29. C 80.65%, H 5.92%, O 13.43%. $C_6H_5CH{=}CHCOOCH_2C_6H_5$. Constituent of storax, Peru and Tolu balsams: Tschirch, Trog, *Arch. Pharm.* **232**, 70 (1894); Tschirch, Oberländer, *ibid.* 559. Prepn: Volwiler, Vliet, *J. Am. Chem. Soc.* **43**, 1672 (1921); Eliel, Anderson, *ibid.* **74**, 547 (1952); Bender, Zerner, *ibid.* **84**, 2550 (1962). Toxicity study: P. M. Jenner *et al.*, *Food Cosmet. Toxicol.* **2**, 327 (1964).

Crystals from 95% ethanol; sweet odor of balsam. mp 39°; also reported as mp 33-34° (Volwiler, Vliet). Dec on distillation at ordinary pressure; $bp_{0.5}$ 154-157°, bp_5 195-200°, bp_{22} 228-230°. Practically insol in water, propylene glycol and glycerin. Sol in alc, ether, oils. LD_{50} orally in rats: 5530 mg/kg (Jenner).

USE: In artificial flavors, in perfumes, mainly as a fixative.

1166. Benzyl Cyanide. *Benzeneacetonitrile;* phenylacetonitrile; α-tolunitrile; ω-cyanotoluene. C_8H_7N; mol wt 117.15. C 82.02%, H 6.02%, N 11.96%. $C_6H_5CH_2CN$. Occurs in garden cress and other plants; made from benzyl chloride, and NaCN: Adams, Thal, *Org. Syn.* vol. 2, 9 (1922); coll. vol. I, 101 (107 in 2nd ed.).

Oily liquid, aromatic odor. d_{15}^{15} 1.0214. mp −23.8°. bp_{760} 233.5°; bp_{100} 161.8°; bp_{20} 119.4°; $bp_{1.9}$ 60°. n_D^{25} 1.52105. Insoluble in water, miscible with alc, ether.

1167. Benzyl Ether. *1,1'-[Oxybis(methylene)]bis[benzene];* dibenzyl ether. $C_{14}H_{14}O$; mol wt 198.26. C 84.81%, H 7.12%, O 8.07%. $(C_6H_5CH_2)_2O$. Prepd: Lachman, *J. Am. Chem. Soc.* **45**, 2356 (1923); Staab, Wendel, *Ber.* **93**, 2902 (1960); Lichtenberger, Tritsch, *Bull. Soc. Chim. France* **1961**, 363. Manuf by reduction of benzaldehyde in the presence of $[Co(CO)_4]_2$: Wender, Orchin, U.S. pat. 2,614,-107 (1952 to U.S.A. as represented by the Secy. of Agr.). Physical properties: Svirbely *et al.*, *J. Am. Chem. Soc.* **71**, 507 (1949); Dreisbach, Martin, *Ind. Eng. Chem.* **41**, 2875 (1949). Miscibility: Jackson, Drury, *ibid.* **51**, 1491 (1959).

Unstable liquid, bp 295-298° (with dec), bp_{21} 173-174°; bp_2 125.5-126.5°. Appears to dec slowly at ordinary temps. d^{35} 1.0341; d_4^{25} 0.99735; d_4^{20} 1.00142; d^{15} 1.0482. n_D^{25} 1.5601 (Svirbely *et al.*), 1.53851 (Dreisbach, Martin); n_D^{20} 1.54057 (Dreisbach, Martin), 1.566 (Lichtenberger, Tritsch). Practically insol in water; miscible with ethanol, ether, chloroform, acetone.

USE: Plasticizer for nitrocellulose; solvent in perfumery.

1168. Benzyl Ethyl Ether. *(Ethoxymethyl)benzene.* $C_9H_{12}O$; mol wt 136.19. C 79.37%, H 8.88%, O 11.75%. $C_6H_5CH_2OC_2H_5$. Preparation from sodium ethoxide and benzyl bromide: Letsinger, Pollart, *J. Am. Chem. Soc.* **78**, 6079 (1956); by reduction of benzaldehyde diethyl acetal with $LiAlH_4$-$AlCl_3$: Eliel, Rerick, *J. Org. Chem.* **23**, 1088 (1958).

Oily liquid, aromatic odor. bp 186°; bp_{10} 65°. d 0.949, n_D^{20} 1.4955. Volatile with steam. Practically insol in water; miscible with alcohol, ether.

1169. Benzyl Formate. *Formic acid phenylmethyl ester; formic acid benzyl ester.* $C_8H_8O_2$; mol wt 136.15. C 70.57%, H 5.92%, O 23.50%. $HCOOCH_2C_6H_5$. Prepn from formic acid and benzyl alcohol: Mailhe, *Chem. Ztg.* **35**, 508 (1911).

Liquid; pleasant fruity odor. d 1.081. bp 203°. Practically insol in water. Sol in alcohol.

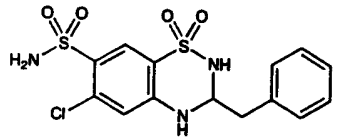
USE: Solvent for cellulose esters; in perfumery.

1170. Benzyl Fumarate. *(E)-2-Butenedioic acid bis(phenylmethyl) ester; fumaric acid dibenzyl ester;* dibenzyl fumarate. $C_{18}H_{16}O_4$; mol wt 296.32. C 72.96%, H 5.44%, O 21.60%. $C_6H_5CH_2OOCCH{=}CHCOOCH_2C_6H_5$. Prepd from fumaric acid and benzyl alcohol: Volwiler, Vliet, *J. Am. Chem. Soc.* **43**, 1672 (1921).

Cryst powder, mp 58.5-59.5°. bp_5 210-211°. Practically insol in water. Sol in alcohol, chloroform, ether, oils.

USE: In room spray deodorant: Kulka, U.S. pat. 3,077,-457 (1963 to Fritzsche Bros.).

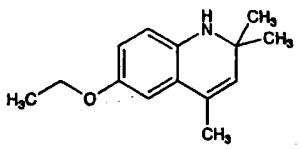
1171. Benzylhydrochlorothiazide. *6-Chloro-3,4-dihydro-3-(phenylmethyl)-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; 3-benzyl-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide;* 6-chloro-7-sulfamoyl-3-benzyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide; 3-benzyl-6-chloro-3,4-dihydro-7-sulfamoyl-1,2,4-benzothiadiazine 1,1-dioxide; Behyd. $C_{14}H_{14}ClN_3O_4S_2$; mol wt 387.87. C 43.35%, H 3.64%, Cl 9.14%, N 10.83%, O 16.50%, S 16.53%. Prepn: Werner *et al.*, *J. Am. Chem. Soc.* **82**, 1161 (1960); Novello *et al.*, *J. Org. Chem.* **25**, 970 (1960); Ugi, U.S. pat. 3,108,097 (1963).



Crystals from acetic acid + water, mp 260-262°. Also reported as crystals from water, mp 269°.

THERAP CAT: Antihypertensive; diuretic.

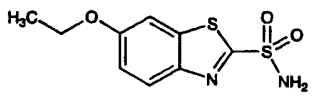
1172. Benzylideneacetone. *4-Phenyl-3-buten-2-one;* benzalacetone; methyl styryl ketone; cinnamyl methyl ketone; acetocinnamone. $C_{10}H_{10}O$; mol wt 146.19. C 82.16%, H 6.89%, O 10.94%. $C_6H_5CH{=}CHCOCH_3$. Prepd by con-



Yellow liquid. bp_2 123-125°. n_D^{25} 1.569-1.672. d_{25}^{25} 1.029-1.031. LD_{50} orally in rats, mice: 1920, 1730 mg/kg (Piul'skaya).

USE: Antioxidant in feed and food; antidegradation agent for rubber.

3801. Ethoxzolamide. *6-Ethoxy-2-benzothiazalesulfonamide;* ethoxyzolamide; Cardrase; Ethamide; Glaucotensil; Redupresin. $C_9H_{10}N_2O_3S_2$; mol wt 258.32. C 41.85%, H 3.90%, N 10.84%, O 18.58%, S 24.83%. Carbonic anhydrase inhibitor. Prepn: Brit. pat. 795,174; J. Korman, U.S. pat. 2,868,800 (1958, 1959 both to Upjohn).

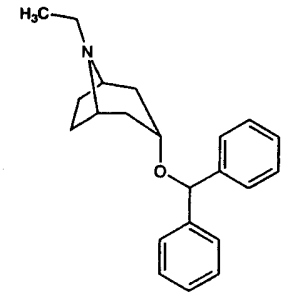


Crystals from ethyl acetate + Skellysolve B, mp 188-190.5°.

THERAP CAT: Diuretic.

THERAP CAT (VET): Diuretic.

3802. Ethybenztropine. *endo-3-(Diphenylmethoxy)-8-ethyl-8-azabicyclo[3.2.1]octane; 3α-(diphenylmethoxy)-8-ethyl-1αH,5αH-nortropane; N*-ethylnortropine benzhydryl ether; tropethydrylin; *N*-ethyl-8-aza-3-bicyclo[3.2.1]octyl benzhydryl ether; *N*-ethylbenztropine; ethylbenzatropine. $C_{22}H_{27}NO$; mol wt 321.46. C 82.20%, H 8.47%, N 4.36%, O 4.98%. Prepn: Brit. pat. 804,837 (1958 to Sandoz); Boehringer *et al.*, Brit. pat. 824,875 (1959 to Boehringer, Ing.). Used as hydrochloride or hydrobromide salts.



Hydrochloride, $C_{22}H_{27}NO.HCl$, *Ponalid.* Crystals from acetone, mp 190-191°.

Hydrobromide, $C_{22}H_{27}NO.HBr$, *Panolid.* Crystals from methanol + ether, mp 226-228°.

THERAP CAT: Anticholinergic.

3803. Ethyl Acetate. *Acetic acid ethyl ester;* acetic ether; vinegar naphtha. $C_4H_8O_2$; mol wt 88.11. C 54.53%, H 9.15%, O 36.32%. $CH_3COOC_2H_5$. Obtained by the slow distillation of a mixture of acetic acid, ethyl alc, and sulfuric acid: Alheritiere, Mercier, U.S. pat. 2,787,636 (1957 to Usines de Melle); Faith, Keyes, & Clark's *Industrial Chemicals,* F. A. Lowenheim, M. K. Moran, Eds. (Wiley-Interscience, New York, 4th ed., 1975) pp 350-354. Toxicity: H. F. Smyth *et al., Am. Ind. Hyg. Assoc. J.* 23, 95 (1962).

Clear, volatile, flammable liq; characteristic fruity odor; pleasant taste when diluted. Slowly dec by moisture, then acquires an acid reaction. Absorbs water (up to 3.3% w/w). d_4^{20} 0.902; d_{25}^{25} 0.898. bp 77°. mp −83°. Flash pt +7.2° (open cup). Ignition temp 800°F. Explosive limits (% vol in air): 2.2 to 11.5. n_D^{20} 1.3719. Vapor density 3.04 (air = 1). One ml dissolves in 10 ml water at 25°; more sol at lower and less sol at higher temps. Misc with alc, acetone, chloroform, ether. Azeotropic mixture with water (6.1% w/w) bp 70.4°. Azeotropic mixture with water (7.8% w/w) and alc (9.0% w/w) bp 70.3°. *Keep tightly closed in a cool place and away from fire.* LD_{50} orally in rats: 11.3 ml/kg (Smyth).

Caution: Potential symptoms of overexposure are irritation of eyes, nose and throat; narcosis; dermatitis. *See NIOSH Pocket Guide to Chemical Hazards* (DHHS/NIOSH 90-117, 1990) p 104.

USE: Pharmaceutic aid (flavor); artificial fruit essences; solvent for nitrocellulose, varnishes, lacquers, and aeroplane dopes; manuf smokeless powder, artificial leather, photographic films and plates, artificial silk, perfumes; cleaning textiles, etc.

3804. Ethyl Acetoacetate. *3-Oxobutanoic acid ethyl ester;* acetoacetic acid ethyl ester; acetoacetic ester; ethyl 3-oxobutanoate. $C_6H_{10}O_3$; mol wt 130.14. C 55.37%, H 7.74%, O 36.88%. $CH_3COCH_2COOC_2H_5$. Only the equilibrium mixture of the keto and enol forms is described here. Prepd from ethyl acetate by the action of sodium, sodium ethoxide, sodamide, or calcium: Inglis, Roberts, *Org. Syn.* coll. vol. I, 235 (2nd ed., 1941); Hansley, Schott, U.S. pat. 2,843,623 (1958 to Natl. Distillers); Scheibler, *Ann.* 565, 176 (1949); Gattermann-Wieland, *Praxis des Organischen Chemikers* (de Gruyter, Berlin, 40th ed., 1961) p 218. Discussion of keto-enol tautomerism: Ward, *J. Chem. Ed.* 39, 95 (1962). Toxicity study: H. F. Smyth *et al., J. Ind. Hyg. Toxicol.* 31, 60 (1949).

Liq. Agreeable odor. d_4^{10} 1.0357; d_4^{17} 1.0288; d_4^{25} 1.0213; d_4^{54} 0.9924; d_4^{75} 0.9703. mp −45°. bp_{760} 180.8°; bp_{400} 158.2°; bp_{200} 138.0°; bp_{60} 106°; bp_{20} 81.1°; bp_5 54.0°; $bp_{1.0}$ 28.5°. n_D^{20} 1.41937. Absorption spectrum: Morton, Rosney, *J. Chem. Soc.* 1926, 711. Flash pt, closed cup: 184°F. Sol in about 35 parts water; misc with the usual organic solvents. LD_{50} orally in rats: 3.98 g/kg (Smyth).

Caution: Moderately irritating to skin, mucous membranes.

3805. Ethyl Acrylate. *2-Propenoic acid ethyl ester;* acrylic acid ethyl ester. $C_5H_8O_2$; mol wt 100.12. C 59.98%, H 8.05%, O 31.96%. $CH_2{=}CHCOOCH_2CH_3$. Prepd from ethylene chlorohydrin or acrylonitrile, ethanol, and sulfuric acid; also by an oxo reaction from acetylene, carbon monoxide, and ethanol in the presence of suitable catalysts. *See* the refs under Methyl Acrylate.

Monomer, liquid, acrid, penetrating odor, retained by clothing. *Lacrimator.* d_4^{20} 0.9405. fp below −72°. bp_{760} 99.4°; $bp_{39.2}$ 20° (polymerizes on distn). n_D^{20} 1.404. Specific heat at −60°: 0.442 cal/g/°C. Heat of vaporization 8.27 kcal/mol; heat of combustion 655.49 kcal/mol. Flash pt, open cup: 60°F (15°C). Vapor density 3.45 (air = 1). Soly in water at 20°: 2 g/100 ml. Soly of water in ethyl acrylate at 20°: 1.5 g/100 g. Sol in alcohol, ether. Azeotropes: 45.0% water = bp 81°; 56.8% ethanol = bp 76°. Easily polymerizes on standing; polymerization process speeded up by heat, light, and peroxides. If pure, the monomer can be stored below +10° without incurring polymerization.

Polymer, transparent, elastic substance. Practically no odor. Little adhesive power. Resists the usual solvents.

Caution: Potential symptoms of overexposure to the monomer are irritation of eyes, respiratory system and skin. *See NIOSH Pocket Guide to Chemical Hazards* (DHHS/NIOSH 90-117, 1990) p 106. *See also Patty's Industrial Hygiene and Toxicology* vol. 2A, G. D. Clayton, F. E. Clayton, Eds. (Wiley-Interscience, New York, 3rd ed., 1981) p 2292-2296. This substance may reasonably be anticipated to be a carcinogen: *Seventh Annual Report on Carcinogens* (PB95-109781, 1994) p 203.

USE: The monomer in the manuf of water emulsion paint vehicles; in production of emulsion-based polymers used in textile and paper coatings, leather finish resins and adhesives. Imparts flexibility to hard films.

3806. Ethyl Alcohol. *Ethanol;* absolute alcohol; anhydrous alcohol; dehydrated alcohol; ethyl hydrate; ethyl hydroxide. C_2H_6O; mol wt 46.07. C 52.14%, H 13.13%, O 34.73%. C_2H_5OH. Manuf: by fermentation of starch, sugar, and other carbohydrates; from ethylene, acetylene, sulfite waste liquors, and synthesis gas (CO + H); by hydrolysis of ethyl sulfate, and oxidation of methane. Toxicity: G. S. Wiberg *et al., Toxicol. Appl. Pharmacol.* 16, 718 (1970). Embryotoxicity in mammals: N. A. Brown *et al.,*

Science **206,** 573 (1979). Possible mechanism for actions of ethanol on the brain: G. Aston-Jones *et al., Nature* **296,** 857 (1982). Ethanol-induced chromosomal abnormalities in mice: M. H. Kaufman, *ibid.* **302,** 258 (1983). Disruption of reproductive function in female primates following alcohol self-administration: N. K. Mello *et al., Science* **221,** 677 (1983). Review of metabolism and toxicity: C. S. Lieber in *Reviews in Biochemical Toxicology* vol. 5, E. Hodgson *et al.,* Eds. (Elsevier, New York, 1983) pp 267-312; of pharmacology: L. Pohorecky, J. Brick, *Pharmacol. Ther.* **36,** 335-427 (1988); of hepatotoxicity: C. S. Lieber, L. M. DeCarli, *J. Hepatol.* **12,** 394-401 (1991). General reviews: P. Baud, "Ethyl Alcohol Industry" in Grignard, *Traité de Chimie Organique* vol. 5 (Masson, 1937) pp 841-975; Zabel, *Chem. Inds.* (now *Chem. Week*) **64,** 212 (1949); Faith, Keyes & Clark's *Industrial Chemicals,* F. A. Lowenheim, M. K. Moran, Eds. (Wiley-Interscience, New York, 4th ed., 1975) pp 355-364; P. D. Sherman, P. R. Kavasmaneck, "Ethanol" in Kirk-Othmer *Encyclopedia of Chemical Technology* vol. 9 (Interscience, New York, 3rd ed., 1980) pp 338-380.

Clear, colorless, very mobile, flammable liquid; pleasant odor; burning taste. Absorbs water rapidly from air. d_4^{20} 0.789. bp 78.5°. mp −114.1°. n_D^{20} 1.361. Flash pt, closed cup: 13°C. Miscible with water and with many organic liquids. *Keep tightly closed, cool, and away from flame!* LD_{50} in young, old rats (g/kg): 10.6, 7.06 orally (Wiberg).

The terms *95% alcohol* and *alcohol* (when used alone) refer to a binary azeotrope having a distillate composition of 95.57% ethyl alcohol (by wt) and bp 78.15°. *Alcohol, USP* is specified as containing not less than 92.3% and not more than 93.8% by weight, corresponding to not less than 94.9% and not more than 96.0% by vol of C_2H_5OH at 15.56°. d_{25}^{25} 0.810; d 0.816 at 15.56° (60°F). *Diluted alcohol,* prepd from equal vols 95% alcohol and water, contains about 41.5% by wt or about 48.9% by vol of C_2H_5OH. d_{25}^{25} 0.931; d 0.936 at 15.56° (60°F). *See U.S.P.* XXI, 22, 1530 (1985).

Caution: Nausea, vomiting, flushing, mental excitement or depression, drowsiness, impaired perception, incoordination, stupor, coma, death may occur, *cf. Clinical Toxicology of Commercial Products,* R. E. Gosselin *et al.,* Eds. (Williams & Wilkins, Baltimore, 5th ed., 1984) Section III, pp 166-171.

USE: Most ethyl alcohol is used in alcoholic beverages in suitable dilutions. Other uses are as solvent in laboratory and industry, in the manufacture of denatured alcohol, pharmaceuticals (rubbing compds, lotions, tonics, colognes), in perfumery, in organic synthesis. Octane booster in gasoline. Pharmaceutic aid (solvent).

THERAP CAT: Antiseptic.

THERAP CAT (VET): Antiseptic. To destroy nerve tissue. Solvent and dehydrating agent.

3807. Ethyl Alcohol, Denatured. Denatured alcohol. Ethyl alcohol to which has been added some substance or substances which, while allowing the use of the alcohol in the most varied industries and arts, renders it entirely unfit for consumption as a beverage. The most commonly used denaturants, either alone or in combination, are the following: Methanol, camphor, Aldehol, amyl alcohol, gasoline, isopropanol, terpineol, benzene, castor oil, acetone, nicotine, aniline dyes, ether, cadmium iodide, pyridine bases, sulfuric acid, kerosene, diethyl phthalate. *Formula 1* is 5 gallons approved wood alcohol added to 100 gal of 95% ethanol. *Formula 2B* is 0.5 gal benzene added to 100 gal of 95% ethanol. Similarly *formula 3A* contains 5 gal commercial methanol, *formula 6B* contains 0.5 gal pyridine bases, *formula 12A* 5 gal benzene, *formula 13A* 10 gal ethyl ether, *formula 19* 4 gal methyl isobutyl ketone and 1 gal kerosene, *formula 20* 5 gal crude chloroform, *formula 23A* 10 gal acetone, *formula 28* 10 gal benzene, *formula 28A* 1 gal gasoline, *formula 30* 10 gal methanol, *formula 32* 5 gal ethyl ether, *formula 33* 30 lbs methyl violet, *formula 35A* 5 gal ethyl acetate, *formula 39C* 1 gal diethyl phthalate; *formula 44* contains 20 gal *n*-butanol. Additional permissible formulas are given in *Appendix to Regulations No. 3, Formulae for Completely and Specially Denatured Alcohol,* published by the U.S. Treasury Dept., Bureau of Industrial Alcohol. Reprinted in N. A. Lange, *Handbook of Chemistry.*

Caution: Denaturants, particularly methanol, may modify and increase toxic symptoms caused by ingestion and exposure to fumes.

3808. Ethylamine. *Ethanamine;* monoethylamine; aminoethane. C_2H_7N; mol wt 45.08. C 53.28%, H 15.65%, N 31.07%. $C_2H_5NH_2$. Prepn from ethyliodide + liq ammonia: Watt, Otto, *J. Am. Chem. Soc.* **69,** 836 (1947); from ethanol + ammonia: Davies *et al.,* U.S. pat. **2,609,394** (1952 to ICI); Lemon, Myerly, U.S. pat. **3,022,349** (1962 to Union Carbide). Toxicity study: H. F. Smyth *et al., Arch. Ind. Hyg. Occup. Med.* **10,** 61 (1954).

Flammable, liq; ammonia odor; strong alkaline reaction. d_{15}^{15} 0.689. bp 16.6°. Solidif −80°. Miscible with water, alcohol, ether. *Keep tightly closed and in cold place.* LD_{50} orally in rats: 0.40 g/kg (Smyth).

Hydrochloride, $C_2H_7N.HCl$, crystals from ethanol + water, mp 110°. d 1.22. Soluble in 0.4 part water; freely sol in alcohol; slightly sol in chloroform or acetone. Practically insol in ether. *Keep well closed.*

Hydriodide, $C_2H_7N.HI$, hygroscopic crystals, mp 188°. d 2.10. Freely sol in water or alcohol. Practically insol in chloroform, ether. *Keep well closed and protected from light.*

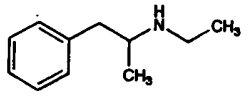
Oleate, $C_2H_7N.C_{18}H_{34}O_2$, *ethanamine (Z)-9-octadecenoate, Etalate.* Commercial prepn is a 5% soln with 2% benzyl alcohol as anodyne.

Caution: Potential symptoms of overexposure are irritation of eyes; skin burns; respiratory irritation; dermatitis. *See NIOSH Pocket Guide to Chemical Hazards* (DHHS/NIOSH 90-117, 1990) p 106.

USE: In resin chemistry; stabilizer for rubber latex; intermediate for dyestuffs, medicinals; in oil refining; in organic syntheses.

THERAP CAT: Oleate as a sclerosing agent.

3809. *N*-Ethylamphetamine. *N-Ethyl-α-methylbenzeneethanamine; N-ethyl-α-methylphenethylamine; N*-ethyl-ω-phenylisopropylamine; 2-ethylamino-1-phenylpropane; Adiparthrol; Apetinil. $C_{11}H_{17}N$; mol wt 163.26. C 80.93%, H 10.50%, N 8.58%. Prepn: Keil, Dobke, Ger. pat. **767,263** (1952 to Theodor H. Temmler); Leonard *et al., J. Am. Chem. Soc.* **80,** 4858 (1958). Separation of isomers: **Brit. pat. 814,339** (1959 to Sterling Drug).



bp_{14} 104.5-106°. n_D^{25} 1.4986.

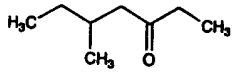
d-Form hydrochloride, $C_{11}H_{17}N.HCl$, mp 154-156°. $[\alpha]_D^{25}$ +17.2° (c = 2 in water).

l-Form hydrochloride, $C_{11}H_{17}N.HCl$, mp 155-156°. $[\alpha]_D^{25}$ −17.3° (c = 2 in water).

Note: This is a controlled substance (stimulant) listed in the U.S. Code of Federal Regulations, Title 21 Part 1308.11 (1995).

THERAP CAT: Anorexic.

3810. Ethyl Amyl Ketone. *5-Methyl-3-heptanone;* amyl ethyl ketone; EAK. $C_8H_{16}O$; mol wt 128.21. C 74.94%, H 12.58%, O 12.48%. *Review:* Buller, *Ind. & Eng. Chem.* **48,** 1323 (1956).



Liquid. Mild fruity odor. d_{20}^{20} 0.820-0.824. One gallon weighs 6.83 lbs at 20°. bp_{760} 157-162°. Flash pt 59° (138°F). Evaporation rate 0.3 (*n*-butyl acetate = 1.0). n_D^{15} 1.4195. Slightly miscible with water. Compatible with alcohols, ketones, ethers, many other organic solvents.

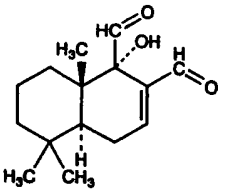
Caution: Narcotic in high concns.

USE: Solvent for nitrocellulose-alkyd, nitrocellulose-maleic, and vinyl resins.

3811. Ethylaniline. *N-Ethylbenzenamine;* ethylphenylamine. $C_8H_{11}N$; mol wt 121.18. C 79.29%, H 9.15%, N

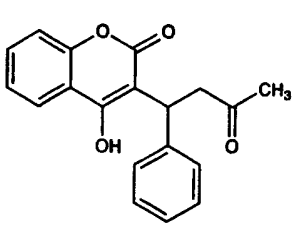
W

10173. Warburganal. *[1S-(1α,4aα,8aβ)]-1,4,4a,5,6,7,8,-8a-Octahydro-1-hydroxy-5,5,8a-trimethyl-1,2-naphthalenedicarboxaldehyde.* $C_{15}H_{22}O_3$; mol wt 250.34. C 71.97%, H 8.86%, O 19.17%. Drimane sesquiterpene with antifeedant activity against the African army worm. Biological activity includes plant growth regulation, cytotoxic, antimicrobial and molluscicidal properties. Isoln from *Warburgia ugandensis, Canellaceae* and structure: I. Kubo *et al., Chem. Commun.* **1976,** 1013. Relationship between structure and antifeedant activity: K. Nakanishi, I. Kubo, *Isr. J. Chem.* **16,** 28 (1977). Synthesis of (±)-warburganal: S. P. Tanis, K. Nakanishi, *J. Am. Chem. Soc.* **101,** 4398 (1979); T. Nakata *et al., ibid.* 4400; A. S. Kende, T. J. Blacklock, *Tetrahedron Letters* **1980,** 3119; P. A. Wender, S. L. Eck, *ibid.* **1982,** 1871; D. M. Hollinshead *et al., J. Chem. Soc. Perkin Trans. I* **1983,** 1579. *See also:* **Japan. Kokai 80 136,238,** and **80 136,240** (both 1980 to Inst. Phys. Chem. Res.); **Japan. Kokai 81 43,236** (1981 to Suntory Ltd.); **Japan. Kokai 83 38,232** (1983 to Teikoku Zoki). Synthesis of (−)-warburganal: H. Okawara *et al., Tetrahedron Letters* **1982,** 1087.



mp 98-99°. uv max (methanol): 224 nm (ε 6300). $[\alpha]_D^{21}$ −260° (c = 0.350 in $CHCl_3$).

10174. Warfarin. *4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one; 3-(α-acetonylbenzyl)-4-hydroxycoumarin;* 1-(4'-hydroxy-3'-coumarinyl)-1-phenyl-3-butanone; 3-α-phenyl-β-acetylethyl-4-hydroxycoumarin; compound 42; WARF compound 42; Co-Rax; Rodex. $C_{19}H_{16}O_4$; mol wt 308.33. C 74.01%, H 5.23%, O 20.76%. The commercial product is the racemic mixture; the *S*(−)-form is more active than the *R*-isomer. Prepd by the Michael condensation of benzylidene-acetone with 4-hydroxycoumarin: Stahmann *et al.,* U.S. pat. **2,427,578** (1947); Schroeder, Link, U.S. pat. **2,765,321** (1956 to Wisconsin Alumni Res. Found.); Link, U.S. pat. **2,777,859** (1957). Resolution and abs configuration: West *et al., J. Am. Chem. Soc.* **83,** 2676 (1961); Preis, *Dissertation Abstr.* **18,** 793 (1958); Preis *et al.,* U.S. pat. **3,239,529** (1966 to Wisconsin Alumni Res. Found.). Mechanism of action: Bell *et al., Biochemistry* **11,** 1959 (1972). Conformation in soln: E. J. Valente *et al., J. Med. Chem.* **20,** 1849 (1977); **21,** 141, 231 (1978). Human metabolism: R. J. Lewis, W. F. Trager, *Ann. N.Y. Acad. Sci.* **179,** 205 (1971). Stereospecific HPLC determn in plasma: C. Banfield, M. Rowland, *J. Pharm. Sci.* **72,** 921 (1983). Antimetastatic effect in lung cancer: L. R. Zacharski *et al., Cancer* **53,** 2046 (1984); in rat adenocarcinoma: B. L. Neubauer *et al., J. Urol.* **135,** 163 (1986). Toxicity studies: E. C. Hagan, J. L. Radomski, *J. Am. Pharm. Assoc.-Sci. Ed.* **42,** 379 (1953); N. Back *et al., Pharmacol. Res. Commun.* **10,** 445 (1978). Review of therapeutic uses: J. V. Lloyd, *Med. J. Aust.* **142,** 197-201 (1985); and pharmacology: J. Hirsh *et al., Chest* **102,** Suppl., 312S-326S (1992). Comprehensive description: S. A. Babhair *et al., Anal. Profiles Drug Subs.* **14,** 423-452 (1985).



Crystals from alc, mp 161°. uv max (water, pH 10): 308 nm (ε 13610). Soluble in acetone, dioxane. Moderately sol in methanol, ethanol, isopropanol, some oils. Freely sol in alkaline aq solns (forms a water-soluble sodium salt). Practically insol in water, benzene, cyclohexane, Skellysolves A and B. Warfarin has an acidic enol which forms metallic salts and an acetate, mp 117-118°, and a ketone which forms an oxime, mp 182-183° and a 2,4-dinitrophenylhydrazone, mp 215-216°.

Sodium salt, $C_{19}H_{15}NaO_4$, *Coumadin, Marevan, Panwarfin, Prothromadin, Tintorane, Warfilone, Waran.* Slightly bitter, crystalline powder. Discolored by light. Very sol in water; freely sol in alcohol; very slightly sol in chloroform, ether. LD_{50} in male rats, female rats, mice, rabbits (mg/kg): 323, 58, 374, ~800 orally (Hagen); also reported as LD_{50} in male, female rats (mg/kg): 100.3, 8.7 orally (Back).

Potassium salt, $C_{19}H_{15}KO_4$, *Athrombin-K.*

Compound with 2-(dimethylamino)ethanol, $C_{23}H_{27}NO_5$, *warfarin-deanol, MD-6134, Adoisine.*

Caution: Potential symptoms of overexposure are hematuria, back pain; hematoma of arms and legs; epistaxis, bleeding lips and mucous membrane hemorrhage; abdominal pain, vomiting and fecal blood; petechial rash; abnormal hematologic indices. *See NIOSH Pocket Guide to Chemical Hazards* (DHHS/NIOSH 90-117, 1990) p 224. *See also Clinical Toxicology of Commercial Products,* R. E. Gosselin *et al.,* Eds. (Williams & Wilkins, Baltimore, 5th ed., 1984) Section III, pp 395-397.

USE: Rodenticide.

THERAP CAT: Anticoagulant.

10175. Water. Hydrogen oxide. H_2O; mol wt 18.02. H 11.19%, O 88.81%. *Reviews:* N. E. Dorsey, *Properties of Ordinary Water-Substance,* A.C.S. Monograph Series no. **81,** (Reinhold, New York, 1940) 673 pp; D. Eisenberg, W. Kauzmann, *The Structure and Properties of Water* (Oxford University Press, New York, 1969) 296 pp; Ebsworth *et al.,* in *Comprehensive Inorganic Chemistry* **vol. 2,** J. C. Bailar, Jr. *et al.,* Eds. (Pergamon Press, Oxford, 1973) pp 741-747.

Pyrogen-free water (water for injection) is distilled water rendered free of fever-producing proteins (bacteria and their metabolic products). Method of prepn: Ishizuka *et al., C.A.* **49,** 15177 (1955).

Liquid. Temp of max density 3.98°. $d^{3.98}$ 1.000000 g/ml (0.999972 g/cc). d_4^{25} 0.997. d^0 (ice) 0.917 g/cc; d_4^0 (liq) 0.999868. Density tables: Bigg, *Brit. J. Appl. Phys.* **18,** 521 (1967); Kell, *J. Chem. Eng. Data* **12,** 66 (1967). Expands on freezing. mp 0°. bp 100°. One liter satd vapor weighs 0.5974 g at 100° and 760 mm. Crit temp 374.2°; crit pressure 218 atm. Sp. heat (liq; 14°) 1.000 cal/g/°C. Latent heat of fusion: 1.436 kcal/mole. Latent heat of vaporization: 9.717 kcal/mole. n_D^{20} 1.3330. Dielectric const (0°) 87.740. Dipole moment (25°) in benzene 1.76; in dioxane 1.86. Ionization const for pure water only: K (25°) 1.008×10^{-14}; at moderate concn of solutes (e.g. 1.0*M* KOH): K (25°) 0.971×10^{-14}. The most universal solvent known.

10176. Water Gas. Blue gas. Obtained by blowing steam through incandescent coke. *Composition:* 6% CO_2; 42% CO; 51% H_2; 1% N_2.

Caution: Asphyxiant. USE: In the manuf of ammonia as source of hydrogen. *Cf.* Producer Gas.

10177. Watermelon. Arbuse. *Citrullus vulgaris* Schrad., *Cucurbitaceae,* cultivated in hot and temperate zones the world over. Contains diuretic principles: Bliss *et al., Am. J. Pharm.* **105,** 53 (1933); Roby *et al., ibid.* **111,** 68 (1939).

10178. Wheat Germ Oil. Cav-Ecol; Myopone; Denamone. Obtained by hydraulic expression or solvent extraction of wheat germ which constitutes ~2% of a wheat grain, the seed of *Triticum aestivum* L. (*T. sativum* Lam., *T. vulgare* Vill.), *Gramineae. Constit.* (of the oil): Linoleic acid 44.1%, oleic acid 30.0%, satd acids 15.1%, linolenic acid 10.8%, unsaponifiable matter 4.7%. The unsaponifiable matter contains vitamin E-active tocopherols (reported as 0.5% of the oil and as 2 international vitamin E units per

Pharmacokinetics of clindamycin HCl administered intravenously, intramuscularly and subcutaneously to dogs

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Lavy, E., Ziv, G., Shem-Tov, M., Glickman, A., Dey, A. Pharmacokinetics of clindamycin HCl administered intravenously, intramuscularly and subcutaneously to dogs *J. vet. Pharmacol. Therap.* **22,** 261–265.

A buffered aqueous solution of clindamycin Hcl (200 mg/mL) was injected intravenously (i.v.) intramuscularly (i.m.) and subcutaneously (s.c.) in a non-randomized, partial cross-over trial involving six male and six female dogs. Blood samples were collected at conventional, predetermined time periods and serum drug concentrations were determined by microbiological assay. Dogs were observed clinically for signs of pain, and activity of serum creatine phosphokinase (CPK) was monitored after i.m. dosing.

The i.v. data from five of the dogs best fitted a two-compartment open-system pharmacokinetic model whereas a non-compartment model was most suitable for analysis of the data from the remaining seven dogs. The mean i.v. elimination half-life ($t_{½\beta}$) and the mean residence time (*MRT*) were 124 and 143 min, respectively. The mean volume of distribution at steady state (V_{ss}) was 0.86 L/kg. Little pain was recorded upon i.m. injection; mean peak serum drug concentration (C_{max}) was 4.4 μg/mL, the elimination half-life ($t_{½el}$) was 247 min and the calculated bioavailability (*F*) was 115% of the i.v dose. Serum CPK activity was elevated to 25-fold the pretreatment level in samples collected 4, 8 and 12 h after i.m. injection. Pain was not recorded after s.c. drug administration; the mean C_{max} of 20.8 μg/mL was significantly greater than the corresponding value for the i.m. route, and *F* was 310%. The s.c. route appears to be superior to the i.m. route in terms of local tolerance and serum drug level; a 10 mg/kg SID treatment regimen is suggested for treatment of canine infections due to clindamycin sensitive bacteria.

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INTRODUCTION

A semisynthetic derivative of lincomycin, clindamycin has been shown to be clinically effective and is recommended for treatment of staphylococcal and anaerobic infections of skin, soft tissue and bone in dogs (Berg *et al.*, 1984; Greene, 1989; Braden *et al.*, 1988; Braden *et al.*, 1987). Clindamycin is available for parenteral administration as the 2-phosphate and hydrochloride. Clindamycin 2-phosphate is microbiologically inactive, but is hydrolized *in-vivo* to clindamycin (Webber *et al.*, 1980). The pharmacokinetics of clindamycin phosphate in dogs were studied after single intravenous (i.v.) and intramuscular (i.m.) administrations at 11 mg/kg clindamycin (Webber *et al.*, 1980) and after single subcutaneous (s.c.) injections at 2.75, 5.5, 11 and 21 mg/kg clindamycin (Webber *et al.*, 1980). Based on the pharmacokinetics of the drug, s.c. dosage regimen of 11 mg/kg of clindamycin free base as clindamycin-2-phosphate/kg body weight every 24 h was recommended (Budsberg *et al.*, 1992). The i.m. administration of clindamycin-2-phosphate solution (50 mg/mL) induced signs of pain and other side-effects and, therefore, this route was not recommended (Budsberg *et al.*, 1992). A buffered 20% aqueous solution of clindamycin hydrochloride (200 mg/mL) is available for pharmacokinetic and clinical testing. The purpose of this study was to determine the concentrations of clindamycin in normal canine serum after single i.v., i.m. and s.c. administrations of clindamycin HCl and compare the derived kinetic variables with those obtained earlier in dogs injected with equal doses of clindamycin phosphate (Webber *et al.*, 1980; Budsberg *et al.*, 1992). Local tolerance and appearance of side-effects following i.m. and s.c. administrations were particularly examined.

MATERIALS AND METHODS

Animals

Twelve adult mixed breed dogs, six males and six females (4–13 kg b.w.) were used in the study. All dogs were housed in the test facility for 3 weeks prior to the study. Dogs had free access to

water and commercial dry dog ration before and during the study. Inclusion criteria included normal findings on physical examination, complete blood count (CBC) serum concentrations of urea nitrogen, creatinine, albumin, total protein glucose, bilirubin, triglycerides, cholesterol, calcium, magnesium, sodium, potassium, chlorides,inorganic phosphorus and serum activities of alkaline phosphataes, alanine aminotransferase, aspartate aminotransferase and amylase were determined. Dogs selected for the study exhibited normal CBC and blood biochemistry test results.

Experimental design

Intravenous protocol

Jugular vein catheters were placed in each dog; patency of each catheter was maintained with heparinized saline. A blood sample was taken prior to the beginning of the trial. Each dog was given the 20% buffered aqueous clindamycin HCl i.v. at 10 mg/kg. Blood samples were obtained at 10, 20, 30, 40, 50, 60, 80, 90, 120, 180, 240, 360, 480 and 600 min post injection. Blood was allowed to clot at 20°C for 2 h and was then centrifuged at $1000 \times \boldsymbol{g}$; the serum was collected and stored at −20°C until it was assayed.

Intramuscular and subcutaneous protocols

A 2 week rest period was allowed for all dogs. All indwelling jugular venous catheter was placed and maintained as for the i.v. protocol, and a baseline blood sample was taken. The injection site (4×4 cm^2 area) on the dorsal aspect of the left and right sites were then shaved to remove short hair. Nine dogs received a single i.m. injection of the 20% clindamycin HCl at 10 mg/kg in the left-side of the neck and the remaining three dogs received a single i.m. injection of 3–5 mL sterile physiological saline in the neck. Two weeks later, nine dogs were prepared by procedures identical to those used before i.m. drug administration. Six dogs received a single s.c. injection of 20% clindamycin HCl at 10 mg/kg in the right-side of the neck. All dogs were observed immediately following i.m. and s.c. injections for evidence of pain, itching or irritation. The injection sites on both sides of the neck were palpated at each blood sampling time and at least two-times per day on the following 2 days and any abnormal finding such as pain, swelling and discoloration were recorded.

Blood samples were obtained at 15, 30, 60, 90, 150, 210, 270, 390, 510, 630, 720 and 1440. min post i.m. injection. Blood samples were collected at 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720 and 1440 min post s.c. injection. Blood samples were processed as for after i.v. injection.

Clindamycin analysis

Clindamycin concentrations were measured by microbiological well/agar plate diffusion assay as previously described (Bennett *et al.*, 1966). The assay organism *S. lutea* ATCC 9341, was inoculated into antibiotic Medium No. 1 (Difco, Detroit, MI, USA) and a 7.0 mm layer seeded medium was added to each Petri Plates. Six wells, 8 mm in diameter, were cut into the agar at equal distances. A 50.0 μL aliquot of samples (and standard clincamycin HCl solution) was alternately added to each well and the plates were incubated at 37°C for 14–16 h. The concentration of drug in each sample was calculated from zone of inhibition diameters using polinomial regression techniques. Sensitivity limit of assay method was 0.1 μg/mL. Standard curves were derived using clindamycin HCl (Sigma Chemical Co. St. Louis, MO, USA) in dog serum. The correlation coefficient of the standard curve from 0.10 to 6.0 μg/mL was 0.99 ($P < 0.001$). Samples with concentrations > 6.0 μg/mL were diluted with antibiotic-free dog serum to bring clindamycin concentrations within the range of the standard curve. The coefficients of variation of repeatedly assayed samples at concentrations ranging between 1–6 μg/mL and 0.1–1.0 μg/mL were 7.5% and 12.5%, respectively. Samples were assayed in duplicate and data are reported as mean ± SD. It was recognized that this assay fails to distinguish between clindamycin and its putative active metabolites and, therefore, results were expressed as serum clindamycin antimicrobial equivalent activity. Thus the term 'clindamycin concentration' where used throughout this report is rather clindamycin antimicrobial equivalent activity.

Serum creatine phosphokinase (CPK)

Serum CPK values were determined, using as enzymatic method (CK-NAC-active creatine kinase EC2.7.3.2, Randox Laboratories Ltd., Crumlin, Northern Ireland), in blood samples collected at 0, 4, 8, 12, 24, 32, 48 and 72 h after nine dogs were injected i.m. with 20% clindamycin HCl solution, three dogs were injected with saline, three dogs were injected s.c. with 20% clindamycin HCl and three dogs were administered saline s.c. As large differences in pretreatment CPK values were found among the dogs examined, serum CPK data were converted to percentage by dividing each post treatment value by the pretreatment value for the corresponding animal. The post treatment CPK data are presented as mean ± SD-fold rise from pretreatment (baseline) CPK value.

Data analysis

Estimates of first-order rate constants and volumes were initially obtained by subjecting mean data to analysis, using iterative least squares regression analysis (Brown & Manno, 1978). The concentrations vs. time data from each dog were then analysed, using a microcomputer program for nonlinear weighted least square regression (Bourne, 1986).

The most appropriate pharmacokinetic model was selected on the basis of the lowest weighted sum of squares and the lowest Akaike's information criterion (AIC) value (Yamaoka *et al.*, 1978) for data from each dog. The i.v., i.m. and s.c. areas under the curves (AUCs) were calculated using trapezoidal approximations between time of drug administration and 1440 min afterwards. Differential calculus methods (Edwards & Penney, 1982) were used to estimate peak serum drug concentrations (C_{max}) and time of C_{max} (t_{max}) after i.m. and s.c. administrations. Kinetic values are presented as mean ± SD; half lives, however, are presented as harmonic mean ± pseudo-SD (Lam *et al*, 1985). The paired Student's *t*-test was used for calculating the significance of the differences in the mean kinetic values for the i.m. and s.c. routes; $P < 0.05$ value was considered significant.

RESULTS

Clinical signs indicative of slight pain were noticed in four to five of the dogs immediately following i.m. injection; the remaining dogs did not exhibit any pain reaction. Signs suggesting pain or discomfort were not shown by any dog after s.c. injection. Palpation of the injection site did not elicit any pain reaction. Local changes could not be felt at the injection site. Thus, the neck side injected with clindamycin HCl could not be differentiated from the side injection with sterile saline solution.

Mean serum clindamycin concentrations after i.v., i.m. and s.c. administrations are presented (Table 1). Data are also presented graphically as mean log10 serum concentrations vs. time plot (Fig. 1). The i.v. data from five of the dogs best fitted a two-compartment open system pharmacokinetic whereas a one-compartment model was most suitable for analysis of data from the remaining seven dogs. Thus, the kinetic values Cp°, A, and $t_{½\alpha}$ presented (Table 2) represent data from these five dogs; it shows a rapid rate of drug distribution from the central to the peripheral body compartment. The elimination half-life ($t_{½\beta}$) and the mean residence time (*MRT*) were 124.0 ± 57.0 min and 143.0 ± 34.0 min, respectively and the steady state volume of distribution (V_{ss}) was 0.86 ± 0.35 L/kg. After i.m. drug administration, the mean C_{max} (4.4 ± 0.5 μg/mL) was significantly ($P < 0.05$) lower than the corresponding value for the s.c. administration (20.8 ± 6.2 μg/mL).

The mean absorption time (*MAT*) of clindamycin HCl solution injected s.c. was significantly shorter than after i.m. administration. The mean $t_{½el}$ i.m. value (427.0 ± 209.0 min) was not significantly different from the mean $t_{½el}$ for the s.c. route (310.2 ± 190.4 min) but these values were significantly longer than the mean i.v. $t_{½\beta}$. The mean s.c. *AUC* was significantly larger than the mean i.m. *AUC* and the resulting calculated bioavailability *(F)* values which were 1.15 and 3.1 for the i.m. and s.c. routes, respectively (Table 3).

Serum CPK activity rose sharply within 8 h after i.m/ injection of clindamycin HCl; activity returned to pretreatment level by 48 h post treatment. A minimal rise in serum CPK activity was observed after s.c. clindamycin injection. The i.m. and s.c. administration of saline did not affect serum CPK activity.

Table 1. Mean serum clindamycin concentrations (μg/mL) after intravenous, intramuscular and subcutaneous injection of clindamycin HCl to dogs at 10 mg/kg body weight

	Treatment					
	Intravenous $n=12$		Intramuscular $n=9$		Subcutaneous $n=6$	
Time (min)	Mean	SD	Mean	SD	Mean	SD
10	13.4	2.3	NS		NS	
15	NS		2.6	0.76	NS	
20	11.3	2.2	NS		NS	
30	10.2	2.3	3.6	0.90	16.8	12.8
40	8.9	2.5	NS		NS	
45	NS		NS		19.5	3.8
50	7.7	1.8	NS		NS	
60	6.8	1.4	4.0	0.60	17.4	6.7
80	5.75	0.6	NS		NS	
90	5.6	1.3	4.0	0.70	13.5	4.1
120	4.1	0.87	NS		12.6	4.6
150	NS		3.5	0.50	NS	
180	3.0	0.7	NS		10.7	3.2
210	NS		3.0	0.40	NS	
240	2.1	0.45	NS		6.9	2.2
270	NS		2.3	0.40	NS	
360	0.82	0.22	NS		4.6	1.4
390	NS		1.7	0.40	NS	
480	0.46	0.10	NS		3.3	1.8
510	NS		1.1	0.40	NS	
600	0.31	0.11	NS		2.7	1.6
630	NS		0.70	0.30	NS	
720	NS		0.60	0.30	1.7	1.2
1440	NS		0.30	0.10	0.3	0.1

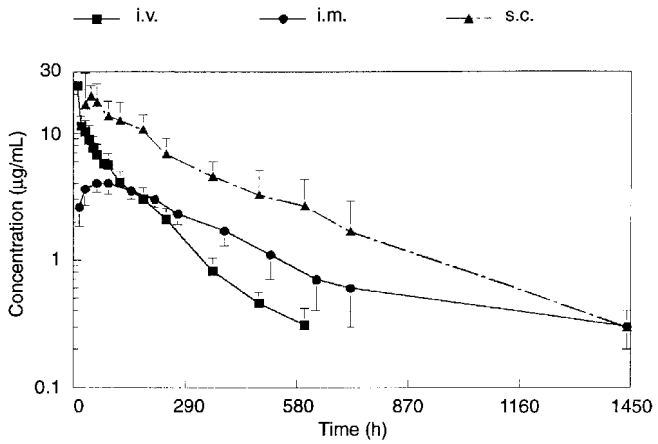

Fig. 1. Serum clindamycin concentrations (μg/mL, log 10 scale) after intravenous, intramuscular and subcutaneous administration of clindamycin HCl to dogs at 10 mg/kg.

DISCUSSION

The clinical manifestations of pain described (Budsberg *et al.*, 1992) following i.m. administration of 5% solution of clindamycin phosphate to dogs were not seen at all after i.m. injection of more concentrated (20%) buffered aqueous solution of clindamycin HCl. We can only speculate on the causes for these differences in local tolerance; they could be due to the type of clindamycin salt, the presence of buffer or a four-fold smaller volume injected using the 20% clindamycin HCl solution. The transient rise in serum CPK activity observed after i.m. injection of clindamycin HCl to dogs (Fig. 2) indicates some degree of muscle tissue damage at the injection site (Steinnes *et al.*, 1978). However, a similar or even higher and more persistent rise has been documented for a long list of apprived veterinary injectable products which are very commonly used in small and large animal practice without any observable pain reactions (Rasmussen, 1980; Svendsen, 1983). A better safety evaluation of i.m. clindamycin HCl therapy must wait until data from multiple injections are available. The present

Table 2. Selected pharmacokinetic values for clindamycin HCl administered intravenously to 12 dogs at 10 mg/kg

Kenetic value & unit	Mean	SD
Cp°, μg/mL	18.75	3.71
A, μg/mL	11.06	3.35
B, μg/mL	7.54	3.35
$t_{½\alpha}$, min	11.00	13.30
$t_{½\beta}$, min	124.00	57.00
MRT, min	143.00	34.00
V_c, L/kg	0.56	0.11
V_{ss}, L/kg	0.86	0.35
AUC, μg/mL.min	1457.00	280.00
Cl_t, mL/min/kg	6.10	1.10
r^2	0.967	0.031

A = ordinal intercept of fastest disposition slope minus the intercept of the slowest disposition slope; B = ordinal intercept of the slowest disposition slope; Cp° = initial serum concentration; $t_{½\alpha}$ = distribution half-life; $t_{½\beta}$, elimination half-life; *MRT* = mean residence time; V_c = volume of the central compartment; V_{ss} = volume of distribution at steady state; *AUC* = area under the concentration-time cutve from zero to 24 h post-treatment; Cl_t = total body clearance; r^2 = correlation coefficient to the line of best fit for a two-compartment open system pharmacokinetic.

Table 3. Selected pharmacokinetic values for clindamycin administered intramuscularly and subcutaneously to dogs at 10 mg/kg

	Treatment			
Kinetic value and unit	Intramuscular $n=9$ Mean	SD	Subcutaneous $n=6$ Mean	SD
$C_{max.}$ μg/mL	4.4	0.5	20.8	6.2
$t_{max.}$ min	73.0	16.0	46.7	20.1
MAT, min	546.0	226.0	224.5	163.5
$t_{½el.}$ min	427.0	209.0	310.2	190.4
MRT, min	700.0	246.0	364.2	147.3
AUC, μg/mL min	1806.0	346.0	5258.0	2161.0
F(*)	1.15	0.19	3.10	0.22

$C_{max.}$ peak maximal serum concentration; t_{max}, time to peak serum concentration; *MAT* = mean absorption time, calculated as $MRT_{non\text{-}i.v.} - MRT_{i.v.}$; F^* = bioavailability, calculated as $AUC_{non\text{-}i.v.}/AUC_{i.v.}$

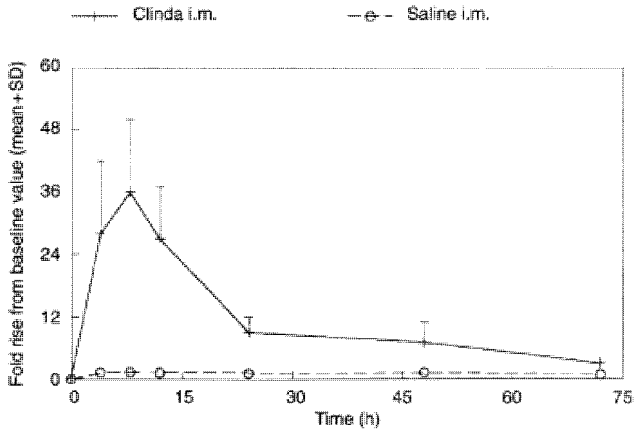

Fig. 2. Serum CPK activity in dogs after intramuscular administration of clindamycin HCl at 10 mg/kg.

findings clearly indicate that the s.c. route is superior to the i.m. route in terms of local tolerance.

Interpretation of data gathered in the course of the present study must take into consideration the assay method used (microbiological). Although a good agreement was shown between microbiological and chemical (GC) test results in dog serum for clindamycin (Ziv & Shem-Tov, unpublished data), there is a slight chance that there are some putative active metabolites. The disposition curves after i.v. administration of clindamycin HCl was best represented as a two-compartment open model in only five of the dogs. The i.v study protocol we used called for collecting the first post treatment blood sample at 10 min.

Because of the rapid distribution rate of the drug in the dog ($t_{½\alpha}$ of 3.5 ± 1.1 min) according to (Budsberg *et al.*, 1992), we probably missed observing the distribution phase. Values for the other major kinetic parameters found in the present study were also different from the values calculated in dogs injected i.v. with clindamycin phosphate (Budsberg *et al.*, 1992). Thus, mean $t_{½\beta}$, *MRT* and *AUC* after clindamycin phosphate administration were 194.6 min, 263.4 min and 2009.5 μg·mL, respectively. Such differences in kinetic values, although small, could result from the rate of appearance of bioactive antibiotic in the serum after i.v. administration of the microbiologically inactive clindamycin phosphate, differences in body-weight (clindamycin phosphate was injected to dogs weighting 20 to 30 kg), (Budsberg *et al.*, 1992) or slightly different methods used for calculating the kinetic variable. On the other hand, mean Cl_t, V_c, and V_{ss} for clindamycin phosphate were very close to the corresponding values for clindamycin HCl estimated in the present study. Regardless of these small differences, the large V_{ss} of clindamycin indicates possible wide distribution in the body fluids and tissues. Direct measurements of tissue clindamycin concentrations in humans (Panzer *et al.*, 1972; Dhawan & Thadepalli, 1982) and cats (Brown *et al.*, 1990) confirmed these assumptions.

The kinetic variables calculates from the i.m. serum drug level data for clindamycin HCl and clindamycin phosphate were in good agreement. The short t_{max} (1h) and average bioavailability of nearly 100% support rapid and complete absorption of the drug from the site of i.m. injection, as was remarked earlier (Budsberg *et al.*, 1992). The kinetic profile of the drug in serum of all dogs after s.c administration of clindamycin HCl is rather unique; mean C_{max} (20.8 μg/mL) was nearly 4.5 times greater than the mean i.m. C_{max}. Moreover, mean serum concentrations during the first 12 h post treatment by the s.c. route were two to three-fold higher than the concentrations found after i.m. drug administration (Fig. 1). After a nearly equivalent dose of the drug (11 mg/kg) was injected s.c. to dogs as clindamycin phosphate (Webber *et al.*, 1980) a mean C_{max} of 6.1 ± 0.3 μg/mL was recorded at t_{max} of 40–60 min. We found that the terminal elimination rate of the drug from serum ($t_{½el}$) after s.c. administration of 20% clindamycin HCl solution (310.2 ± 190.4 min) was considerably longer than the reported (Budsberg *et al.*, 1992) $t_{½el}$ of 234.8 ± 27.3 min after an equivalent dose was given to dogs i.m. as clindamycin phosphate. A $t_{½el}$ of 13.9 h was calculated (Webber *et al.*, 1980) from the serum clindamycin data of dogs injected s.c. with clindamycin phosphate.

It appears therefore, that s.c. administration of clindamycin HCl allows for rapid, complete drug absorption and, at the same time, acts as a depot which limits the rate of drug elimination from serum. A entero-hepatic circulation effect was suggested to operate in dogs treated orally with clindamycin HCl (Lavy *et al.*, 1999) contributing to the prolongation of $t_{½el}$ to nearly 6 h, and for calculated oral bioavailability values exceeding 100%. Whatever the pharmacokinetic processes involved, it appears from the present study that the s.c. route is superior to the i.m. in practical terms by permitting a longer treatment interval.

Earlier studies (Braden *et al.*, 1987; Budsberg *et al.*, 1992) attempted to establish i.v. and i.m. dosing recommendations using average serum concentrations at steady state with the accompanying peak (Cp_{max}) and through (Cp_{max}) concentrations as means for calculating (Gibaldi, 1982; Riviere, 1988) dosage regimens for clindamycin phosphate in dogs. The calculated dosage schedule was eventually found to be in agreement with the currently recommended oral dosage schedule of 11 mg/kg, q 12 h (Budsberg *et al.*, 1992). We have tried to use a similar approach for selecting desirable, potentially antibacterial effective, serum drug concentrations in dogs given clindamycin HCl by s.c. route. In relating the minimal inhibitory concentration (*MIC*) of clindamycin to its pharmacokinetic properties it has been assumed (Webber *et al.*, 1980; Budsberg *et al.*, 1992; Brown *et al.*, 1990; Riviere, 1988) that: (a) tissue drug concentration at least equal to the MIC is maintained throughout the entire dose interval; (b) the drug is minimally bound to serum protein and serum concentrations are equal to, or even slightly lower than, the concentration in major target sites of the body (excluding bone): (c) the kinetic profiles of the drug in serum and the target tissue on multiple dosing are very similar; and (d) the *MIC* for *Staphylococcus aureus/intermedius* ranges from 0.04 to 0.4 μg/mL and for most anaerobic bacteria, the *MIC* ranges from 0.1 to 3.1 μg/mL but the *MIC* 90 is in effect > 1.6 μg/mL (Greene, 1989; Budsberg *et al.*, 1992; Brown *et al.*, 1990). Using the mean serum concentration values, we observed that a single s.c. 10 mg/kg SID dosage regimen appears to be appropriate for clindamycin HCl for the treatment of staphylococcal soft tissue infections. For anaerobic infections, however, this treatment should be given BID. A more intensive course of clindamycin therapy is apparently required for the treatment of staphylococcal bone infections in the dog (Braden *et al.*, 1987; Braden *et al.*, 1988; Budsberg *et al.*, 1991).

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Disposition Kinetics of Difloxacin After Intravenous, Intramuscular and Subcutaneous Administration in Calves

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ABSTRACT

The pharmacokinetics of difloxacin (Dicural) was studied in a crossover study using three groups ($n = 4$) of male and female Friesian calves after intravenous (i.v.), intramuscular (i.m.) and subcutaneous (s.c.) administrations of 5 mg/kg body weight. Drug concentration in plasma was determined by high-performance liquid chromatography using fluorescence detection. The plasma concentration–time data following i.v. administration were best fitted to a two-compartment open model and those following i.m. and s.c. routes were best fitted using one-compartment open model. The collected data were subjected to a computerized kinetic analysis. The mean i.v., i.m. and s.c. elimination half-lives ($t_{1/2\beta}$) were 5.56 ± 0.33 h, 6.12 ± 0.42 h and 7.26 ± 0.6 h, respectively. The steady-state volume of distribution (V_{dss}) was 1.12 ± 0.09 L/kg and total body clearance (Cl_B) was 2.19 ± 0.1 ml/(min. kg). The absorption half lives ($t_{1/2ab}$) were 0.38 ± 0.027 h and 2.1 ± 0.09 h, with systemic bioavailabilities (F) of 96.5% ± 6.4% and 84% ± 5.5% after i.m. and s.c. administration, respectively. After i.m. and s.c. dosing, peak plasma concentrations (C_{max}) of 3.38 ± 0.13 μg/ml and 2.18 ± 0.12 μg/ml were attained after (t_{max})1.22 ± 0.20 h and 3.7 ± 0.52 h. The MIC_{90} of difloxacin for *Mannheimia haemolytica* was 0.29 ± 0.04 μg/ml. The AUC/MIC_{90} and C_{max}/MIC_{90} ratios for difloxacin following i.m. administration were 120 and 11.65, respectively and following s.c. administration were 97.58 and 7.51, respectively. Difloxacin was 31.7–36.8% bound to calf plasma protein. Since fluoroquinolones display concentration-dependent activities, the doses of difloxacin used in this study are likely to involve better pharmacodynamic characteristics that are associated with greater clinical efficacy following i.m. administration than following s.c. administration.

Keywords: difloxacin, Dicural, female calves, male calves, minimum inhibitory concentration, pharmacodynamics, pharmacokinetics, *Mannheimia haemolytica*

Abbreviations: $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life (i.v.); k_{12}, first-order rate constant for transfer from central to peripheral compartment; k_{21}, first-order rate constant for transfer from peripheral to central compartment; V_c, apparent volume of the central compartment; V_{dss}, volume of distribution at steady state; Cl_B, total body clearance; $AUC_{0-\infty}$, area under curve from zero time to infinity; MRT, mean residence time; k_{ab}, first-order absorption rate constant; $t_{1/2ab}$, absorption half-life; k_{el}, first-order elimination rate constant; $t_{1/2el}$, elimination half-life (i.m. or s.c.); MAT, mean absorption time; C_{max}, maximum plasma concentration; t_{max}, time to peak plasma concentration; F, systemic bioavailability; MIC_{90}, minimum inhibitory concentration; cfu, colony—forming unit

INTRODUCTION

Difloxacin is a difluoroquinolone antimicrobial agent with high *in vitro* activity against a wide range of Gram-positive bacteria, Gram-negative bacteria and mycoplasmas (Digranes and Dibb, 1986; Mader *et al.*, 1987; Fernandes, 1988; Brown, 1996). Similarly to that of other fluoroquinolones, the bactericidal activity of difloxacin is mediated by inhibition of subunit A of DNA topoisomerases II (gyrase), an enzyme that is essential for DNA synthesis and repair (Wolfson and Hooper, 1989; Drlica and Zhao, 1997). Difloxacin differs in particular from other fluoroquinolones on account of its *p*-fluorophenyl ring at position

N-1 of the quinoline nucleus, which reportedly to gives it enhanced activity against Gram-positive bacteria (Walker, 2000).

Difloxacin is rapidly absorbed and has high systemic bioavailabilities following oral administration in chickens, pigs (Inui *et al.*, 1998) and dogs (Frazier *et al.*, 2000) as well as i.m. administration in rabbits (Abd el Aty *et al.*, 2005). In addition, it has good distribution characteristics and long elimination half-life in goats (Atef *et al.*, 2002).

Difloxacin is indicated for treatments of bovine respiratory disease caused by *Pasteurella* spp. and or *Mycoplasma* spp. Although the pharmacokinetics of difloxacin has been investigated in a number of species, including chickens, pigs, dogs and goats (Inui *et al.*, 1998; Frazier *et al.*, 2000; Atef *et al.*, 2002; Heinen, 2002; Abd El Aty *et al.*, 2005), there are no reports evaluating the pharmacokinetics of the drug in calves.

The purpose of this study was to investigate the pharmacokinetic and pharmacodynamic correlation following intravenous (i.v.), intramuscular (i.m.) and subcutaneous (s.c.) administration of difloxacin in calves at the dose rate (5 mg/kg body weight) recommended by the manufacturer in many countries.

MATERIAL AND METHODS

Experimental design

A three-period crossover study was undertaken in 6 male and 6 female healthy Friesian calves (10.5 months of age with a mean weight ± SEM of 257.8 ± 18.7 kg), such that each calf received difloxacin (Dicural 10% injectable solution, Fort Dodge Animal Health, Holland) at a dose of 5 mg/kg body weight by i.v., i.m. or s.c. route. Each calf was housed in an individual pen and fed on antibiotic-free pelleted concentrates (Zagazig Ration, Zagazig Company, El Sharqia, Egypt), hay and alfalfa. Food and water were provided *ad libitum*.

Calves were randomly assigned into three groups, with each group containing four animals, two of each sex. In period 1 of the study, animals of group 1 received 5 mg/kg difloxacin intravenously into the right jugular vein and animals of group 2 received the same dose into the thigh muscle; animals of group 3 received the same dose subcutaneously in the neck region. Subsequent treatments in periods 2 and 3 were administered according to a Latin square design so that each calf received each treatment in sequence.

Blood samples (5 ml/per sample) were obtained by venepuncture of the jugular vein into heparinized tubes just before administration of the drug by different routes and at 5, 10, 15 and 30 min and at 1, 2, 4, 6, 8, 10, 12, 24 and 36 h after i.v., i.m. or s.c. dosing. Samples were allowed to stand protected from light at room temperature (approximately 24°C) for 20 min and then centrifuged at 1500*g* for 10 min to harvest plasma. Plasma was aliquoted and stored at −70°C pending analysis.

Difloxacin assay

Instrumentation. Drug concentration in plasma was determined using reversed-phase high-performance liquid chromatography (HPLC) according to the method previously described

by Frazier and colleagues (2000). The HPLC system consisted of a Model 616 solvent delivery pump (Waters, Milford, MA, USA), a Waters Model 600 S controller, a Model 717-plus autosampler equipped with a temperature-controlled rack (Waters), and a variable-wavelength fluorescence UV detector (Spectrofluorometric detector Rf 10, Shimadzu)

Chromatographic conditions. The chromatographic conditions included a mobile phase of acetonitrile in 0.05 mol/L sodium phosphate buffer (pH $\approx$ 3.5) (25:75 v/v) at a flow rate of 1 ml/min through a reversed-phase C_{18} column (Discovery, Supelco, 5 μm, 4.6 × 150 mm). Fluorescence detection was at an excitation wavelength of 295 nm and emission wavelength of 500 nm.

Calibration curve. For preparation of the calibration curves, plasma of antibiotic-naive calves was spiked with 0.02, 0.05, 0.1, 0.5, 5 and 10 μg/ml difloxacin. Quality control samples were prepared in large volume from an independent weighing of reference standards, aliquoted into the same type of vials used for storage of plasma samples, and frozen until the day of the assay. A calibration curve was obtained by plotting the peak height ratio versus the nominal concentrations. The equation was calculated by the least-squares method using linear regression. The minimum quantitative limit of the assay was 0.02 μg/ml. The standard curve of difloxacin in calf plasma was linear between 0.02 and 5 μg/ml. the value of correlation coefficients (r) was >0.97. The peak height ratio of an unknown specimen (peak height of difloxacin/peak height of internal standard) was compared with that of the standard; over the concentration range 0.02–5 μg/ml, the concentration of difloxacin was directly related to the peak height ratio. Any sample concentrations of difloxacin greater than 5 μg/ml were diluted 1:1 or 1:10 and the analysis was repeated. Dilution of quality control samples that exceeded the ranges of the standard curve with drug-free plasma was confirmed not to interfere with accurate drug quantification.

Sample extraction. The plasma samples or calibration standards to be assayed (500 μl) were placed in centrifuge tube and spiked with 50 μl of internal standard (ofloxacin 5 μg/ml in 0.05 mol/L phosphate buffer) and vortexed. Dichloromethane (6 ml) was added, samples were vortexed, the aqueous layer was removed by aspiration, and the organic layer was evaporated to dryness. The residue was reconstituted using HPLC mobile phase (250 μl) and transferred to an autosampler vial for injection.

Validation of the assay method. The precision and accuracy of the method were evaluated by repetitive analysis of the plasma samples ($n = 12$) spiked with 0.02, 0.05, 0.1, 0.5 and 5 μg/ml difloxacin. Intra-day precision and accuracy were obtained by analysis of these samples on one day by the same operator. Inter-day precision and accuracy were obtained by assay of these samples ($n = 12$) on different days by two operators. Stability of analytes was determined by comparing peak heights in quality control samples with those in freshly prepared standards in plasma. The recoveries were calculated by comparison of plasma and aqueous samples ($n = 6$).

The intra-assay coefficient of variation for plasma was <3.2% and the intra-assay accuracy was >94.7%. The inter-assay coefficient of variation for plasma was <4.1% and the inter-assay accuracy was >95%. Recovery of difloxacin from plasma was found to be 94%.

Estimation of protein binding

The extent of plasma protein binding was determined *in vitro* by to a method previously described (Singhvi *et al*., 1977). Plasma from each calf was spiked with 0.05, 0.1, 0.5, 1, 5 and 10 μg/ml difloxacin and 1 ml was added to a commercial ultrafiltration device (Centrifree 4104, Amicon Corp., Danvers, MA, USA). The ultrafiltration device was centrifuged at a fixed angle (28°) (Sorvall, RC-5B Refrigerated super speed centrifuge, GSA rotor, DuPont Instruments, Newtown, Connecticut) at 1200g for 30 min at 37°C. This resulted in an ultrafiltrate volume of at least 200 μl. The ultrafiltrate was frozen until assayed for difloxacin. The percentage of protein-bound fraction (B) was calculated according to the equation $B = \{$[initial plasma (difloxacin)−ultrafiltrate (difloxacin)]/[initial plasma (difloxacin)]$\} \times 100$. The coefficients of variation for this method were <4.7%.

Minimum inhibitory concentration

The minimum inhibitory concentrations (MIC_{90}) of difloxacin for *Mannheimia haemolytica* isolated from diseased calves (12 isolates) were determined by broth microdilution technique (Jones *et al*., 1985). Ten replicates of twofold dilutions of difloxacin (0.062, 0.125, 0.25, 0.5, 1.0, 2.0 μg/ml) were used. Fifty microlitres of each concentration was added to each well. One well in each row contained only Muller–Hinton (MH) broth to serve as an inoculation and growth control. Clinical isolates (24-hour-old cultures) were subcultured to MH broth at a density of approximately 10^8 colony-forming units (cfu)/ml, compared with density of a 0.5 McFarland standard. This suspension was further diluted to 10^5 cfu/ml in MH broth. Fifty microlitres of the suspension was delivered to each well. The MIC was the lowest concentration of difloxacin for which no visible growth was observed after 18 h of incubation at 37°C. The coefficients of variation for this method were <3.6%.

Pharmacokinetic analysis

Following intravenous administration, the plasma concentration–time data were fitted to a two-compartment open model system (Baggot, 1978) according to the biexponential equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, where C_t is the plasma concentration of difloxacin; t is time after intravenous administration; A and α are the intercept and slope, respectively, of the distribution phase; B and β are the intercept and slope of the elimination phase; and e is the base of natural logarithms. Pharmacokinetic variables were obtained by use of a computer program (R Strip, Micromath, UT, USA). The distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$), the volume of distribution at steady state (V_{dss}), the volume of the central compartment (V_c), the total body clearance (Cl_B) and the two-compartment

microconstants k_{12}, k_{21} were computed according to standard equations (Gibaldi and Perrier, 1982).

Following i.m. and s.c. administration, plasma concentration data were analysed by compartmental and non-compartmental methods based on statistical moment theory (Gibaldi and Perrier, 1982). In compartmental analysis, data were found to be best fitted using a one-compartment open model and first-order absorption rate constant according to the equation $C_t = Ee^{-K_{el}t} - Ce^{-K_{ab}t}$, where C_t is the plasma concentration of difloxacin; t is time after i.m. or s.c. administration, k_{el} is the elimination rate constant and k_{ab} is the first-order absorption rate constant. The terminal elimination half-life ($t_{1/2el}$) and absorption half life ($t_{1/2ab}$) were calculated as $(\ln 2)/k_{el}$ or $(\ln 2)/k_{ab}$, respectively. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) and the area under the first moment curve ($AUMC_{0-\infty}$) were calculated by the trapezoidal rule for all measured data with extrapolation to infinity using C_{36}/k_{el} or C_{36}/β, where C_{36} is the plasma concentration at 36 h divided by the β (elimination rate constant for two-compartment model) or k_{el} in the case of the one-compartment open model. The mean residence time (MRT) was calculated as $MRT = AUMC_{0-\infty}/AUC_{0-\infty}$. The mean absorption time (MAT) was calculated as $MAT = MRT_{(s.c.ori.m.)} - MRT_{(i.v.)}$ The peak plasma concentration (C_{max}) and time to maximum concentration (t_{max}) were taken from the plot of each calf's concentration–time curve. Bioavailability (F; fraction of drug absorbed systemically) was calculated as $F = AUC_{(i.mors.c.)}/AUC_{i.v} \times 100$.

Pharmacodynamic efficacy of difloxacin was determined by calculation of C_{max}/MIC_{90} and $AUC_{0-\infty}/MIC$ ratios following i.m and s.c. administration of the drug using MIC_{90} for *Mannheimia haemolytica* determined in the present study.

Statistical analysis

Results are presented as mean ± standard error (SE). All data were subjected to analysis of variance with a significant level of 0.05 and the homogeneity of variances was tested by Bartlett test. Testing for homogeneity of the variances suggested the use of the non-parametric Mann–Whitney test for comparing the pharmacokinetic parameters after i.m. and s.c. administration, p-values <0.05 were considered significant. All the previously mentioned tests were performed using the SPSS 6.1.3 software package (SAS Inc., Cary, NC, USA)

RESULTS

Following i.v administration of difloxacin, the plasma concentration–time curve of the drug was decreased in a biphasic manner, as characterized by a two-compartment open model. Figure 1 illustrates the mean plasma concentrations as a function of time following all routes of administration. Table I summarizes the pharmacokinetic parameters for difloxacin following i.v. administration. The distribution half-life ($t_{1/2\alpha}$) was 0.24 ± 0.021 h and the elimination half life ($t_{1/2\beta}$) was 5.56 ± 0.33 h. The V_{dss} was 1.12 ± 0.09 L/kg and MRT was 7.32 ± 0.56 h.

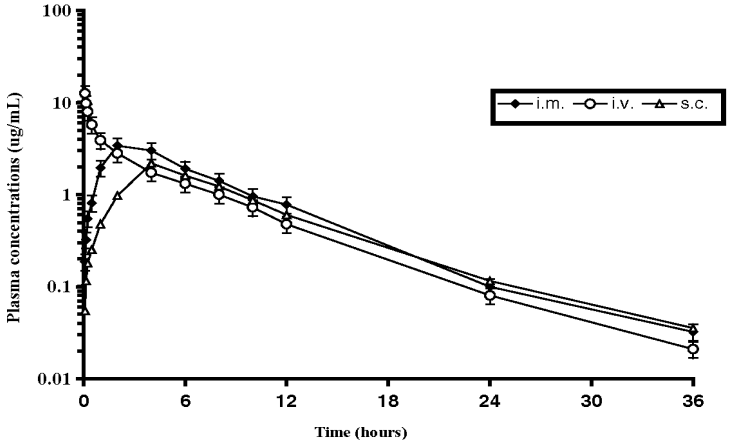

Figure 1. Semilogarithmic graph depicting the time–plasma concentration course of difloxacin in calves following intravenous, intramuscular and subcutaneous administration of 5 mg/kg body weight ($n = 12$)

TABLE I
Kinetic parameters (mean I SE) of difloxacin following a single i.v. injection of 5 mg/kg body weight in calves ($n = 12$)

Parameter[a]	Unit	Value
α	h^{-1}	2.82 ± 0.16
$t_{1/2\alpha}$	h	0.24 ± 0.021
β	h^{-1}	0.12 ± 0.01
$t_{1/2\beta}$	h	5.56 ± 0.33
k_{12}	h^{-1}	1.51 ± 0.07
k_{21}	h^{-1}	1.21 ± 0.08
V_c	L/kg	0.47 ± 0.034
V_{dss}	L/kg	1.12 ± 0.09
Cl_B	ml/(min.kg)	2.19 ± 0.1
$AUC_{0-\infty}$	µg/(ml.h)	36.0 ± 2.6
MRT	h	7.32 ± 0.56

[a] α, β, hybrid rate constants representing the slopes of distribution and elimination phases, respectively; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$ elimination half-life; k_{12}, first-order rate constant for transfer from central to peripheral compartment; k_{21}, first-order rate constant for transfer from peripheral to central compartment; V_c, apparent volume of the central compartment V_{dss}, volume of distribution at steady state; Cl_B, total body clearance; $AUC_{0-\infty}$, area under curve from zero time to infinity; MRT, mean residence time

TABLE II
Mean ± SE kinetic parameters of difloxacin following single i.m. and s.c. injections of 5 mg/kg body weight in calves ($n = 12$)

Parameter[a]	Unit	Value	
		i.m.	s.c.
k_{ab}	h^{-1}	1.78 ± 0.09	0.33 ± 0.01**
$t_{1/2ab}$	h	0.38 ± 0.027	2.1 ± 0.09**
k_{el}	h^{-1}	0.11 ± 0.009	0.09 ± 0.006
$t_{1/2el}$	h	6.12 ± 0.42	7.26 ± 0.6
$AUC_{0-\infty}$	μg/(ml.h)	34.82 ± 1.9	28.3 ± 1.5**
MRT	h	8.71 ± 0.62	13.65 ± 1.1**
MAT	h	1.38 ± 0.11	6.33 ± 0.52**
C_{max}	μg/mL	3.38 ± 0.13	2.18 ± 0.12**
t_{max}	h	1.22 ± 0.2	3.7 ± 0.52**
F	%	96.5 ± 6.4	84 ± 5.5

[a] k_{ab}, first-order absorption rate constant; $t_{1/2ab}$, absorption half-life; k_{el}, first-order elimination rate constant; $t_{1/2el}$, elimination half-life; $AUC_{0-\infty}$, area under curve from zero time to infinity; MRT, mean residence time; MAT, mean absorption time; C_{max}, maximum plasma concentration; t_{max}, time to peak plasma concentration; F, systemic bioavailability
*$p < 0.01$; **$p < 0.001$

Following i.m. and s.c. administration, the plasma concentration – time data were best fitted by a one-compartment open model and the drug was detected in plasma for 36 h following administration. Table II displays the pharmacokinetic parameters following i.m. and s.c. administration. The absorption half-life, mean absorption time and mean residence time following i.m. administration ($t_{1/2ab} = 0.38 \pm 0.027$ h; MAT = 1.38 ± 0.11 h; MRT = 8.71 ± 0.62 h) were significantly shorter than those following s.c. administration ($t_{1/2ab} = 2.1 \pm 0.09$ h; MAT = 6.33 ± 0.52 h; MRT = 13.65 ± 1.1 h). A significantly higher maximum plasma concentration was attained earlier following i.m. administration than that following the s.c. route. The elimination half-life ($t_{1/2el}$) and systemic bioavailability were not significantly different between the two routes. The extent of plasma protein binding varied between 31.7% and 36.8% of difloxacin spiked into plasma of antimicrobial-naive calves. The MIC_{90} for *Mannheimia haemolytica* was 0.29 ± 0.04 μg/ml. The AUC/MIC_{90} and C_{max}/MIC_{90} ratios for difloxacin following i.m. administration were 120 and 11.65, respectively, and following s.c. administration were 97.58 and 7.51, respectively.

DISCUSSION

In the present study, serum concentration of difloxacin was determined by HPLC method. Sarafloxacin, the active metabolite of difloxacin in most animal species (Heinen, 2002), was not quantified in the present study because previous investigation had indicated that

sarafloxacin constitutes a very small proportion in serum and its peak (0.033 μg/ml) was close to the lower quantification level in the earlier study as well as in the present study (Chu *et al*., 1985).

Difloxacin is a well-tolerated drug in calves; no adverse effect was reported following i.v., i.m. or s.c. administration of the drug at a dose of 5 mg/kg body weight. Following i.v administration of difloxacin in calves, the disposition kinetic curve declined in a biphasic manner, suggesting that the drug disposition followed a two-compartment open model. This finding is in agreement with previous reports in goats (Atef *et al*., 2002). Difloxacin has good distribution characteristics represented by short distribution half-life and large volume of distribution ($t_{1/2\alpha}$ = 0.24 hour; V_{dss} = 1.12 L/kg). Similar findings have been reported for difloxacin in goats (Atef *et al*., 2002) and for other fluoroquinolones (enrofloxacin and danofloxacin) in calves (Davidson *et al*., 1986; Mann and Frame, 1992). The elimination half-life in calves (5.56 h) was slightly shorter than that reported in goats (Atef *et al*., 2002). The total body clearance of difloxacin (2.19 ml/(min.kg)) in calves was very similar to that in goats (2.16 ml/(min.kg)) (Atef *et al*., 2002). In contrast, a much higher clearance of difloxacin was reported in rabbits (9.83 ml/(min.kg)) (Abd el Aty *et al*., 2005). Such differences could be attributable to interspecies variations in drug metabolism and elimination.

The extent of plasma protein binding varied between 31.7% and 36.8% of difloxacin spiked into plasma of antimicrobial-naive calves; a higher plasma protein binding has been reported for difloxacin (46–52%) in humans (Granneman *et al*., 1986).

Following i.m administration, difloxacin was rapidly absorbed with a short $t_{1/2ab}$ of 0.38 h; rapid absorption is also reflected by short MAT of 1.38 h. Rapid absorption ($t_{1/2ab}$ = 0.37 h) following i.m. administration has been reported for difloxacin in goats (Atef *et al*., 2002). Conversely, difloxacin was slowly absorbed following s.c. administration and MAT was significantly longer as a result of continued absorption from the site of injection. Following i.m. injection, maximum plasma concentration of the drug (C_{max} = 3.38 μg/ml) was significantly higher and was achieved earlier (t_{max} = 1.22 h) than with s.c. injection (C_{max} = 2.18 μg/ml, t_{max} = 3.7 h); these values are comparable to that of 3.7 μg/ml attained after 1.25 h following i.m. administration to goats at a dose rate similar to that used in the present study (Atef *et al*., 2002).

A longer elimination half life for difloxacin was recorded following s.c. administration than following the i.m. route. However, this difference was not significant and could be attributed mainly to the slow release of the drug from the injection site following s.c. injection.

The systemic bioavailability reported in the current study indicates excellent absorption of the drug (96.5%) following i.m. injection. These values are similar to that reported following i.m. administration of the drug in goats (Atef *et al*., 2002). A smaller proportion of the drug (84%) reached the systemic circulation following administration by the s.c. route.

With i.m. and s.c. administration of difloxacin in calves, plasma concentration was above the MIC_{90} for *Mannheimia haemolytica* for 12 h following administration. From previous studies with fluoroquinolone and ciprofloxacin, it has been proposed that treatment should be optimized by providing an AUC/MIC ratio of at least 125 (Forrest *et al*., 1993; Sullivan *et al*., 1993) and a C_{max}/MIC ratio of 10 or greater. These two breakpoints are essential for

clinical efficacy of fluoroquinolones (Vogelman *et al.*, 1988; Dalhoff and Ullmann, 1990). In the present study, by incorporating C_{max} and AUC data following i.m. and s.c. administration of difloxacin together with the MIC_{90} of 0.29 for *Mannheimia haemolytica*, the following values were obtained. The AUC/MIC_{90} and C_{max}/MIC_{90} ratios for difloxacin following i.m. administration were 120 and 11.65, respectively, and following s.c. administration were 97.58 and 7.51, respectively. The reported ratios following i.m. administration are close to the recommended breakpoints, whereas those following s.c. administration fall short of these breakpoints.

Since fluoroquinolones display concentration-dependent activities, the dose of difloxacin used in this study are likely to involve better pharmacodynamic characteristics that are associated with greater clinical efficacy following i.m. administration rather than following s.c. route. Additionally, there is a need for further investigation that correlates the pharmacokinetics and pharmacodynamics of difloxacin in plasma, exudates, transudate and bronchial secretion following s.c. administration in calves naturally infected with *Mannheimia haemolytica* to extend the *ex vivo* data obtained in this study.

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Tamoxifen-resistant Fibroblast Growth Factor-transfected MCF-7 Cells Are Cross-Resistant *in Vivo* to the Antiestrogen ICI 182,780 and Two Aromatase Inhibitors[1]

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ABSTRACT

Although the antiestrogen tamoxifen has been the mainstay of therapy for estrogen receptor (ER)-positive breast cancer, successful treatment of responsive tumors is often followed by the acquisition of tamoxifen resistance. Subsequently, only 30–40% of patients have a positive response to second hormonal therapies. This lack of response might be explained by mechanisms for tamoxifen resistance that sensitize ER pathways to small amounts of estrogenic activity present in tamoxifen or that bypass ER pathways completely. To elucidate one possible mechanism of tamoxifen resistance, we treated ovariectomized tumor-bearing mice injected with fibroblast growth factor (FGF)-transfected MCF-7 breast carcinoma cells with the steroidal antiestrogen ICI 182,780 or one of two aromatase inhibitors, 4-OHA or letrozole. These treatments did not slow estrogen-independent growth or prevent metastasis of tumors produced by FGF-transfected MCF-7 cells in ovariectomized nude mice. FGF-transfected cells had diminished responses to ICI 182,780 *in vitro*, suggesting that autocrine activity of the transfected FGF may be replacing estrogen as a mitogenic stimulus for tumor growth. ER levels in FGF transfectants were not down-regulated, and basal levels of transcripts for estrogen-induced genes or of ER-mediated transcription of estrogen response element (ERE) luciferase reporter constructs in the FGF expressing cells were not higher than parental cells, implying that altered hormonal responses are not due to down-regulation of ER or to FGF-mediated activation of ER. These studies indicate that estrogen independence may be achieved through FGF signaling pathways independent of ER pathways. If so, therapies directed at the operative mechanism might produce a therapeutic response or allow a response to a second course of antiestrogen treatment.

INTRODUCTION

Because conventional therapy is not usually curative in clinical breast cancer, development of tamoxifen resistance, in which breast tumors previously growth-inhibited by tamoxifen become refractory, represents an important therapeutic dilemma. However, the development of tamoxifen resistance is not necessarily associated with progression to an ER[3]-negative phenotype. In many cases of clinical tamoxifen resistance, ER expression may be retained (1–4), implying that the resistance is due an alteration in activity of the tamoxifen/ER complex. Tamoxifen resistance in such a case could result from three possible mechanisms that, according to present knowledge, would not preclude successful treatment with an alternative hormonal therapy. First, alterations in the ER could arise, which might diminish or extinguish inhibitory responses to tamoxifen, leaving only its partial agonist effects to predominate (5–8). Second, tamoxifen resistance arising in the setting of an intact ER could be a result of altered intratumoral tamoxifen metabolism, which might produce more estrogenic metabolites locally (7, 9–11). Third, available tamoxifen could be sequestered by an increase in antiestrogen binding sites not associated with ERs (12). As mentioned, in each of these three instances, substitution of a hormonal therapy different from tamoxifen might result in a clinical response. Two such alternative therapies used in this report are steroidal estrogen antagonists, such as ICI 182,780, which lack the partial agonist activity of tamoxifen, and aromatase inhibitors, which inhibit endogenous estrogen production by all tissues, depriving the ER of its ligand.

Although the mechanisms of tamoxifen resistance de-

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[3] The abbreviations used are: ER, estrogen receptor; FGF, fibroblast growth factor; IMEM, improved minimal essential medium; X-gal, 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside; FBS, fetal bovine serum; 4-OHA, 4-hydroxyandrostenedione; NK, natural killer; CCS, charcoal-stripped calf serum; ERE, estrogen response element; CAT, chloramphenicol acetyltransferase; RT, reverse transcription.

scribed above should be amenable to alternative hormonal therapy, early results for small numbers of tamoxifen-resistant patients have shown that only about 30–40% of such patients have a positive response to subsequent ICI 182,780 or aromatase inhibitor therapy (13–20). These data imply alternative mechanisms for tamoxifen resistance. Constitutive production of autocrine growth factor(s) or growth factor receptors by tumor cells has been proposed as a mechanism for tamoxifen resistance that may or may not involve ER pathways. Evidence supporting this hypothesis is gained from the acquisition of estrogen-independent growth in tumor models, including the one used in this report, in which growth factors or growth factor receptors have been overexpressed in estrogen-dependent breast carcinoma cell lines (21–26). In addition, recent clinical data showing decreased efficacy of tamoxifen in treating tumors overexpressing *c-erbB2* (27) supports a role for growth factor signaling in clinical tamoxifen resistance. Because some growth factor signaling pathways, including the ERB-B pathway, have been shown to interact with ER signaling pathways (25, 28–32), increased growth factor signaling could be one mechanism by which cells could become sensitive to previously ineffective amounts of estrogenic stimulation produced by the partial agonist activity of tamoxifen itself or its estrogenic metabolites, above. In cases in which such interactions have been demonstrated, the growth factor and ER pathways may act collaboratively (25), making the final outcome susceptible to pharmacological manipulations of either pathway and implying that second line hormonal therapies might have an effect. However, increased autocrine or intracrine growth factor signaling might also bypass the need for ER-mediated growth stimulation in tumor cells or affect stromal components of the tumor, such as endothelial or immune cells (33–36), to alter the tumor environment in ways conducive to tumor growth. In either case, alternative hormonal therapies might not be effective.

Recently, cell-specific coactivators and corepressors have been identified for steroid hormone receptors, including the ER, which may influence steroid receptor-induced transcription positively or negatively (37, 38). Thus, the activity of tamoxifen in inhibiting or even stimulating tumor growth might depend on the relative expression of various stimulatory or inhibitory cofactors in a particular tumor (39, 40). However, transient transfection experiments suggest that tamoxifen-resistant tumors produced by such mechanisms should still be sensitive to pure antiestrogens (40).

FGFs and their receptors have been shown to be present with high frequency in breast cancer specimens (41–50). Evidence for a possible role for FGF signaling in the estrogen-independent growth of breast tumors is gained from study of clonal and polyclonal FGF-transfected MCF-7 cell lines, which are capable of forming large, progressively growing tumors in ovariectomized or tamoxifen-treated nude mice. Moreover, the FGF-transfected cells are metastatic, forming micrometastases in lymph nodes, lungs, and other organs (21, 22, 51). The estrogen-independent and tamoxifen-resistant growth of FGF-transfected MCF-7 cells suggests an interaction between FGF signaling pathways and ER-activated pathways that could occur at the level of the ER itself or at the end point of both pathways, where they impinge on growth mechanisms. If FGF-mediated growth pathways bypass the ER pathway to affect growth directly, we would expect that growth would be unaffected by hormonal treatments devoid of agonist activity. We therefore sought to determine the sensitivity of the estrogen-independent tumor growth of FGF-transfected MCF-7 cells to ICI 182,780 or aromatase inhibitors. In contrast to what was seen with ERB-B signaling pathways, we report that FGF-mediated pathways appear to provide an alternative growth stimulatory signal that is not dependent on ER activation.

MATERIALS AND METHODS

Cell Lines. FGF-transfected MCF-7 cell lines have been described previously (21, 22, 51, 52). Briefly, the ML-20 clonal cell line is a MCF-7-derived cell line that is stably transfected with a *lacZ* expression vector. The *in vitro* and *in vivo* growth characteristics of ML-20 cells are indistinguishable from wild-type MCF-7 cells (51), and >90% of the cells routinely stain positive for β-galactosidase expression by X-gal staining (52). MKL-F (FGF-4-transfected; Ref. 52) and FGF-1 clone 18 (FGF-1-transfected) cells (22) resulted from the stable transfection of the ML-20 clonal cell line with expression vectors for FGF-4 (also known as hst-1/K-FGF) and FGF-1 (also known as acidic FGF or aFGF), respectively. Both cell lines continue to stably express β-galactosidase, allowing effects of FGF overexpression on metastatic capability to be assessed by X-gal staining of organs and tissues of tumor-bearing mice. The MKL-4 cell line was derived by transfecting wild-type MCF-7 cells (of similar passage number used for the ML-20 transfection) with an expression vector for FGF-4, which produced the clonal MKS-1 cells (21). These cells were then retransfected with an expression vector for *lacZ*, yielding MKL-4 cells (51). Cells were maintained in IMEM (Biofluids, Rockville, MD) supplemented with 5% FBS in a humidified, 37°C, 5% CO_2 incubator in routine culture until used for tumor cell injection.

Drugs. ICI 182,780 was kindly donated by Dr. Alan Wakeling of Zeneca Pharmaceuticals (Macclesfield, England), and was administered s.c. at a dose of 5 mg in 0.1 ml of vehicle every week. For the experiment depicted in Fig. 1, powdered drug was first dissolved in 100% ethanol and spiked into warmed peanut oil (Eastman Kodak, Rochester, NY) to give a final concentration of 50 mg/ml. For the experiments depicted in Fig. 1, *B* and *C*, 50 mg/ml preformulated drug in a vehicle of 10% ethanol, 15% benzyl benzoate, 10% benzyl alcohol, brought to volume with castor oil, was supplied by B. M. Vose (Zeneca Pharmaceuticals). 4-OHA was donated by Angela Brodie (University of Maryland, Baltimore, MD) and was administered s.c. at a dose of 1 mg/mouse/day 6 days of the week in a vehicle of 0.3% hydroxypropylcelluose. Letrozole was donated by Dr. Ajay Bhatnagar (Novartis, Ltd., Basil, Switzerland) and was administered via gavage at a dose of 1 mg/mouse/day 6 days of the week in a vehicle of 0.3% hydroxypropylcellulose. Sustained-release (60 day) pellets containing 5 mg of tamoxifen were obtained from Innovative Research of America (Sarasota, FL) and implanted s.c. in the interscapular area at the time of tumor cell injection.

Tumor Cell Injection. The procedure for tumor cell injection has been described previously (21). Briefly, tumor cells were scraped into their normal growth medium, and viable cells were quantified using trypan blue exclusion. The cells were

resuspended in their normal growth medium at a density of 66.7×10^6 cells/ml, and 0.15 ml (containing 10 million cells) were used to inject ovariectomized mice (nude or *beige/nude/xid*) into the mammary fat pad. For the experiment involving MKL-4 cells and nude mice (Fig. 1*A*), each mouse was injected bilaterally into the thoracic mammary fat pads (two injections per mouse). There were seven mice in the vehicle group and five mice in each treatment group. For the experiments involving MKL-4 cells and *beige/nude/xid* mice (Fig. 2), four tumor cell injections were given, two on each side in the thoracic mammary fat pad and two in the inguinal mammary fat pad; treatment groups consisted of four mice. For the experiments involving MKL-F and FGF-1, clone 18 cells (Fig. 1, *B* and *C*), each mouse was injected once in the right thoracic mammary fat pad. There were seven mice in the each vehicle group, and treatment groups consisted of five or six mice each. Tumors resulting from the injections were measured twice weekly in three dimensions using calipers. Tumor volume is the product of the largest dimension, the orthogonal measurement, and the tumor depth, as described previously (21). Because the FGF-1-transfected clone 18 cell line produces tumors that in some cases are surrounded by a fluid-filled sac that confounds tumor measurements (22), these tumors were measured postmortem by weighing them.

Determination of Metastasis. Organs were harvested from tumor-bearing animals, fixed briefly, and stained with X-gal as reported previously (51) and viewed through a dissecting microscope (Olympus SZH). Clusters of blue-staining cells were identified as micrometastases. In accordance with previous results, no macrometastases were identified (21, 22, 51, 53).

Growth Assays. Anchorage-dependent and anchorage-independent growth assays were performed as described (21). Briefly, for anchorage-dependent growth, cells were plated in 24-well culture dishes at a density of 10,000 cells/well for the time course experiments (Fig. 4) and 20,000 or 30,000 cells/well for the concentration-response experiments (Fig. 5). For growth in FBS, following overnight attachment, treatments were added at the indicated concentrations, and cells were counted on the indicated days. For growth assays under estrogen-depleted conditions, cells were stripped of estrogens during a 24-h period the day following plating by changing the medium four times to phenol red-free IMEM supplemented with 5% CCS (21). We have found that this stripping procedure allows complete removal of estrogens without substantial proliferation of cells before treatments are added. Following the stripping procedure, on day 0, treatments were added, and counting of cells was done as above.

Doubling times were determined according to the following equation: doubling time $= t_2 - t_1/3.32\log(N_2/N_1)$, where N_2 and N_1 are the number of cells at times t_2 and t_1, respectively. N_1 and N_2 are the means of quadruplicate determinations.

Anchorage-independent assays in FBS-containing medium were done as described previously (21). For experiments using estrogen-depleted conditions, cells were stripped of estrogens over a 24-h period as described above before being plated in soft agar. Colonies greater than 60 μm were counted using an Omnicon 3600 Image Analysis system.

ER Assays. [^{3}H]Estradiol binding has been described previously (54, 55). Briefly, cells grown to 70% confluence were stripped with twice daily medium changes over 4 days with 5% CCS in phenol red-free IMEM. The prolonged stripping method allows ERs to become up-regulated to maximal levels. Cells were harvested, washed sequentially at 4°C with serum-free, phenol red-free IMEM followed by TEG (10 mM Tris, pH 7.4, 1 mM EDTA, 10% glycerol), and resuspended in 1 ml of TEG plus 1 mM DTT, 0.5 M NaCl and a cocktail of protease inhibitors (1 mg/ml leupeptin, 77 μg/ml aprotinin, 1 μg/ml pepstatin A). A whole-cell extract was prepared by homogenization with 40 strokes in a Teflon-glass Dounce homogenizer followed by centrifugation at $105,000 \times g$ for 30 min. Protein content of the supernatant was determined by the method of Bradford (56), and protein concentrations were adjusted to 2 mg/ml. Extracts were incubated with 10 nM [^{3}H]17β-estradiol with or without a 100-fold excess of unlabeled estradiol for 16 h at 4/C. Unbound ligand was removed by absorption with dextran-coated charcoal followed by centrifugation. Aliquots of the supernatant were counted in a Beckman liquid scintillation counter.

Northern Blots. Cells were grown to 50% confluence in IMEM supplemented with 5% FBS and then stripped of estrogens as described for the growth assays, above. Treatments of 0.1% ethanol (vehicle) or 10^{-8} M 17β-estradiol in the same medium were added. Cultures were harvested after 3 days of treatment, and RNA was extracted using RNAzol B (Tel-Test, Inc.) according to the manufacturer's directions. Thirty μg of each RNA were subjected to electrophoresis in a 1.2% formaldehyde/agarose gel and transferred to nylon (Hybond-N, Amersham Corp., Arlington Heights, IL) by capillarity. ^{32}P-labeled antisense riboprobes for pS-2, GAPDH, and cathepsin D were prepared and sequentially hybridized to the membrane overnight at 65°C [hybridization buffer was 50% formamide, 50 mM Na_2HPO_4, 0.8 M NaCl, 10 mM EDTA, 2.5 × Denhardt's solution (1× Denhardt's = 0.02% polyvinylpyrrolidone, 0.02% BSA), 0.2% SDS, 400 μg/ml yeast tRNA, and 400 μg/ml sonicated salmon sperm DNA with 10^6 DPM/ml of the appropriate probe]. The membrane was washed three times in 0.1% SDS/0.1× SSC at 80°C for the PS-2 and cathepsin D probes, and 75°C for the GAPDH probe. Autoradiograms and PhosphorImager (Molecular Dynamics Model 445SI) quantitation of individual hybridization signals were obtained between the sequential hybridizations. For the results depicted in Fig. 7, *A* and *B*, PhosphorImager values obtained for PS-2 or cathepsin were normalized to those obtained for GAPDH.

Progesterone Receptor mRNA Determination by RT-PCR. The primers for human progesterone receptor that produce a 205-bp PCR product have been described previously (57). The human GAPDH primers that produce a 437-bp PCR product are as follows: 5′-AAG GTC GGT GTG AAC GGA TTT G-3′ (sense) and 5′-TGG TGC AGG ATG CAT TGC TG-3′ (antisense). RT-PCR was performed with 0.1 μg of test RNAs, except T47D cells, where 0.02 μg was used, using the GeneAmp RNA PCR kit (PE Applied Biosystems, Foster City, CA) according to the manufacturer's instructions with the following modifications: the RT reaction was primed with 0.0625 μM random hexamers in a volume of 40 μl, with 2 μl each of ^{35}S-labeled UTP and ^{35}S-labeled ATP (each 3000 Ci/mmol, 10 μCi/μl, Amersham Corp.) substituted for water in the reaction. Then, 20 μl of each RT reaction were transferred into two tubes for separate GAPDH and progesterone receptor PCR reactions.

Cycle analyses using RNA from ML-20, estradiol-treated cells (the highest expressors of progesterone receptor) revealed that amplification remained logarithmic at 35 cycles for the GAPDH reaction and 40 cycles for the progesterone receptor reaction, making these assays semiquantitative. The GAPDH PCR reaction was performed using standard reagent conditions recommended by the manufacturer and cycles of 95°C for 45 s and 50°C for 45 s for 35 cycles. For the progesterone receptor PCR reaction, final $MgCl_2$ concentrations were adjusted to 1.25 mM, and 0.25 M acetamide was included. Cycles were of 95°C for 45 s and 50°C for 45 s for 40 cycles. GAPDH and progesterone receptor reaction products were first visualized by ethidium bromide staining following electrophoresis in a 2% agarose gel. Products were then electrophoresed on a 4–20% acrylamide gel that was subjected to both autoradiography and PhosphorImager quantitation as described above.

Transient Transfection, Luciferase, and CAT Reporter Assays. ML-20 and clone 18 cells were plated in 6-well plates, allowed to attach overnight, and stripped of estrogens in a procedure similar to that for the growth assays (see above). Following stripping, cells were transfected by the calcium phosphate, low-CO_2 method (58). The luciferase plasmids pGLB-MERE or pGLB-MNON were obtained by inserting an approximately 1.48-kb fragment containing a glucocorticoid response element-deleted mouse mammary tumor virus promoter with either a substituted double consensus ERE (MERE) or the same sequence with the ERE palindromes scrambled (MNON) (59) into the *Hin*dIII site of pGLB (Promega, Madison, WI). Each dish received 2.5 μg of either pGLB-MERE or pGLB-MNON and 1.0 μg pCMV-CAT, which directs constitutive expression of CAT, cotransfected as a control for transfection efficiency. Following transfection, each well was washed twice with PBS and incubated for 48 h in medium containing vehicle (0.01% ethanol), 10^{-9} M estradiol, 10^{-7} M ICI 182,780, a combination of E_2 and ICI, 10 ng/ml FGF-1 plus 10 μg/ml heparin, or a combination of FGF, heparin, and ICI 182,780. (Duplicate samples of each treatment were used.) Cells were lysed and assayed for luciferase activity using the Luciferase Reporter Gene Assay (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's instructions. Luciferase values, expressed as relative light units, for each sample were corrected for background by subtracting the value of lysates of untransfected cells prepared in parallel. CAT expression was assayed using the CAT ELISA (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's instructions. Protein content of the lysates was determined using the BCA Protein Assay Reagent (Pierce, Rockford, IL). Luciferase and CAT values, normalized for protein, were used to calculate mean specific relative light units/ng CAT.

Statistical Analyses. Statistical methods used for tumor growth have been described previously (53, 60). For Figs. 1 and 2, only mice surviving at the end of the experiment were included in the analysis. When no tumor developed from a particular injection, tumor volume was recorded as zero. The repeated measures ANOVA (60) was used to compare tumor volumes among the treatment groups using measurements taken over the entire time course of the experiment. In addition, final tumor volumes (or weights in the case of clone 18) were compared among treatment groups at the end of each experiment using ANOVA. For analysis of metastasis in Table 1, for each transfectant, analysis of covariance was used to compare the effects of treatment on total metastases, total distant metastases (lung metastases plus other metastases), lymph node metastases, lung metastases, and other metastases. The analyses were all conducted with final tumor volume (or weight for the clone 18 cells) included in the model as a covariate. The analyses considered the effects of all treatments simultaneously, as well as the effects of individual treatment comparisons (which were adjusted for multiple comparisons using Dunnett's method). For each transfectant, the effect of final tumor volume (or weight for clone 18) on the number of metastases was evaluated using linear regression (for each of the categories of metastasis described above). In Fig. 3, paired *t* tests were performed comparing control and transfected cells under different conditions of treatment. For the anchorage-dependent growth assays depicted in Fig. 4, we examined the effect of treatment on the rate of cell growth, using linear regression with an interaction between time and treatment. To compare cell growth rates and doubling times among the cell lines under specific treatment conditions, nested linear regression models were used. For Fig. 6, ANOVA was used to determine significant differences in ER binding among cell lines.

RESULTS

Estrogen-independent Growth of Tumors Produced by FGF-transfected MCF-7 Cells Is Not Inhibited by Treatment with a Pure Antiestrogen or with Aromatase Inhibitors. We have previously shown that both FGF-1- and FGF-4-transfected MCF-7 cells form progressively growing tumors in ovariectomized nude mice, as well as in similar mice treated with tamoxifen (21, 22, 53). Although ovariectomized mice could be expected to have substantially lower levels of estrogenic compounds than reproductively intact mice, some estrogens are synthesized at extraovarian sites, such as adrenal gland, liver, fat, or possibly the tumor itself. The transfected cells evidently still possess ERs, because they respond to estrogen and tamoxifen administered to the mice, as well as to these compounds used in tissue culture (21, 22). To test the hypothesis that growth of the FGF-transfected cells in ovariectomized or tamoxifen-treated nude mice is due to increased sensitivity to the small amounts of estrogens still present in ovariectomized nude mice, we tested the ability of a pure antiestrogen, ICI 182,780, and two aromatase inhibitors, 4-OHA and letrozole, to inhibit the estrogen-independent tumor growth produced by these FGF-transfected cell lines.

In a first experiment to test the above hypothesis, FGF-4-transfected MKL-4 cells were injected as before, and the mice were treated with vehicle, tamoxifen, or ICI 182,780. There were no significant differences in tumor volume among the treatment groups considered over the entire time course of the experiment ($P = 0.72$) or at the final time point (Fig. 1*A*; $P = 0.72$). Treatment with ICI 182,780 did not inhibit tumor growth below that achieved in vehicle-treated mice ($P = 0.675$). Thus, the failure of ICI 182,780 to inhibit the estrogen-independent growth exhibited by this cell line supports the hypothesis that such growth does not result from small amounts of estrogenic

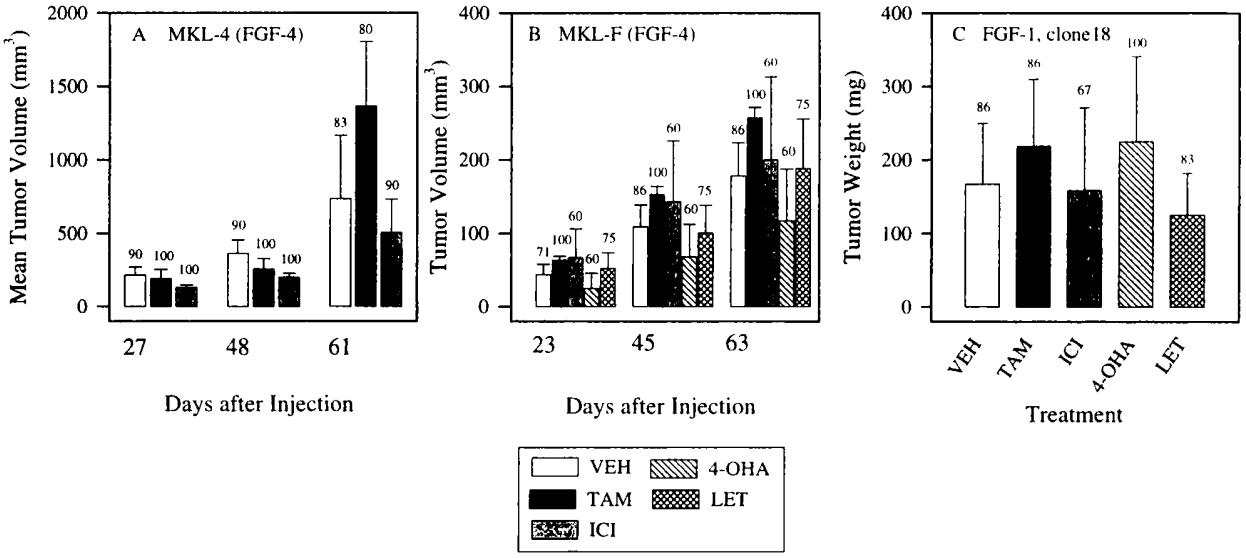

Fig. 1 Growth of FGF-transfected MCF-7 cells in ovariectomized nude mice is not inhibited by treatment with ICI 182,780, 4-OHA, or letrozole. Ten million cells from the indicated cell lines were injected into the mammary fat pads of ovariectomized nude mice treated with vehicle (*VEH*); a 5-mg, 60-day-release tamoxifen pellet (*TAM*); ICI 182,780, 5 mg s.c. every week (*ICI*); 1 mg of 4-OHA s.c. per day 6 days of the week (*4-OHA*); or 1 mg of letrozole per day via gavage 6 days of the week (*LET*). *Columns*, group mean; *bars* SE. *Numbers* above each column are the percentages of injections resulting in measurable tumors at that time point. *A*, volumes of tumors produced by one clonal FGF-4-transfected MCF-7 cell line, MKL-4, at the indicated number of days following tumor cell injection. *B*, volumes of tumors produced by a second clonal FGF-4-transfected MCF-7 cell line, MKL-F, at the indicated number of days following tumor cell injection. *C*, weights of tumors produced by a clonal FGF-1-transfected MCF-7 cell line, FGF-1, clone 18, weighed after sacrifice of the animals 28 days after tumor cell injection. (Because the FGF-1 producing MCF-7 cells may form fluid-filled sacs around the tumor, confounding tumor measurements before sacrifice, only postmortem weights are presented here.)

growth stimulation achieved by extraovarian estrogen production.

We wished to assess the effect of ICI 182,780 on metastasis as well as on tumor growth. In spite of its retention of the transfected *lacZ* expression plasmid, the MKL-4 cell line becomes heterogeneous over time with respect to β-galactosidase expression, such that a few cells have high expression, but most are negative (52). We therefore used a second clonal FGF-4-transfected MCF-7 cell line, MKL-F, the β-galactosidase expression of which is stable, for a subsequent experiment involving FGF-4-transfected MCF-7 cells. Because FGF-1 has also been shown to produce estrogen-independent *in vivo* growth when transfected into MCF-7 cells (22), we also included a clone of FGF-1-transfected cells designated clone 18, the β-galactosidase expression of which is also stable. For these experiments, two aromatase inhibitors, 4-OHA (61, 62) and letrozole (63), were also used to inhibit extraovarian synthesis of estrogens.

In agreement with the experiment using MKL-4 cells depicted in Fig. 1*A*, when the FGF-4-transfected MKL-F cells were used, there were no differences in tumor volume among treatment groups over all time points ($P = 0.382$), and ICI 182,780 did not decrease tumor growth below that obtained in vehicle-treated animals (Fig. 1*B*; $P = 0.837$ for the last time point). In addition, neither 4-OHA nor letrozole decreased tumor growth below vehicle-treated levels ($P = 0.571$ and 0.931 for the last time point, respectively).

FGF-1-transfected clone 18 cells form tumors that are sometimes surrounded by a fluid-filled sac (22, 53), preventing accurate tumor volume measurements during the course of the experiment. Consequently, when these cells were used (Fig. 1*C*), only terminal tumor weights were analyzed with ANOVA. As with the MKL-4 and MKL-F cells, ICI 182,780 did not inhibit estrogen-independent tumor growth in the clone 18 cells ($P = 0.977$). Administration of ICI 182,780 to animals injected with ML-20 cells, a clonal line of β-galactosidase-transfected wild-type MCF-7 cells (51), also produced no effect when compared with vehicle-treated animals [*i.e.*, no progressively growing tumors were obtained in either case (data not shown)]. In other, separate experiments, a polyclonal population of control vector-transfected ML-20 cells that forms progressively growing tumors in estrogen-supplemented mice (22) did not form tumors in either untreated or ICI 182,780-treated animals.[4] Thus, the continued progressive *in vivo* growth of FGF-transfected cells in ovariectomized animals treated with either a pure antiestrogen or aromatase inhibitors demonstrates that the estrogen-independent growth of these cells in untreated ovariectomized nude mice is not due to estrogenic activity produced at extraovarian sites.

Because ICI 182,780, 4-OHA, and letrozole were without effect in the experiments described above, we injected reproductively intact female mice for 2 weeks with these compounds at the same doses used in the above experiments to observe for activity in preventing effects of endogenous estrogens on the

[4] Unpublished results.

Table 1 Metastasis of FGF-transfected MCF-7 cells is not inhibited by treatment with ICI 182,780 or aromatase inhibitors

Mice were sacrificed and tumors and organs were subjected to X-gal staining as described previously (51). Mice bearing tumors produced by injection of MKL-4 cells were sacrificed at 61 days; for MKL-F tumors, mice were sacrificed after 64 days: and for FGF-1 clone 18 tumors, mice were sacrificed after 28 days.

		Metastatic site		
Injected cells/ treatment	No. of tumor-bearing mice	Positive lymph nodes/ lymph nodes examined	Lung	Other
MKL-4				
Vehicle	3	3/10	3	7
TAM[a]	4	5/18	2	2
ICI 182,780	5	4/23	3	4
MKL-F				
Vehicle	6	0/27	3	1
TAM	5	4/20	3	0
ICI 182,780	3	0/14	1	0
4-OHA	3	0/13	0	0
LET	3	1/12	0	0
FGF-1 clone 18				
Vehicle	6	5/24	2	0
TAM	6	3/23	3	3
ICI 182,780	4	2/13	3	1
4-OHA	5	5/18	2	1
LET	5	4/22	3	0

[a] TAM, tamoxifen; LET, letrozole.

endometrium. Uteri harvested from mice injected with either ICI 182,780, 4-OHA, and letrozole weighed less than those from control mice and exhibited a complete lack of endometrial glandular structures (data not shown). Thus, these compounds retained activity, although they had no effect on tumor growth in our experiments.

Metastatic Frequency of Tumors Produced by FGF-transfected MCF-7 Cells in Mice Treated with ICI 182,780 or Aromatase Inhibitors Is Not Affected by Treatment. Because the FGF-4-transfected MKL-F cells and the FGF-1-transfected clone 18 cells stably express bacterial β-galactosidase by virtue of *lacZ* transfection, we were able to detect distant micrometastases from tumors produced by these cells. Although the MKL-4 cells become heterogeneous over time with respect to β-galactosidase expression, some high-expressing cells do remain (52), so animals from the experiment depicted in Fig. 1*A* were also analyzed. Table 1 shows the frequency of metastasis detected for each organ examined. However, there were no significant effects of treatment on reduction of total metastases, distant metastases, lymph node, lung, or other metastases for tumors produced by any of the cell lines. Because we have previously shown that the degree of metastasis in this tumor system is correlated with tumor size in tumors produced by both the MKL-4 and MKL-F cells (51, 53), we evaluated the correlation of individual tumor size with frequency of metastasis in individual mice for the clone 18 cells and found that tumor size and incidence of metastasis were indeed significantly correlated ($P = 0.014$).

Effects of FGF and/or Estrogen on the Residual Immune System of Nude Mice Is Not Responsible for the Estrogen-independent Growth of FGF-transfected MCF-7 Cells. Although nude mice have a T-cell defect, they retain NK cell activity. It has been postulated that the residual NK activity in nude mice is responsible for some xenograft rejection and poor metastatic ability of xenografts (35). Estrogen and tamoxifen have been shown to decrease NK cell activity in nude mice (36), but estrogen increases the ability of NK cells to kill MCF-7 cells in cytotoxicity assays (64). In addition, transforming growth factor β, which might be secreted by MCF-7 cells in response to tamoxifen treatment (65), can decrease NK cell activity (33, 34). FGF-2 (also known as basic FGF or bFGF) has been shown to negatively affect NK activity by decreasing endothelial cell adhesion molecule expression (66), raising the possibility that FGF-1 and/or FGF-4 might have the same effect. In addition, B-cell maturation of nude mice is defective because it lacks appropriate help from T-cells (35). Because of the complexity of possible interactions of estrogen, FGFs, and MCF-7 cells with the immune system, and because of the possibility that the estrogen-independent or tamoxifen-stimulated *in vivo* phenotype of the MKL-4 cells is due to an effect of the transfected FGF and/or estrogen on the remaining immunocompetence in the nude mouse, we injected the MKL-4 clonal line of FGF-4-transfected MCF-7 cells into triply deficient *beige/nude/xid* mice. These mice exhibit intermediate NK activity coupled with defects in maturation of both B and T lymphocytes (35). Tumor growth in this host was somewhat slower than in the athymic nude mice because tumors measured at 74 days (Fig. 2*A*) were smaller than tumors using the same cells in nude mice measured at 61 days (Fig. 1*A*). However, estrogen-independent and tamoxifen-resistant growth was again seen in these animals. Pulmonary and lymphatic micrometastases were present in two of two tumor-bearing mice examined (data not shown). Injection of the clonal MCF-7 cell line ML-20 into this host produced much smaller tumors in estrogen-treated animals, as depicted in Fig. 2*B*. Tumor nodules produced by ML-20 cells in animals treated with tamoxifen were quite small and static, and ultimately they regressed, as has been previously shown in nude mice (21, 67). Although tumor growth was slower and the differences between treatment groups did not reach significance in this host for either cell line, the tumorigenic, tamoxifen-resistant, metastatic phenotype of MKL-4 cells was not altered in this host, and estrogen-independent growth of control ML-20 cells did not occur. We therefore conclude that the residual immunocompetence remaining in nude mice is not important in the estrogen-independent, tamoxifen-resistant *in vivo* growth of these transfectants.

FGF-transfected MCF-7 Clonal Cell Lines Have Diminished *in Vitro* Responses to ICI 182,780. Because ICI 182,780 did not affect the estrogen-independent growth of the FGF-transfected MCF-7 cells *in vivo* and because we have previously shown that the FGF transfectants do not respond to 4-hydroxytamoxifen in estrogen-containing medium to the same extent as the parental cells (21, 22), we determined their growth responses to ICI 182,780 *in vitro*.

In anchorage-independent growth assays using FBS-con-

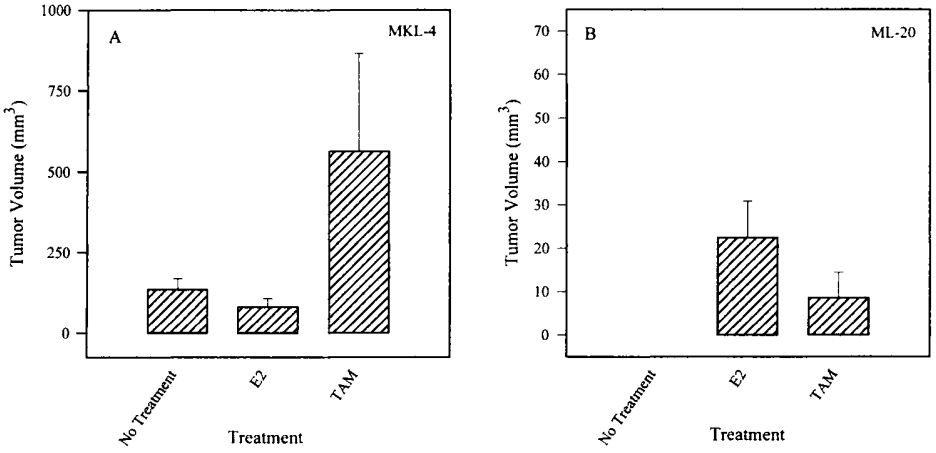

Fig. 2 Tumorigenicity of FGF-4-transfected MCF-7 cells is not increased by injection into *beige/nude/xid* mice. Ten million MKL-4 cells were injected into the mammary fat pads of ovariectomized *beige/nude/xid* mice (four sites per mouse). Tumors were measured as in Fig. 1. The measurements shown are from 74 days after tumor cell injection *A*, volumes of tumors produced by FGF-4-transfected MKL-4 cells; *B*, volumes of tumors produced by ML-20 cells (a clonal line of MCF-7 cells).

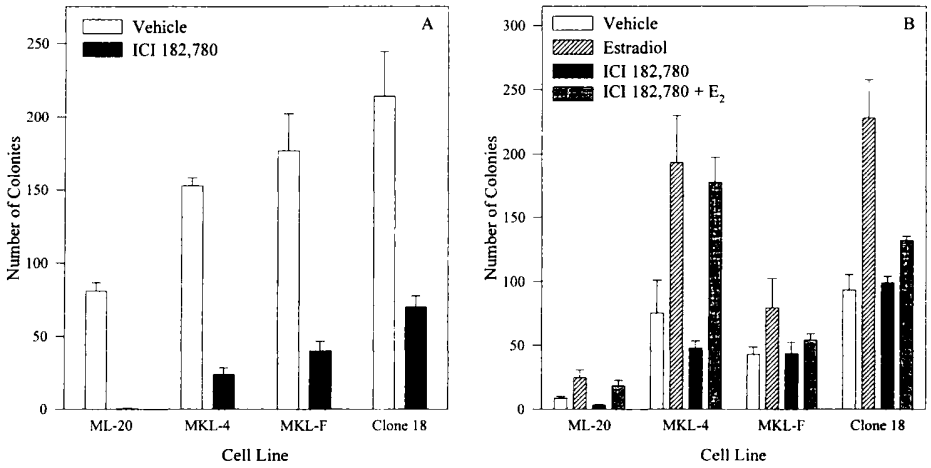

Fig. 3 Effect of ICI 182,780 on anchorage-independent growth of FGF-transfected cells. *A*, for assay of anchorage-independent growth in FBS-containing soft agar, 20,000 cells from each cell line were plated in top agar in 35-mm dishes as described (21). After 8 days of growth, colonies greater than 60 μm in diameter were quantitated using an Omnicon Image Analysis system. *Columns*, mean of triplicate dishes; *bars*, SE. *B*, for assay of anchorage-independent growth in estrogen-depleted medium, cells from each cell line were subjected to a 24-h stripping procedure using 5% CCS in IMEM as described. Twenty thousand stripped cells were plated into top agar in 35-mm dishes, and after 14 days of growth, colonies were quantitated as above. *Columns*, mean of triplicate dishes; *bars*, SE.

taining medium (Fig. 3*A*), as previously reported (21, 22, 74), the baseline colony formation of the FGF transfectants is higher than that of the parental cells ($P < 0.03$). Moreover, when 10^{-7} M ICI 182,780 was added to this medium, the control ML-20 cells and the FGF transfectants were all growth inhibited, but the FGF transfectants still exhibited a higher rate of colony formation than the control ML-20 cells ($P < 0.008$). Whereas colony formation by control transfected cells was inhibited by 99% by ICI 182,780 treatment, the inhibition of colony formation of the FGF-transfected cells ranged from 67 to 84%. For all cell lines tested, addition of 10^{-8} M estradiol to the ICI 182,780-containing medium reversed the inhibition produced by ICI 182,780 (data not shown). Thus, in this assay, the FGF transfectants retained an increased ability for anchorage-independent growth in spite of treatment with ICI 182,780.

In agreement with what we have previously reported (21, 22, 74), in estrogen-depleted medium (Fig. 3*B*), FGF-transfected clonal lines again had significantly greater baseline colony formation than ML-20 cells ($P < 0.05$), with the exception of the MKL-4 line, which just missed significance ($P = 0.06$). As in FBS-containing medium, when ICI 182,780 was added to the medium, the FGF transfectants had significantly increased colony formation when compared with the control ML-20 cells ($P < 0.015$), indicating that the increased colony formation in estrogen-depleted medium is not due to increased sensitivity to residual estrogens remaining after the stripping process. Colony formation in the presence of ICI 182,780 was variably increased by the addition of 10^{-8} M estradiol. Thus, in estrogen-depleted medium, the FGF transfectants again had increased ability for anchorage-independent growth in the presence of ICI 182,780.

Although the anchorage-dependent growth rate of the FGF transfectants did not differ substantially from ML-20 cells in

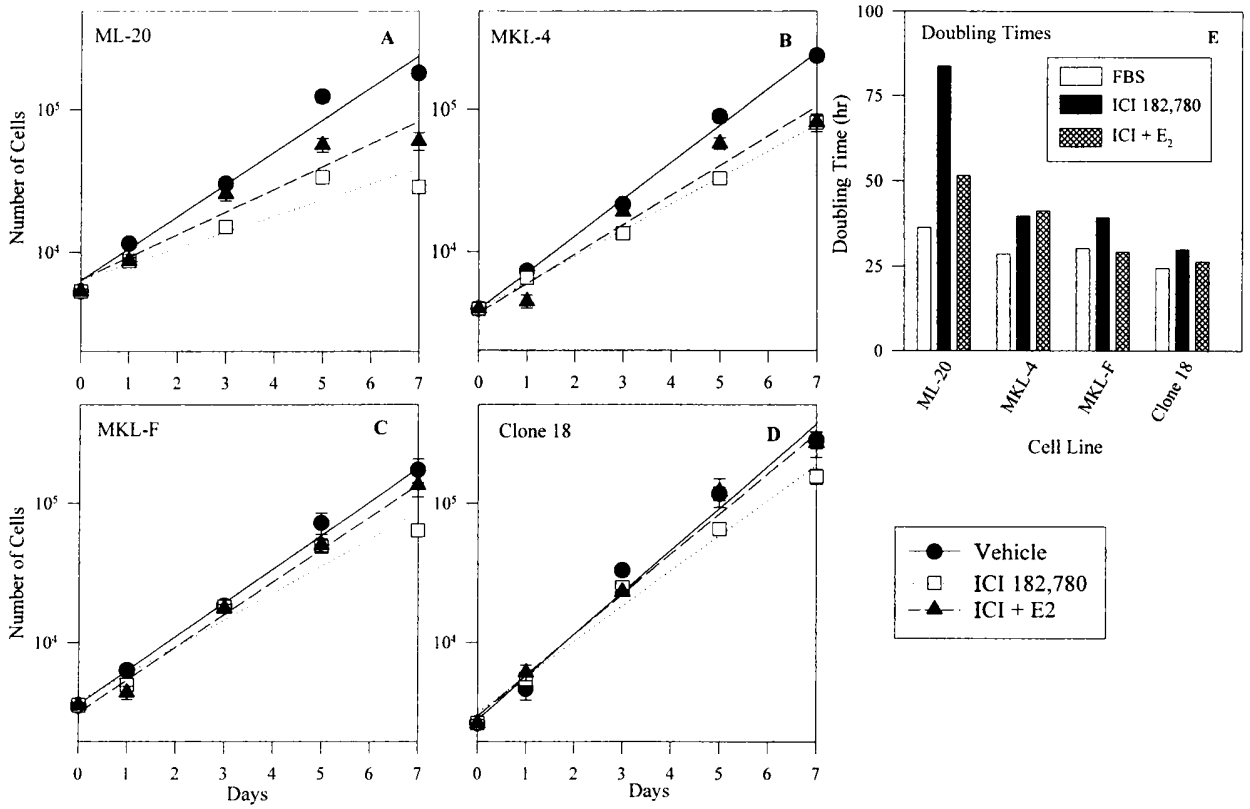

Fig. 4 Effect of ICI-182,780 on anchorage-dependent growth of FGF-transfected MCF-7 cells in 10% FBS. *A*, ML-20 (parental) cells; *B*, FGF-4-transfected MKL-4 cells; *C*, FGF-4-transfected MKL-F cells; *D*, FGF-1-transfected clone 18 cells. *E*, doubling times calculated from the experiments depicted in *A–D*. Cells were plated in 24-well plates at 10,000 cells/well and allowed to attach overnight. The following day (day 0), media were changed to the indicated treatments. Treatment concentrations were as follows: vehicle, 0.1% ethanol; ICI 182,780, 10^{-7} M; estradiol, 10^{-8} M. Cells were harvested and counted at the indicated time points. Linear regression was performed on the data points for each treatment and the lines obtained are shown as indicated. This is a representative experiment of two.

FBS-containing medium (doubling time for ML-20 cells was 36.3 h, *versus* 24.4–30.2 h for the FGF transfectants), in medium supplemented with 10^{-7} M ICI 182,780, their growth rate was slowed to a much lesser extent than the control cells (Fig. 4). The doubling time for ML-20 cells in ICI 182,780-containing medium (83.6 h) was more than twice the doubling times for the FGF transfectants (29.9–39.7 h; Fig. 4*E*), and all of the FGF transfectants had significantly higher growth rates in the presence of ICI 182,780 than ML-20 cells ($P = 0.001$ for MKL-4, 0.007 for MKL-F, and 0.0001 for clone 18). The effect of ICI 182,780 was partially reversed by 10^{-8} M 17β-estradiol in all cell lines tested. Thus, in this assay, as in the anchorage-independent growth assay, the FGF transfectants grew better in the presence of ICI 182,780 than the control ML-20 cells.

Because others have shown that MCF-7 cells that have acquired estrogen independence exhibit increased sensitivity to estradiol or to the partial agonist properties of tamoxifen (68, 69), we determined the concentration-response relationships for 17β-estradiol, ICI 182,780, and 4-hydroxytamoxifen for the control ML-20 cells and the three FGF transfectants. In estrogen-depleted medium, estradiol stimulated growth with approximately the same potency (Table 2 and Fig. 5*A*) in all four cell lines, in agreement with published results for MCF-7 cells (70). As previously shown (Ref. 22 and this report), the maximal effect of estradiol is diminished in stimulating growth of the FGF transfectants, which had a maximal response about 70% of that of the control ML-20 cells (Fig. 5*A*). When ICI 182,780 was added to estrogen-depleted medium supplemented with 10^{-10} M estradiol, its potency was slightly lower for the FGF-transfected cells than for the control cells, with IC_{50} values ranging from 2.5 to 5 times less than that of the parental cells (Table 2 and Fig. 5*B*). In accordance with our previous results for 4-hydroxyta-

Table 2 Potencies of 17β-estradiol in stimulation of growth and ICI 182,780 in inhibition of estradiol stimulation of growth

Potencies were determined graphically from the concentration-response relationships depicted in Fig. 5.

Cell line	EC_{50}, 17β-Estradiol (M)	IC_{50}, ICI 182,780 (M)
ML-20	2×10^{-11}	2×10^{-9}
MKL-4	0.5×10^{-11}	5×10^{-9}
MKL-F	2×10^{-11}	8×10^{-9}
Clone 18	2×10^{-11}	10×10^{-9}

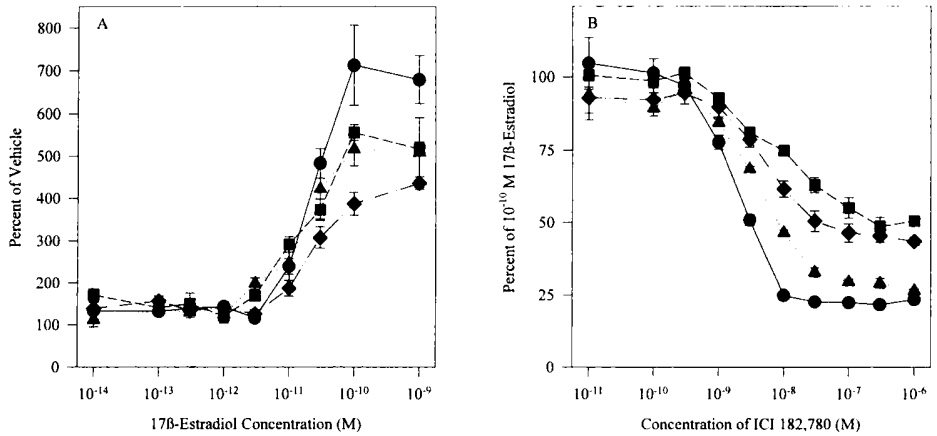

Fig. 5 Concentration-response relationships for 17β-estradiol and ICI 182,780. *A*, 30,000 cells/well of the indicated cell lines were plated in 24-well dishes. After overnight attachment, the cells were stripped with four changes of estrogen-depleted medium over 24 h, after which the indicated concentrations of estradiol were added in fresh estrogen-depleted medium. Cells were harvested after 5 days of growth and counted on a Coulter counter. *Data points* (●, ML-20; ■, MKL-F; ▲, MKL-4; ◆, clone 18), mean of quadruplicate determinations; *bars*, SE. *B*, 20,000 cells/well were plated and stripped as for *A*. Treatments consisted of 10^{-10} M 17β-estradiol alone or with the addition of the indicated concentrations of ICI 182,780. Cells were harvested and counted after 5 days. *Data points* (●, ML-20; ■, MKL-F; ▲, MKL-4; ◆, clone 18), mean of quadruplicate determinations; *bars*, SE.

moxifen (21, 22), the maximal growth-inhibitory effect of ICI 182,780 was less for the FGF transfectants than for the parental cells. All four cell lines exhibited similar small growth stimulation when treated with varying concentrations of 4-hydroxytamoxifen in estrogen-depleted medium (data not shown), in agreement with published reports (68). We conclude that the FGF transfectants do not exhibit substantially increased sensitivity to ER agonists but may be slightly less sensitive to ICI 182,780 when compared with the control ML-20 cells.

FGF-transfected MCF-7 Cells Have Numbers of ERs Similar to the Parental Cells. Others have shown that heregulin-induced growth factor signaling in MCF-7 cells results in down-regulated ERs (25, 26). Because FGF-transfected MCF-7 cells still respond to some extent to estrogen, tamoxifen, and ICI 182,780 *in vivo* and *in vitro* (Figs. 2–5 and Refs. 21 and 22), it seems obvious that they still have ERs. Nonetheless, we measured ER binding on the four cell lines used in these experiments to see whether there were differences between cell lines. Fig. 6 shows ER binding data observed for ML-20, MKL-4, MKL-F, and clone 18 cell lines. ANOVA used with these data revealed no significant differences among cell lines in numbers of binding sites for [^{3}H]estradiol (P = 0.566). Moreover, each cell line contains ample numbers of ERs that are functional, at least with respect to estrogen binding. Although it is difficult to make a direct comparison of these results with those obtained in other laboratories, it would seem that transfections with FGF-1 and FGF-4 produce a different result than transfection of MCF-7 cells with heregulin, which down-regulated ER number.

ERs Are Not Constitutively Activated in FGF-transfected MCF-7 Cells and Remain Capable of Inducing Transcription When Activated with Estrogen. As mentioned, others have reported that growth factor signaling by IGF, EGF, and heregulin can activate ER (25, 71–73). We therefore sought to determine whether ER was constitutively activated in FGF-

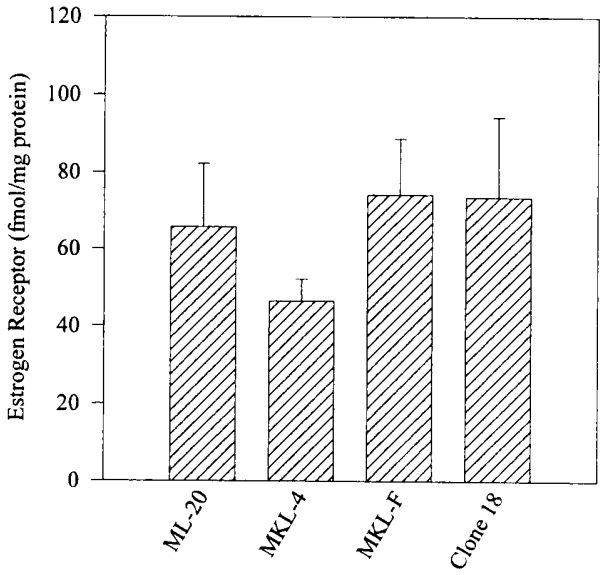

Fig. 6 ER levels by ligand binding assay. Ligand binding was performed using [^{3}H]17β-estradiol on cells stripped of estrogens prior to the assay. *Columns*, means of four separate determinations; *bars*, SE.

transfected MCF-7 cells by determining whether basal levels of expression of estrogen-inducible genes, encoding pS2, cathepsin D, and progesterone receptor were elevated. We also evaluated the capability of the ER expressed in these cells to induce increased levels of these genes when activated by estrogen (Fig. 7). Although basal levels of expression of pS2 and cathepsin D (Fig. 7*B*) or progesterone receptor mRNA (Fig. 7*D*) varied between the cell lines, the FGF-transfected lines did not have consistently elevated levels of expression, which would be ex-

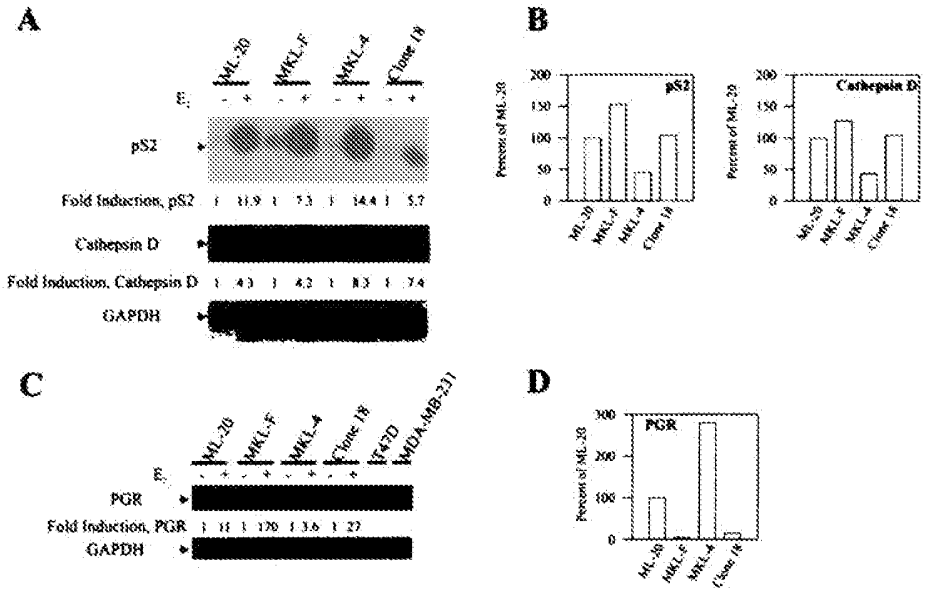

Fig. 7 Basal and estrogen-induced levels of transcripts for estrogen-responsive genes. RNA (0.1 μg) extracted from cells growing in phenol red-free IMEM supplemented with 5% CCS and either 0.1% ethanol or 10^{-8} M 17β-estradiol was subjected to Northern blot analysis for pS2, cathepsin D, and GAPDH transcripts using 30 μg of total RNA (*A*) or semiquantitative RT-PCR for progesterone receptor (*PGR*) and GAPDH transcripts using 0.1 μg of total RNA as template for RT (*C*). RNA from T47D cells (0.02 μg), which express high levels of progesterone receptor, was used as a positive control, and 0.1 μg of RNA from MDA-MB 231 cells, which do not express progesterone receptor, was used as a negative control. Reactions that contained no RNA or no reverse transcriptase yielded no amplified bands (data not shown). Transcript/GAPDH ratios obtained by PhosphoImager analysis were analyzed for fold induction produced by 17β-estradiol (*A* and *C*) or comparison of basal expression with that of control ML-20 cells (*B* and *D*).

pected if the ER were constitutively activated by virtue of the FGF transfection. Similarly, the degree of induction for pS2, cathepsin D, and progesterone receptor (Fig. 7, *A* and *C*) attained by estrogen treatment was variable between cell lines, but the transfected cells did not exhibit consistently increased or decreased sensitivity to estrogen when compared with controls. Thus, the differences in basal expression or degree of estrogen induction for these estrogen-induced genes between the different cell lines is probably due to clonal or experimental variability, rather than being an effect of transfection.

Transient transfection of control and an FGF-overexpressing cell line with an ERE-containing luciferase reporter construct also indicated that ER was not constitutively activated by virtue of FGF transfection. We measured the ability of ERs expressed by the control ML-20 and one FGF-transfected cell line to direct transcription of the luciferase reporter gene driven by an ERE-containing promoter under basal and estrogen or FGF-1 stimulated conditions (Fig. 8). Neither the clone 18 nor the control ML-20 cells exhibited transcriptional activity greater than background, as determined by a similar reporter plasmid in which the ERE sequences were scrambled, under any experimental conditions other than estrogen treatment. In particular, transcription of the reporter was not elevated in the FGF-1-transfected clone 18 cells under estrogen-depleted conditions or decreased when treated with ICI 182,780. Moreover, treatment of the control ML-20 cell line with FGF-1 did not induce transcription of the reporter. We conclude that ERs in the FGF-transfected clone 18 line do not exhibit constitutive transcriptional activity and that activation of FGF receptors of untransfected cells by FGF-1 does not induce ER-mediated transcriptional activity. Thus, taken together, our results indicate that the transfected FGFs are stimulating growth by a mechanism that bypasses the ER-mediated growth-stimulatory pathway.

DISCUSSION

In this report, we have shown that the estrogen-independent *in vivo* growth of FGF-transfected MCF-7 cells is not affected by ICI 182,780 or by either of two aromatase inhibitors. This treatment failure cannot be attributed to an estrogen-, tamoxifen-, or FGF-induced decrease in the immunocompetence remaining in nude mice. The persistence of estrogen-independent growth despite pharmacological strategies to abrogate all estrogenic activity supports the hypothesis that the effect of FGF transfection in promoting such growth is due to a direct effect of the transfected FGF. These findings are supported by our data showing normal numbers of ERs present in the FGF transfectants, which are able to direct expression of known estrogen-induced genes and interact with an ERE-containing promoter in a reporter plasmid but which are not constitutively activated in the FGF-transfected cell lines. The direct effect of FGF transfection on tumor growth may be to promote mitogenesis of the transfected cells by autocrine or intracrine FGF receptor activation. This viewpoint is supported by the generally increased proliferation rate and colony-forming ability of the FGF transfectants under estrogen-depleted tissue culture conditions. However, in addition to having a mitogenic effect on tumor cells, the

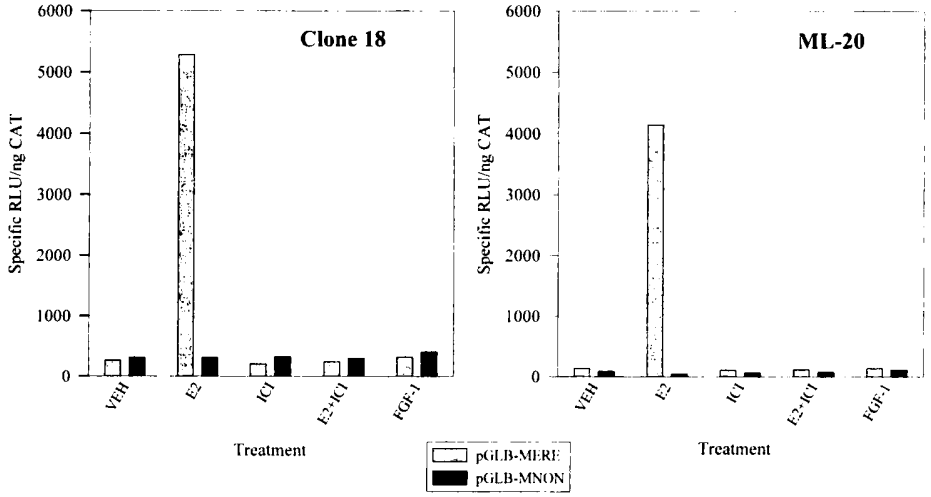

Fig. 8 ER is not constitutively activated in FGF-1-overexpressing or FGF-1-treated MCF-7 cells. Cells stripped of estrogens were transiently transfected with a luciferase reporter plasmid (pGLB, Promega) driven by a mouse mammary tumor virus promoter that contained two ERE sequences (pGLB-MERE) or the same plasmid with the ERE sequences scrambled (pGLB-MNON). Luciferase results were corrected for protein content of lysates, transfection efficiency by comparison with a cotransfected constitutively expressed CAT reporter plasmid (pCMV-CAT), and background luciferase activity.

transfected FGF may also stimulate tumor growth via effects on stromal components of the tumor, such as fibroblasts or endothelial cells. Investigations concerning the relative contributions of autocrine and/or paracrine effects of the transfected FGF-1 to tumor growth of the clone 18 cells have revealed that autocrine or intracrine effects are important for estrogen-independent tumor growth but do not seem to be necessary for either estrogen-stimulated or tamoxifen-resistant tumor growth (74). Moreover, the insensitivity of the estrogen-independent *in vivo* growth of the FGF transfectants to ICI 182,780 or the aromatase inhibitors implies that clinical tamoxifen resistance due to FGF receptor-mediated signaling may not respond to a second hormonal therapy.

The mechanism(s) determining whether a given clinical case of antiestrogen resistance will be responsive to a second hormonal manipulation has not been elucidated and may be multifactorial. Because only 30–40% of patients with acquired tamoxifen resistance have a positive response to a second hormonal therapy, with an additional 30% showing no immediate disease progression after switching therapies (13, 14, 19, 20), ER alterations that render the receptor unresponsive or differentially responsive to hormones or utilization of alternative pathways for growth that do not involve the ER may be responsible for 30–70% of acquired tamoxifen resistance. ER mutations and/or splice variants have been shown to be present in only a low percentage of clinical breast cancer cases (2, 11, 75, 76). Only a subset of these alterations results in receptors capable of producing tamoxifen resistance, and one of these, an exon 5 deletion, is not present more frequently in tamoxifen-resistant patients (77). Therefore, this mechanism is probably not a common mode of tamoxifen resistance.

The recent discovery of the *ERβ* gene (78) has raised the question of whether responses to antiestrogens for this gene product differ from those of the previously studied *ERα*. To date, transcription driven by ERβ at a consensus ERE in response to various antiestrogens does not seem to be different from that of ERα (79). However, AP-1-mediated transcription can be influenced by ER activation independent of ER-ERE interactions (71, 80). Both ICI 182,780 and tamoxifen were shown to activate transfected ERβ-induced transcription at AP-1 sites in a transient transfection assay using MCF-7 cells (79). However, native MCF-7 cells have not been shown to express substantial levels of ERβ (81). Therefore, if our ML-20 cells are representative of native MCF-7 cells described in the literature, we would not expect that the effects of FGF transfection on *in vivo* and *in vitro* growth are due to ERβ-mediated stimulation of transcription at AP-1 sites.

Ligand-independent activation of the ER by growth factors has been shown to occur when activated mitogen-activated protein kinase phosphorylates a serine residue within the AF1 domain of the ER (30–32). This phosphorylation also appears to increase the agonistic effects of tamoxifen but does not alter the antagonistic properties of pure antiestrogens, such as ICI 182,780 (30). Thus, growth factor pathways that result in activation of the ER might be expected to produce a situation in which tumor growth becomes supersensitive to low concentrations of hormonal agonists. Under such circumstances, the partial agonist activity of tamoxifen in promoting growth might be enhanced, whereas a pure antiestrogen would remain growth inhibitory. In one such example, overexpression of ERB-B2 in MCF-7 cells has been shown to result in estrogen-independent and tamoxifen-insensitive growth *in vitro* and *in vivo* (23, 30). Moreover, clinical studies show decreased benefit of tamoxifen treatment in node-negative ERB-B2-overexpressing breast tumors (27). In support of the possibility that activation of this particular signaling pathway results in down-regulation of ERs, similar to the effect of agonist activation, it has been found that activation of ERB-B2 signaling pathways in MCF-7 cells by the ligand, heregulin, activates the ER by phosphorylation (25) and down-regulates ER number (25, 26). This implies that an interaction between the activated ERB-B2 signal transduction pathway and the ER is responsible for the estrogen independence and decreased tamoxifen sensitivity of the ERB-B2 transfectants. This interpretation is further supported by the observation that the effects of added heregulin on ER activation in parental MCF-7 cells can be blocked with ICI 182,780, which also

blocks activation resulting from ERB-B2 overexpression (25). The results using these transfected cell systems, therefore, support the view that interactions between these particular growth factor pathways and the ER can produce tamoxifen resistance but may still be at least partially sensitive to ICI 182,780. Our data in this report suggest that this is not the case with FGF signaling, further suggesting that there are alternative growth-stimulating pathways that bypass the ER.

In vitro growth assays with the FGF transfectants demonstrate an increased estrogen-independent growth and reduced effectiveness of a pure antiestrogen, under both anchorage-dependent and anchorage-independent conditions, and suggest that increased growth is not due to increased potency of residual low levels of estrogen. Because separate experiments using pooled FGF-1 transfectants, as compared with pooled control transfectants, also demonstrate reduced sensitivity to ICI 182,780 (data not shown) similar to that seen with the clonal cell lines used in this study, this effect is unlikely to be due to clonal variation. Moreover, when autocrine FGF-1 signaling in the FGF-1-transfected clone 18 cells is abrogated by subsequent transfection with a dominant negative FGF receptor, sensitivity to ICI 182,780 is restored (74), implying that the reduced sensitivity seen in these experiments is due to FGF receptor activation by the transfected FGF.

Despite the activation of endogenous FGF receptors (82) by the transfected ligand, we did not observe a down-regulation of ERs in these cells, as was reported for the ERB-B2 transfectants, above. Although our data showing a slightly decreased potency of ICI 182,780 in inhibiting estradiol-stimulated growth could be interpreted as showing a slight effect of FGF receptor pathway activation on the affinity of the ER for ICI 182,780, the similar potency of 17β-estradiol in all cell lines argues against sensitization of the ER to small amounts of estradiol being responsible for the estrogen-independent growth of these cells and suggests that FGF overexpression does not alter the affinity of ER for 17β-estradiol. In addition, ICI 182,780 did not reduce anchorage-independent growth to levels of the parental cells, as one would expect if such growth were due to ligand-independent activation of the ER by the transfected FGF (Fig. 3). Moreover, we do not observe enhanced levels of mRNA estrogen-responsive genes, such as *pS2, cathepsin D,* or *progesterone receptor* under estrogen-depleted conditions in our transfectants. Finally, transcriptional assays using an ERE-containing reporter did not show high basal levels of transcriptional activity in the FGF transfectants. When taken together, these data provide evidence for a mechanism by which FGF-stimulated estrogen-independent growth bypasses the ER signal transduction pathway. Moreover, the algebraically additive effects of tamoxifen and estrogen to the estrogen-independent *in vivo* growth of some of the FGF transfectants (21, 22) and continued high frequency of colony formation in ICI 182,780-containing medium argues for an additive effect of ER signaling to that produced by the FGF. Studies to further investigate interactions between ER and FGF receptor signaling pathways in these transfectants are under way in our laboratory.

Previous laboratory attempts to mimic tamoxifen resistance have produced varied results with respect to cross-resistance to steroidal antiestrogens and evidence of interaction of growth factor receptor-activated and ER-activated growth pathways. MCF-7 cells selected for growth in estrogen-depleted medium have acquired supersensitivity to estrogen *in vitro* and *in vivo* and remain sensitive to steroidal antiestrogens (69). When the LCC1 cell line, derived from MCF-7 cells by progressive *in vivo* and *in vitro* selection under estrogen-depleted conditions (83), was subjected to a subsequent *in vitro* selection in tamoxifen, the resulting LCC2 cell line remained sensitive to ICI 182,780 (84). However, a second cell line, designated LCC9, derived from the same LCC1 parent but selected instead with ICI 182,780, is cross-resistant to tamoxifen (85). Other cell lines selected for resistance to the steroidal pure antiestrogens ICI 164,384 or ICI 182,780 are cross-resistant to the other steroidal antiestrogen but not to tamoxifen (86). Additionally, a MCF-7-derived cell line selected for estrogen-independent growth in nude mice exhibits decreased numbers of ERs, is growth stimulated *in vivo* by tamoxifen, and exhibits increased AP-1-mediated transcriptional activation independent of ER activation but retains sensitivity to ICI 182,780 (87). However, tumors produced by MCF-7 cells selected *in vivo* for resistance to ICI 182,780 have shown only weak responses to tamoxifen (88). MCF-7 or T47D cells that inducibly overexpress cyclin D have been found to exhibit resistance to both tamoxifen and steroidal antiestrogens (89). Because cyclin D has been shown to be at the convergence of growth factor and ER pathways that stimulate growth (90), these results could be pertinent to our model system. Together, these diverse data imply heterogeneity for the mechanism of antiestrogen resistance and predict that clinical response to a second hormonal therapy in a given case of breast cancer will depend on the characteristics of that particular tumor.

In summary, our studies implicate direct action by FGFs in the estrogen-independent growth produced by transfection of either FGF-4 or FGF-1 into MCF-7 cells, and they rule out effects resulting from increased sensitivity of the transfectants to small amounts of extraovarian estrogen production. Our data also imply that effects of the transfected FGFs do not involve a direct interaction with the ER itself or ER signal transduction pathways, which ultimately stimulate growth, although the two pathways may still converge or interact at common downstream targets (90). We demonstrate that FGF activity at its receptor is capable of producing an increased proliferation rate of the transfectants under estrogen-depleted conditions *in vitro*, and this effect may be partly responsible for estrogen-independent growth *in vivo*. We and others have found FGF family members to be expressed in breast tissue and/or breast tumors (41–48). Moreover, FGF receptors are rather ubiquitously expressed, have been shown to be present in clinical breast cancer (49, 50), and can be activated by multiple FGF family members as well as heparin, cell adhesion molecules, or activating mutations (91). Thus, it is likely that FGF receptor-mediated signaling is operative in a significant proportion of ER-positive breast tumors. Therefore, the model described in this report might be pertinent to a number of clinical cases of tumor growth that is refractory to therapy with antiestrogens. In contrast to some of the models mentioned above, which may mimic tamoxifen-resistant breast tumors that would respond to a second hormonal therapy, we predict that tumors in which FGF receptor-mediated signaling drives autonomous growth would be refractory to alternative hormonal therapies, as well as to tamoxifen. Therapy of such tumors with agents directed against the autocrine or paracrine effects of

FGFs (53) might result in beneficial effects in such cases and might result in the restoration of antiestrogen sensitivity.

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CHEMICAL ABSTRACTS, vol. 109, no. 3, 18th July 1988, page 73, abstract no. 17199p, Columbus, Ohio, US; N. FROEHLANDER et al.: "Growth hormone and somatomedin C during post-menopausal replacement therapy with estrogen alone and in combination with an antiestrogen", & MATURITAS 1988, 9(4), 297-302

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Description

This invention relates to a therapeutic product for use in a new method of medical treatment and, more particularly, it relates to a product comprising an oestrogen and a pure antioestrogen for use in a new method for the treatment or prophylaxis of perimenopausal or postmenopausal conditions, particularly perimenopausal or postmenopausal osteoporosis. The invention also relates to a pharmaceutical composition comprising an oestrogen and a pure antioestrogen and to the use thereof in the manufacture of a new medicament for use in the treatment or prophylaxis of perimenopausal or postmenopausal conditions.

When a female animal, particularly a human female, enters the perimenopausal stage the animal's ovaries begin to secrete less of the female sex hormones, particularly oestradiol. Symptoms in women at this stage include the following: vasomotor disturbances (hot flushes), urogenital atrophy (particularly affecting the vagina and distal urethra), psychosomatic complaints, changes in lipid metabolism and osteoporosis. The rate of decline of ovarian function and the severity of the above-mentioned symptoms are highly variable between individual women but in a substantial number of individuals the symptoms are sufficiently severe that treatment is required. Oestrogen replacement therapy has been used in women and it is generally recognised to be effective in combatting the typical perimenopausal and post-menopausal symptoms (British Medical Journal, 1987, 295, 914; American Journal of Obstet. and Gynecol., 1987, 156, 1298 and 1347). However oestrogen replacement therapy can also cause uterine hyperplasia, irregular vaginal menstruation and, in a small proportion of women, endometrial cancer (American Journal of Obstet. and Gynecol., 1987, 156, 1313).

To combat the continuous unopposed stimulation of oestrogen-responsive tissues an oestrogen and a progestogen are normally co-administered for part of each treatment period thereby causing regular vaginal menstruation. (American Journal of Obstet. and Gynecol., 1987, 156, 1304). However the continuation of menstrual periods is unattractive to many postmenopausal women and, in addition, progestogens can cause side effects, for example oedema, premenstrual irritability and breast tenderness.

Alternative therapies are therefore required.

It has recently been shown that compounds demonstrating a mixture of oestrogenic and antioestrogenic properties in warm-blooded animals, including humans, may be of use in the treatment of postmenopausal conditions (European Patent Specification No..0178862). Particular compounds stated to have such activity include clomiphene and tamoxifen. Comprehensive reviews of the clinical usage of these compounds are available, for example a review of clomiphene by Clark et al. in Pharmacology and Therapeutics, 1982, Volume 15, pages 467 to 519, and a review of tamoxifen by Furr et al. in Pharmacology and Therapeutics, 1984, Volume 25, pages 127-205.

It has also recently been shown that a treatment regime comprising the dosing to postmenopausal women of the oestrogen ethinyloestradiol led to an increase in serum growth hormone (GH) levels whereas the periodic dosing of both ethinyloestradiol and the antioestrogen tamoxifen led to a reduction of GH levels to pre-treatment levels [N. Froehlander et al., Maturitas, 1988, 9(4), 297 (Chem. Abstracts, 109, 17199p)].

It has also recently been shown that a treatment regime comprising the dosing of a small amount of an oestrogen, for example oestrone sulphate or natural conjugated oestrogens, followed by the dosing of an antioestrogen, for example tamoxifen or clomiphene led to the partial inhibition of the maximum oestrogen-induced stimulation of uterine endometrial tissue (A. Kauppila et al., Gynecol. obstet. Invest., 1988, 25, 58 and Arch. Gynecol., 1983, 234, 49).

It has now been found that administration of an oestrogen and a pure antioestrogen, whether simultaneously, sequentially or separately, results in the oestrogen being selectively effective in some oestrogen-responsive tissues, for example bone, and being selectively opposed in other oestrogen-responsive tissues, for example the endometrium of the uterus, and this is the basis of the present invention.

A pure antioestrogen is a compound which possesses antioestrogenic activity and no oestrogenic activity. This may be demonstrated in rats by the effect of the compound in antagonising the increase in weight of the uterus of an immature female rat produced by administering oestradiol benzoate to said rat. Thus, when each of a pure antioestrogen and oestradiol benzoate are administered for 3 days to such a rat, a smaller increase in uterine weight is produced than the substantial increase which would be produced by the administration of oestradiol benzoate alone. Unlike the known antioestrogens tamoxifen and clomiphene, when a pure antioestrogen is administered alone to a rat no increase in uterine weight whatsoever is observed.

It is disclosed in European Patent Specification No. 138504 that certain preferred steroidal antioestrogens are pure antioestrogens. It is also disclosed in European Patent No. 124369 that certain preferred non-steroidal antioestrogens are pure antioestrogens.

According to the present invention there is provided a product comprising an oestrogen and a pure antioestrogen as a combined preparation for simultaneous, sequential or separate use in selective oestrogen therapy of perimenopausal or postmenopausal conditions, the pure antioestrogen being selected from

N-n-butyl-N-methyl-, N-1H,1H-heptafluorobutyl-N-methyl- or N,N-(3-methylpentamethylene)-11-(3,17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl)undecanamide;

N-n-butyl- or N-1H,1H-heptafluorobutyl-3-p-[4-(3,17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl)butyl]-phenylpropionamide;

7α-(10-p-chlorophenylthiodecyl)-, 7α-(10-p-chlorophenylsulphinyldecyl)-, 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]-, 7α-[10-(4,4,4-trifluorobutylsulphinyl)decyl]- or 7α-[10-(p-chlorobenzylsulphinyl)decyl]-oestra-1,3,5(10)-triene-3,17β-diol;

7α-(9-n-heptylsulphinylnonyl)oestra-1,3,5(10)-triene-3,17β-diol; and

a compound of the formula:-

NU-A-X-R^1

wherein NU is 6-hydroxy-2-p-hydroxyphenylnaphth-1-yl and A is $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ or $-(CH_2)_5-$(1,4-phenylene)-$(CH_2)_2-$;

or NU is 1,2,3,4-tetrahydro-6-hydroxy-2-p-hydroxyphenylnaphth-1-yl (either 1RS,2RS or 1RS,2SR isomer), or 1,2,3, 4-tetrahydro-6-hydroxy-2-p-hydroxyphenyl-2-methylnaphth-1-yl (either the 1RS,2RS or 1RS,2SR isomer), and A is $-(CH_2)_{10}-$, $-(CH_2)_{11}-$or $-(CH_2)_4-$(1,4-phenylene)-$(CH_2)_2-$;

or NU is (1RS,2RS)-5-hydroxy-2-p-hydroxyphenylindan-1-yl or (1RS,2RS)-5-hydroxy-2-p-hydroxyphenyl-2-methylindan-1-yl and A is $-(CH_2)_{10}-$, $-(CH_2)_{11}-$or $-(CH_2)_4-$(1,4-phenylene)-$(CH_2)_2-$;

and wherein XR^1 is $-CONR^1R^2$ wherein R^2 is hydrogen or methyl and R^1 is n-butyl, 1H,1H-heptafluorobutyl, n-pentyl or n-hexyl, or XR^1 is $-SR^1$, SOR^1 or $-SO_2R^1$ wherein R^1 is n-pentyl, n-hexyl, 4,4,5,5,5-pentafluoropentyl or 1H,1H,2H,2H,3H,3H-heptafluorohexyl.

In a particular product of the invention the oestrogen component of a product of the invention is oestradiol, ethinyloestradiol, oestriol, oestrone, natural conjugated oestrogens, piperazine oestrone sulphate, mestranol, chlorotrianisene, dienoestrol, stilboestrol or hexoestrol or a pharmaceutically-acceptable ester thereof.

A pharmaceutically-acceptable ester of the oestrogen component of a product of the invention is, for example, an alkyl or aryl ester each of up to 12 carbon atoms. It will be appreciated that an ester of a steroidal oestrogen may be formed at the 3-position, the 17-position or at both of these positions. It will also be appreciated that an ester may be formed at one or both of the phenolic groups in some non-steroidal oestrogens, for example stilboestrol and hexoestrol. A suitable alkyl ester of up to 12 carbon atoms is, for example, an acetate, propionate, butyrate, valerate, hexanoate, heptanoate, octanoate, cyclopentylpropionate, nonanoate, decanoate, undecanoate or dodecanoate. A suitable aryl ester of up to 12 carbon atoms is, for example, a benzoate, toluate or naphthoate. A preferred pharmaceutically-acceptable ester of the oestrogen component of a product of the invention includes, for example, oestradiol benzoate, oestradiol cyclopentylpropionate, oestradiol dipropionate, oestradiol heptanoate, oestradiol undecanoate, oestradiol valerate and stilboestrol dipropionate.

In a further particular product of the invention the pure antioestrogen is

N-n-butyl-, N-n-butyl-N-methyl-, N-n-pentyl, N-(1H,1H-heptafluorobutyl)-or N-(1H,1H-heptafluorobutyl)-N-methyl-3-p-[5-(6-hydroxy-2-p-hydroxyphenylnaphth-1-yl)pentyl]phenylpropionamide;

N-methyl-N-(1H,1H-heptafluorobutyl)-p-[4-[(1RS,2RS)-6-hydroxy-2-p-hydroxphenyl-2-methyl-1,2,3,4-tetrahydronaphth-1-yl]-butyl] phenylpropionamide;

(1RS,2RS)-1-[4-[p-(2-n-hexylthioethyl)phenyl]butyl]-2-p-hydroxyphenyl-1,2,3,4-tetrahydronaphth-6-ol or the corresponding 4,4,5,5,5-pentafluoropentylthio derivative, or the corresponding hexylsulphinyl, hexylsulphonyl or pentafluoropentylsulphinyl derivatives;

2-p-hydroxyphenyl-1-[5-[p-(2-n-hexylthioethyl)phenyl]pentyl]naphth-6-ol or the corresponding hexylsulphinyl derivative; or

(1RS, 2RS)-1-[4[p-(2-n-hexylthioethyl)phentyl]butyl]-2-p-hydroxyphenyl-2-methyl-1,2,3,4-tetrahydronaphth-6-ol or the corresponding 4,4,5,5,5-pentafluoropentylthio derivative, or the corresponding hexylsulphinyl or pentafluoropentylsulphinyl derivative, or the corresponding (1RS,2SR) isomers of both the hexylthio and hexylsulphinyl derivatives.

A preferred product of the invention comprises an oestrogen and a pure antioestrogen for use as stated above wherein the oestrogen is oestradiol or ethinyloestradiol, or a pharmaceutically-acceptable ester thereof, and the pure antioestrogen is 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol or (1RS,2RS)-2-p-hydroxyphenyl-2-methyl-1-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]-

1,2,3,4-tetrahydronaphth-6-ol.

A particularly preferred product of the invention comprises an oestrogen and a pure antioestrogen for use as stated above wherein the oestrogen is oestradiol, oestradiol benzoate, oestradiol valerate or oestradiol undecanoate and the pure antioestrogen is 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]-oestra-1,3,5(10)-triene-3,17β-diol.

According to a further feature of the invention there is provided a process for the manufacture of a product comprising an oestrogen and a pure antioestrogen as a combined preparation for simultaneous, sequential or separate use in selective oestrogen therapy of perimenopausal or postmenopausal conditions, which process comprises bringing together said oestrogen and said pure antioestrogen.

In a further feature of the invention there is provided a process for the manufacture of a product comprising an oestrogen and a pure antioestrogen for simultaneous use in selective oestrogen therapy of perimenopausal or postmenopausal conditions, which process comprises bringing into admixture said oestrogen and said pure antioestrogen.

A product of the invention may be administered to a warm-blooded animal, including a human, in the form of a pharmaceutical composition. Thus according to a further feature of the present invention there is provided a pharmaceutical composition which comprises the product of the invention together with a pharmaceutically-acceptable diluent or carrier.

As mentioned above a product of the invention is useful for selective oestrogen therapy of perimenopausal or postmenopausal conditions. It will be understood that there is no absolute requirement that the oestrogen and pure antioestrogen components of the product of the invention must be dosed simultaneously. Sequential or separate use of these components may also provide selective oestrogen therapy and such use is to be understood to fall within the definition of a product of the invention. Thus it will be appreciated that a pharmaceutical composition according to the present invention includes a composition comprising an oestrogen, a pure antioestrogen and a pharmaceutically-acceptable diluent or carrier. Such a composition conveniently provides the product of the invention for simultaneous use in selective oestrogen therapy of perimenopausal or postmenopausal conditions. A pharmaceutical composition according to the present invention also includes separate compositions comprising a first composition comprising an oestrogen and a pharmaceutically-acceptable diluent or carrier, and a second composition comprising a pure antioestrogen and a pharmaceutically-acceptable diluent or carrier. Such a composition conveniently provides the product of the invention for sequential or separate use in selective oestrogen therapy of perimenopausal or postmenopausal conditions.

The compositions of the invention may be in a form suitable for oral use (for example as tablets, capsules, aqueous or oily suspensions, emulsions or dispersible powders or granules), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions; for example for use within a transdermal patch), for parenteral administration (for example as a sterile aqueous or oily solution or suspension for intravenous, subcutaneous, intramuscular or intravascular dosing), or as a suppository for rectal dosing or as a pessary for vaginal dosing.

The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art.

Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or alginic acid; binding agents such as gelatin or starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl p-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal tract, or to improve their stability and/or appearance, in either case using conventional coating agents and procedures well known in the art.

Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions generally contain the active ingredient in finely powdered form together with one or more suspending agents, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as lecithin or condensation products of an alkylene oxide with fatty acids (for example polyoxethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation

products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives (such as ethyl or propyl p-hydroxybenzoate, anti-oxidants (such as ascorbic acid), colouring agents, flavouring agents, and/or sweetening agents (such as sucrose, saccharine or aspartame).

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil (such as arachis oil, castor oil, sesame oil or coconut oil) or in a mineral oil (such as liquid paraffin). The oily suspensions may also contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set out above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water generally contain the active ingredient together with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, such as sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as castor oil, soya bean oil or arachis oil, or a mineral oil, such as, for example, liquid paraffin or a mixture of any of these. Suitable emulsifying agents may be, for example, naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides (for example sorbitan monooleate) and condensation products of the said partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring and preservative agents.

The pharmaceutical compositions may also be in the form of sterile injectable aqueous or oily suspensions, which may be formulated according to known procedures using one or more of the appropriate dispersing or wetting agents and suspending agents which have been mentioned above. A sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example a solution in 1,3-butanediol, in a vegetable oil (such as arachis oil, castor oil or coconut oil) or in a mineral oil (such as liquid paraffin).

Conveniently the subcutaneous or intramuscular injection of an aqueous suspension or an oily solution or suspension of a pharmaceutical composition of the invention provides a depot of the active ingredients at the injection site from which those ingredients may leach out over a period of time to provide the sustained release thereof.

Suppository formulations may be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

Topical formulations, such as creams, ointments, gels and aqueous or oily solutions or suspensions, may generally be obtained by formulating an active ingredient with a conventional, topically acceptable, vehicle or diluent using conventional procedure well known in the art.

According to a further feature of the invention there is provided a process for the manufacture of a pharmaceutical composition as defined above which comprises bringing into admixture a product as defined above together with a pharmaceutically-acceptable diluent or carrier.

The invention also provides the use of a product as defined above for the manufacture of a combined preparation for use simultaneously, sequentially or separately in selective oestrogen therapy of perimenopausal or postmenopausal conditions.

It will be appreciated that the definition of the product of the invention and the pharmaceutical composition of the invention includes only those products or compositions which are useful in a new method for the treatment or prophylaxis of perimenopausal or postmenopausal condition. Pharmaceutical compositions comprising an oestrogen and a pure antioestrogen, together with a pharmaceutically-acceptable diluent or carrier, are novel. In European Patent Sepcifications Nos. 138504 and 124369 it is disclosed that the antioestrogenic activity of the compounds disclosed therein may be demonstrated by the co-administration of a test compound and oestradiol benzoate to an immature female rat. Antioestrogenic activity is demonstrated by antagonism of the increase in weight of the uterus of the rat which is produced when oestradiol benzoate alone is administered to said rat. It is to be noted that, during those tests, the oestradiol benzoate was given by subcutaneous injection whereas the test compound was given separately either orally or subcutaneously.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising an oestrogen and a pure antioestrogen together with a pharmaceutically-acceptable diluent or carrier.

The pharmaceutical compositions of this feature of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients well known in the art such as, for example, those disclosed above.

This aspect of the invention also provides a process for the manufacture of a pharmaceutical composition as defined immediately above which comprises bringing into admixture an oestrogen and a pure antioestrogen together with a pharmaceutically-acceptable diluent or carrier.

The invention also provides the use of a pharmaceutical composition as defined immediately above for the manufacture of a new medicament for use in selective oestrogen therapy of perimenopausal or postmenopausal conditions.

As stated above a product of the invention is of use in selective oestrogen therapy of perimenopausal or postmenopausal conditions. Selective oestrogen therapy may be demonstrated using the standard procedure set out below:-

a) an in vivo assay measuring the antioestrogenic activity of a compound and any oestrogenic activity possessed by that compound. This may be demonstrated in rats by the effect of the compound in antagonising the increase in weight of the uterus of an immature female rat produced by administering oestradiol benzoate to said rat. Thus, when each of a pure antioestrogen and oestradiol benzoate are administered for 3 days to such a rat, a smaller increase in uterine weight is produced than the substantial increase which would be produced by the administration of oestradiol benzoate without the pure antioestrogen. Unlike the known antioestrogens tamoxifen and clomiphene, when a pure antioestrogen is administered alone to a rat no increase in uterine weight whatsoever is observed.

The oestrogenic activity of a compound may be demonstrated in rats by the effect of the compound when it is administered alone to said rat on the uterine weight of the animal.

b) An in vivo assay in mature rats measuring the antioestrogenic activity of a compound by the effect of the compound when dosed during a test period of 28 days in antagonising the protective effect on the animals' bone density of their endogenous oestrogens. The bone density of a group of ovariectomised rats in which endogenous oestrogen levels are much reduced serves as a control for the effect expected to be produced by a fully effective antioestrogen.

The antioestrogenic activity of the compound in mature rats can also be measured in the same assay by measuring the effect of the compound in antagonising the effect of the animals' endogenous oestrogens which serve to increase the weight of their uteri.

A comparison of the potencies of the antioestrogenic effects of a compound as measured by its effects on the animals' bone density and uterine weights allows the selectivity of the antioestrogenic effects of the compound to be measured.

Although the pharmacological properties of a product of the invention vary with the structures of the oestrogenic and antioestrogenic components and with the route of administration, in general a product of the invention comprises:-

(i) an oestrogen which possesses oestrogenic activity in the above test (a) at doses in the range, for example, 0.002-2.0 mg/kg orally or in the range, for example, 0.0001-0.1 mg/kg subcutaneously;

(ii) a pure antioestrogen which possesses antioestrogenic activity in the above tests (a) and (b) at doses in the range, for example, in test (a): ED_{50} 0.05-5 mg/kg orally or ED_{50} 0.01-1.0 mg/kg subcutaneously; in test (b): antiuterotrophic effect:- ED_{50} < 20 mg/kg/day orally, < 2 mg/kg/day subcutaneously or intramuscularly and < 10 mg/kg/injection when dosed as an intramuscular depot injection; reduction in bone density:- ED_{50} > 20 mg/kg/day orally, > 5 mg/kg/day subcutaneously or intramuscularly and > 10 mg/kg/injection when dosed as an intramuscular depot injection.

A product of the invention is thereby seen to be surprisingly selective as the activity of the pure antioestrogen component is expressed to a high degree within uterine tissue but to a lesser degree on bone.

The size of the dose, for therapeutic or prophylatic purposes, of a product of the invention as defined above will naturally vary according to the nature and severity of the conditions presented, the age and menopausal state of the animal and the route of administration.

In general the minimum quantity of the oestrogenic component of a product of the invention as defined above will be chosen so as to provide a beneficial effect with regard to the nature and severity of the conditions presented. The quantity of the pure antioestrogenic component is then chosen to antagonise to a substantial degree the effect of the oestrogenic component on the uterine tissue. Methods of evaluating the condition of uterine tissue are well known to the man skilled in the art, for example, by examination of a

specimen of endometrial tissue taken by, for example, suction or, for example, by way of a biopsy.

So far as the oestrogenic component of a product of the invention as defined above is concerned the size of the dose and routes of administration conventionally utilised in oestrogen replacement therapy may be used. Thus, for example, a tablet containing, for example, 0.5 to 2 mg of oestradiol, oestradiol benzoate, natural conjugated oestrogens or oestradiol valerate may be administered daily. Alternatively a tablet containing 10 to 100 μg of ethinyloestradiol may be administered daily. Alternatively the oestrogenic component may be administered by, for example, intramuscular injection utilising, for example, 1 to 10 mg of oestradiol benzoate dissolved in an oil such as ethyl oleate; for example, transdermal means utilising, for example, 10-100 μg of oestradiol contained within a transdermal patch; or, for example, vaginal application utilising, for example, daily application of 0.5 to 2 mg of natural conjugated oestrogens contained within 0.5 to 5 ml of a cream.

So far as the antioestrogenic component of a product of the invention as defined above is concerned the size of the dose is chosen such that the effect of the oestrogenic component on uterine tissue is antagonised to a substantial degree whereas the beneficial effect of the oestrogenic component on bone is substantially unopposed. Thus, for example, the antioestrogenic component may be formulated in like manner to the oestrogenic component, for example as a tablet, an oily solution suitable for intramuscular injection, within a transdermal patch, or within a cream suitable for vaginal application.

The daily administration of one or more tablets containing conveniently 50 mg to 5 g, and preferably 50 mg to 500 mg, of a pure antioestrogen may be used. Preferably the pure antioestrogen may be administered by the periodic intramuscular injection of, for example, an aqueous suspension or an oily solution or suspension containing 50 mg to 5 g of the pure antioestrogen. Preferably an oily solution, for example a solution containing arachis or castor oil, an alcohol such as benzyl alcohol and 50 mg to 500 mg of the pure antioestrogen is employed. Such an injection provides a depot of the pure antioestrogen which thereafter leaches out from the injection site to provide a selective antioestrogenic effect for a period of, for example, one to six weeks.

As mentioned above a product of the invention is useful for selective oestrogen therapy of peri-menopausal or postmenopausal conditions. As previously mentioned perimenopausal and postmenopausal conditions include, for example, vasomotor disturbances (hot flushes), urogenital atrophy (particularly affecting the vagina and the distal urethra), psychosomatic complaints, changes in the lipid metabolism and oesteoporosis. The selective antioestrogenic effect of the pure antioestrogenic component of a product of the invention, as demonstrated by a greater antioestrogenic effect on the uterus of a rat than on the bone of the rat, allows the beneficial effect of the oestrogenic component of the product of the invention to be selectively applied to the bone and prevents the detrimental effect of an unopposed oestrogenic effect on the uterus. The utero-selective effect of the pure antioestrogenic component of a product of the invention will allow the beneficial effect of the oestrogenic component of a product of the invention to be applied to other oestrogen-responsive tissues, for example those causing vasomotor disturbances, pyschosomatic complaints and changes in lipid metabolism.

The invention will now be illustrated in the following non-limiting Examples.

Example 1

Assay in Mature Rats of the Selective Antioestrogenic Activity of a Pure Antioestrogen

The pure antioestrogen used was (1RS,2RS)-2-p-hydroxyphenyl-2-methyl-1-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]-1,2,3,4-tetrahydronaphth-6-ol.

The compound was given subcutaneously as a solution in arachis oil at doses of 2 mg/kg/day and 10 mg/kg/day to two groups of 5 mature rats for a total of 28 days. Further groups of 5 mature rats served as an untreated control group. A further group of 5 mature rats was ovariectomised to serve as another control group. At the end of the treatment period the weights of the uteri of the test and control groups of rats were determined. In addition the femurs were dissected, weighed and their volumes were determined using Archimedes Principle. The femurs were then burned and the residual ash was weighed. From these data, gross femur density and bone mineral density were calculated as follows:-

Gross Femur Density = Femur Weight/Femur Volume
Bone Mineral Density = Femur Ash Weight/Femur Volume

The results shown below in Tables I and II demonstrate that at a dose of 2 mg/kg/day subcutaneously the test compound selectively inhibits the action of the animals' endogenous oestrogen on their uteri (90%

inhibition of uterine weight) whereas there was no significant inhibition of either bone mineral density or of gross femur density.

TABLE I

Treatment	Uterine Weight (mg)	Calculated Inhibition
Untreated Controls	382 ± 34	
Ovariectomised Controls	111 ± 14	
Test Compound at 2 mg/kg/day s.c.	135 ± 8	91%
Untreated Controls	369 ± 47	
Ovariectomised Controls	99 ± 5	
Test Compound at 10 mg/kg/day s.c.	125 ± 4	90%

TABLE II

Treatment	Gross Femur Density (g/ml)	Calculated Inhibition	Bone Mineral Density (g/ml)	Calculated Inhibition
Untreated Controls	1.612 ± 0.010		0.742 ± 0.009	
Ovariectomised Controls	1.569 ± 0.010		0.685 ± 0.010	
Test Compound at 2 mg/kg/day s.c.	1.604 ± 0.006	19%*	0.730 ± 0.007	21% *
Untreated Controls	1.629 ± 0.014		0.766 ± 0.005	
Ovariectomised Controls	1.571 ± 0.007		0.704 ± 0.005	
Test Compound at 10 mg/kg/day s.c.	1.580 ± 0.004	84%	0.727 ± 0.005	63%

* This level of inhibition was not statistically significant.

Example 2

The experiment described in Example 1 was repeated except that the pure antioestrogen used was 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol. This compound was given at a series of doses as a daily intramuscular injection, the compound having been dissolved in a mixture of propylene glycol: ethanol: water: poloxamer 407. The formulation contained 25 mg of test compound, 100 mg of ethanol (96%), 100 mg of water, 20 mg of poloxamer 407 and sufficient propylene glycol to bring the solution to a volume of 1 ml.

The results shown below in Tables III and IV demonstrate that at all doses tested the compound selectively inhibits the action of the animals' endogenous oestrogen on their uteri whereas there was no significant inhibition of gross femur density.

TABLE III

Treatment	Uterine Weight (mg)	Calculated Inhibition
Untreated Controls	302 ± 36	
Ovariectomised Controls	70 ± 1.3	
Test Compound (mg/kg)		
0.1	208 ± 17	41
0.3	174 ± 16	55
1	94 ± 9	90
3	103 ± 2	86

TABLE IV

Treatment	Gross Femur Density (g/ml)	Calculated Inhibition
Untreated Controls	1.523 ± 0.008	
Ovariectomised Controls	1.491 ± 0.006	
Test Compound at (mg/kg)		
0.1	1.528 ± 0.005	0%
0.3	1.528 ± 0.008	0%
1	1.532 ± 0.005	0%
3	1.533 ± 0.005	0%

Example 3

The pure antioestrogen used was 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol.

Each of a series of selected doses of this compound was dissolved in a mixture of castor oil and benzyl alcohol and given by intramuscular injection to a group of 5 mature rats. The formulation contained 50 mg of the test compound, 400 mg of benzyl alcohol and sufficient castor oil to bring the solution to a volume of 1 ml. In each case a second dose was administered two weeks after the first dose. Two weeks after the second dose the weights of the uteri of the test groups of rats were determined. In addition the femurs were dissected and analysed for Gross Femur Density as in Example 1.

A further group of rats, given two injections of castor oil separated by a two week period, served as an intact control group. A further group of rats was ovariectomised to serve as another control group.

The results shown below in Tables V and VI demonstrate that at all doses tested the compound selectively inhibits the action of the animals' endogenous oestrogen on their uteri whereas at the two higher test doses there was no significant inhibition of gross femur density.

TABLE V

Treatment	Uterine Weight (mg)	Calculated Inhibition
Intact Controls	318 ± 31	
Ovariectomised Controls	76 ± 4	
Test Compound (mg/rat/dose)		
0.75	202 ± 23	48
1.25	180 ± 15	57
2.5	123 ± 12	81

TABLE VI

Treatment	Gross Femur Density (g/ml)	Calculated Inhibition
Intact Controls	1.584 ± 0.007	
Ovariectomised Controls	1.521 ± 0.005	
Test Compound (mg/rat/dose)		
0.75	1.562 ± 0.004	35
1.25	1.576 ± 0.004	13*
2.5	1.569 ± 0.007	23*

* This level of inhibition was not statistically significant.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A product comprising an oestrogen and a pure antioestrogen as a combined preparation for simultaneous, sequential or separate use in selective oestrogen therapy of perimenopausal or postmenopausal conditions, the pure antioestrogen being selected from

N-n-butyl-N-methyl-, N-1H,1H-heptafluorobutyl-N-methyl- or N,N-(3-methylpentamethylene)-11-(3,17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl)undecanamide;

N-n-butyl- or N-1H,1H-heptafluorobutyl-3-p-[4-(3,17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl)butyl]-phenylpropionamide;

7α-(10-p-chlorophenylthiodecyl)-, 7α-(10-p-chlorophenylsulphinyldecyl)-, 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]-, 7α-[10-(4,4,4-trifluorobutylsulphinyl)decyl]- or 7α-[10-(p-chlorobenzylsulphinyl)decyl]-oestra-1,3,5(10)-triene-3,17β-diol;

7α-(9-n-heptylsulphinylnonyl)oestra-1,3,5(10)-triene-3,17β-diol; and

a compound of the formula:-

NU-A-X-R^1

wherein NU is 6-hydroxy-2-p-hydroxyphenylnaphth-1-yl and A is -$(CH_2)_{10}$-, -$(CH_2)_{11}$- or -$(CH_2)_5$-(1,4-phenylene)-$(CH_2)_2$-;

or NU is 1,2,3,4-tetrahydro-6-hydroxy-2-p-hydroxyphenylnaphth-1-yl (either 1RS,2RS or 1RS,2SR isomer), or 1,2,3,4-tetrahydro-6-hydroxy-2-p-hydroxyphenyl-2-methylnaphth-1-yl (either the 1RS,2RS or 1RS,2SR isomer), and A is -$(CH_2)_{10}$-, -$(CH_2)_{11}$-or -$(CH_2)_4$-(1,4-phenylene)-$(CH_2)_2$-;

or NU is (1RS,2RS)-5-hydroxy-2-p-hydroxyphenylindan-1-yl or (1RS,2RS)-5-hydroxy-2-p-hydroxyphenyl-2-methylindan-1-yl and A is -$(CH_2)_{10}$-, -$(CH_2)_{11}$-or -$(CH_2)_4$-(1,4-phenylene)-$(CH_2)_2$-;

and wherein XR^1 is -$CONR^1R^2$ wherein R^2 is hydrogen or methyl and R^1 is n-butyl, 1H,1H-heptafluorobutyl, n-pentyl or n-hexyl, or XR^1 is -SR^1, SOR^1 or -SO_2R^1 wherein R^1 is n-pentyl, n-hexyl, 4,4,5,5,5-pentafluoropentyl or 1H,1H,2H,2H,3H,3H-heptafluorohexyl.

2. A product as claimed in claim 1 wherein the oestrogen is oestradiol, oestradiol benzoate, oestradiol valerate or oestradiol undecanoate and the pure antioestrogen is 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol.

3. A process for the manufacture of a product comprising an oestrogen and a pure antioestrogen as a combined preparation for simultaneous, sequential or separate use in selective oestrogen therapy of perimenopausal or postmenopausal conditions as claimed in any one of claims 1 and 2, which process comprises bringing together said oestrogen and said pure antioestrogen.

4. A pharmaceutical composition comprising a product as claimed in any one of claims 1 and 2 together with a pharmaceutically-acceptable diluent or carrier.

5. The use of a product as claimed in any one of claims 1 and 2 for the manufacture of a combined preparation for use simultaneously, sequentially or separately in selective oestrogen therapy of

perimenopausal or postmenopausal conditions.

Claims for the following Contracting States : ES, GR

1. A process for the manufacture of a product comprising an oestrogen and a pure antioestrogen as a combined preparation for simultaneous, sequential or separate use in selective oestrogen therapy of perimenopausal or postmenopausal conditions, the pure antioestrogen being selected from

N-n-butyl-N-methyl-, N-1H, 1H-heptafluorobutyl-N-methyl- or N,N-(3-methylpentamethylene)-11-(3,17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl)undecanamide;

N-n-butyl- or N-1H,1H-heptafluorobutyl-3-p-[4-(3, 17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl)butyl]-phenylpropionamide;

7α-(10-p-chlorophenylthiodecyl)-, 7α-(10-p-chlorophenylsulphinyldecyl)-, 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]-, 7α-[10-(4,4,4-trifluorobutylsulphinyl)decyl]- or 7α-[10-(p-chlorobenzylsulphinyl)decyl]-oestra-1,3,5(10)-triene-3,17β-diol;

7α-(9-n-heptylsulphinylnonyl)oestra-1,3,5(10)-triene-3,17β-diol; and

a compound of the formula:-

NU-A-X-R^1

wherein NU is 6-hydroxy-2-p-hydroxyphenylnaphth-1-yl and A is -$(CH_2)_{10}$-, -$(CH_2)_{11}$- or -$(CH_2)_5$-(1,4-phenylene)-$(CH_2)_2$-;

or NU is 1,2,3,4-tetrahydro-6-hydroxy-2-p-hydroxyphenylnaphth-1-yl (either 1RS,2RS or 1RS,2SR isomer), or 1,2,3, 4-tetrahydro-6-hydroxy-2-p-hydroxyphenyl-2-methylnaphth-1-yl (either the 1RS,2RS or 1RS,2SR isomer), and A is -$(CH_2)_{10}$-, -$(CH_2)_{11}$-or -$(CH_2)_4$-(1,4-phenylene)-$(CH_2)_2$-;

or NU is (1RS,2RS)-5-hydroxy-2-p-hydroxyphenylindan-1-yl or (1RS,2RS)-5-hydroxy-2-p-hydroxyphenyl-2-methylindan-1-yl and A is -$(CH_2)_{10}$-, -$(CH_2)_{11}$-or -$(CH_2)_4$-(1,4-phenylene)-$(CH_2)_2$-;

and wherein XR^1 is -$CONR^1R^2$ wherein R^2 is hydrogen or methyl and R^1 is n-butyl, 1H,1H-heptafluorobutyl, n-pentyl or n-hexyl, or XR^1 is -SR^1, SOR^1 or -SO_2R^1 wherein R^1 is n-pentyl, n-hexyl, 4,4,5,5,5-pentafluoropentyl or 1H,1H,2H,2H,3H,3H-heptafluorohexyl,

which process is characterised by bringing together said oestrogen and said pure antioestrogen.

2. A process as claimed in claim 1 wherein the oestrogen is oestradiol, oestradiol benzoate, oestradiol valerate or oestradiol undecanoate and the pure antioestrogen is 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol.

3. A process for the manufacture of a pharmaceutical composition which comprises bringing into admixture a product as defined in any one of claims 1 and 2 together with a pharmaceutically-acceptable diluent or carrier.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Produkt, das ein Östrogen und ein reines Antiöstrogen als Kombinationspräparat zur gleichzeitigen, aufeinanderfolgenden oder voneinander getrennten Verwendung zur selektiven Östrogen-Therapie von Beschwerden während der oder nach den Wechseljahren enthält, wobei das reine Antiöstrogen aus folgendem ausgewählt ist:

N-n-Butyl-N-methyl-, N-1H,1H-Heptafluorbutyl-N-methyl- oder N,N-(3-Methylpentamethylen)-11-(3,17β-dihydroxyöstra-1,3,5(10)-trien-7α-yl)undecanamid;

N-n-Butyl- oder N-1H,1H-Heptafluorbutyl-3-p-[4-(3,17β-dihydroxyöstra-1,3,5(10)-trien-7α-yl)butyl]-phenylpropionamid;

7α-(10p-Chlorphenylthiodecyl)-, 7α- (10-p-Chlorphenylsulfinyldecyl)-, 7α-[9-(4,4,5,5,5-Pentafluorpentylsulfinyl)nonyl]-, 7α-[10-(4,4,4-Trifluorbutylsulfinyl)decyl]- oder 7α-[10-(p-Chlorbenzylsulfinyl)decyl] -östra-1,3,5 (10) -trien-3,17ß-diol;

7α- (9-n-Heptylsulfinylnonyl)östra-1,3,5 (10-trien-3,17β-diol; und

einer Verbindung mit der Formel: -

NU-A-X-R^1

in der NU für 6-Hydroxy-2-p-hydroxyphenylnaphth-1-yl steht und A für $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ oder $-(CH_2)_5$-(1,4-Phenylen)-$(CH_2)_2-$ steht;
oder in der NU für 1,2,3,4-Tetrahydro-6-hydroxy-2-p-hydroxyphenylnapthth-1-yl (entweder das 1RS,2RS- oder 1RS,2SR-Isomer) oder für 1,2,3,4-Tetrahydro-6-hydroxy-2-p-hydroxyphenyl-2-methylnaphth-1-yl (entweder das 1RS,2RS- oder 1RS,2SR-Isomer) steht, und A für $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ oder $-(CH_2)_4$-(1,4-Phenylen)-$(CH_2)_2-$ steht;
oder in der NU für (1RS,2RS)-5-Hydroxy-2-p-hydroxyphenylindan-1-yl oder (1RS,2RS)-5-Hydroxy-2-p-hydroxyphenyl-2-methylindan-1-yl steht und A für $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ oder $-(CH_2)_4$-(1,4-Phenylen)-$(CH_2)_2-$ steht;
und in der XR^1 für $-CONR^1R^2$ steht, wobei R^2 für Wasserstoff oder Methyl steht und R^1 für n-Butyl, 1H,1H-Heptafluorbutyl, n-Pentyl oder n-Hexyl steht, oder in der XR^1 für $-SR^1$, SOR^1 oder $-SO_2R^1$ steht, wobei R^1 für n-Pentyl, n-Hexyl, 4,4,5,5,5-Pentafluorpentyl oder 1H,1H,2H,2H,3H,3H-Heptafluorhexyl steht.

2. Produkt nach Anspruch 1, wobei es sich bei dem Östrogen um Östradiol, Benzoesäureöstradiolester, Valeriansäureöstradiolester oder Undecansäureöstradiolester handelt und bei dem reinen Antiöstrogen um 7α-[9-(4,4,5,5,5-Pentafluorpentylsulfinyl)nonyl)östra-1,3,5(10)-trien-3,17β-diol.

3. Verfahren zur Herstellung eines Produkts, das ein Östrogen und ein reines Antiöstrogen als Kombinationspräparat zur gleichzeitigen, aufeinander folgenden oder voneinander getrennten Verwendung zur selektiven Östrogen-Therapie von Beschwerden während der oder nach den Wechseljahren enthält, nämlich nach einem der Ansprüche 1 und 2, wobei bei dem Verfahren das Östrogen und das reine Antiöstrogen zusammengebracht werden.

4. Pharmazeutische Zusammensetzung, die ein Produkt nach einem der Ansprüche 1 und 2 zusammen mit einem pharmazeutisch geeigneten Verdünnungsmittel oder Träger enthält.

5. Verwendung eines Produkts nach einem der Ansprüche 1 und 2 zur Herstellung eines Kombinationspräparats zur gleichzeitigen, aufeinander folgenden oder voneinander getrennten Verwendung in der selektiven Östrogen-Therapie von Beschwerden während der oder nach den Wechseljahren.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Herstellung eines Produkts, das ein Östrogen und ein reines Antiöstrogen als Kombinationspräparat zur gleichzeitigen, aufeinanderfolgenden oder voneinander getrennten Verwendung in der selektiven Östrogen-Therapie von Beschwerden während der oder nach den Wechseljahren enthält, wobei das reine Antiöstrogen aus folgendem ausgewählt ist:
N-n-Butyl-N-methyl-, N-1H,1H-Heptafluorbutyl-N-methyl- oder N,N-(3-Methylpentamethylen)-11-(3,17β-dihydroxyöstra-1,3,5(10)-trien-7α-yl)undecanamid;
N-n-Butyl- oder N-1H,1H-Heptafluorbutyl-3-p-[4-(3,17β-dihydroxyöstra-1,3,5(10)-trien-7α-yl)butyl]-phenylpropionamid;
7α-(10p-Chlorphenylthiodecyl)-, 7α-(10-p-Chlorphenylsulfinyldecyl)-, 7α-[9-(4,4,5,5,5-Pentafluorpentylsulfinyl)nonyl]-, 7α-[10-(4,4,4-Trifluorbutylsulfinyl)decyl]- oder 7α-[10-(p-Chlorbenzylsulfinyl)decyl]östra-1,3,5(10)-trien-3,17β-diol;
7α-(9-n-Heptylsulfinylnonyl)östra-1,3,5(10-trien-3,17β-diol; und
einer Verbindung mit der Formel: -

NU-A-X-R^1

in der NU für 6-Hydroxy-2-p-hydroxyphenylnaphth-1-yl steht und A für $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ oder $-(CH_2)_5$-(1,4-Phenylen)-$(CH_2)_2-$ steht;
oder in der NU für 1,2,3,4-Tetrahydro-6-hydroxy-2-p-hydroxyphenylnapthth-1-yl (entweder das 1RS,2RS- oder 1R2,2SR-Isomer) oder für 1,2,3,4-Tetrahydro-6-hydroxy-2-p-hydroxyphenyl-2-methylnaphth-1-yl (entweder das 1RS,2RS- oder 1RS,2SR-Isomer) steht, und A für $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ oder $-(CH_2)_4$-(1,4-phenylen)-$(CH_2)_2-$ steht;
oder in der NU für (1RS,2RS)-5-Hydroxy-2-p-hydroxyphenylindan-1-yl oder (1RS,2RS)-5-Hydroxy-2-p-hydroxyphenyl-2-methylindan-1-yl steht und A für $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ oder $-(CH_2)_4$-(1,4-Phenylen)-$(CH_2)_2-$ steht;

und in der XR^1 für $-CONR^1R^2$ steht, wobei R^2 für Wasserstoff oder Methyl steht und R^1 für n-Butyl, 1H,1H-Heptafluorbutyl, n-Pentyl oder n-Hexyl steht, oder in der XR^1 für $-SR^1$, SOR^1 oder $-SO_2R^1$ steht, wobei R^1 für n-Pentyl, n-Hexyl, 4,4,5,5,5-Pentafluorpentyl oder 1H,1H,2H,2H,3H,3H-Heptafluorhexyl steht,
wobei das Verfahren dadurch gekennzeichnet ist, daß das Östrogen und das reine Antiöstrogen zusammengebracht werden.

2. Verfahren nach Anspruch 1, wobei es sich bei dem Östrogen um Östradiol, Benzoesäureöstradiolester, Valeriansäureöstradiolester oder Undecansäureöstradiolester handelt und bei dem reinen Antiöstrogen um 7α-[9-(4,4,5,5,5-Pentafluorpentylsulfinyl)nonyl)östra-1,3,5(10)-trien-3,17β-diol.

3. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, bei dem ein wie in einem der Ansprüche 1 und 2 definiertes Produkt mit einem pharmazeutisch geeigneten Verdünnungsmittel oder Träger gemischt wird.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Produit comprenant un oestrogène et un antioestrogène pur sous forme d'une préparation mixte pour une utilisation simultanée, séquentielle ou distincte dans une thérapie sélective par oestrogènes de troubles périménopausiques ou postménopausiques, l'anti-oestrogène pur étant choisi entre

   le N-n-butyl-N-méthyl-, N-1H,1H-heptafluorobutyl-N-méthyl- ou N,N-(3-méthylpentaméthylène)-11-(3,17β-dihydroxyoestra-1,3,5(10)-triène-7α-yl)undécanamide ;

   le N-n-butyl- ou N-1H,1H-heptafluorobutyl-3-p-[4-(3,17β-dihydroxyoestra-1,3,5(10)-triène-7α-yl)-butyl]phénylpropionamide ;

   le 7α-(10-p-chlorophénylthiodécyl)-, 7α-(10-p-chlorophénylsulfinyldécyl)-, 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]-, 7α[10-(4,4,4-trifluorobutylsulfinyl)-décyl]- ou 7α-[10-(p-chlorobenzylsulfinyl)-décyl]-oestra-1,3,5(10)-triène-3,17β-diol ;

   le7α-(9-n-heptylsulfinylnonyl)oestra-1,3,5(10)-triène-3,17β-diol ; et

   un composé de formule :

   NU-A-X-R^1

   dans laquelle NU représente un groupe 6-hydroxy-2-p-hydroxyphénylnapht-1-yl et A représente un groupe $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ ou $-(CH_2)_5$-(1,4-phénylène)-$(CH_2)_2-$;

   ou bien NU représente un groupe 1,2,3,4, -tétrahydro-6-hydroxy-2-p-hydroxyphénylnapht-1-yle (isomère 1RS, 2RS ou bien 1RS, 2SR), ou un groupe 1,2,3,4-tétrahydro-6-hydroxy-2-p-hydroxyphényl-2-méthylnapht-1-yle (isomère 1RS, 2RS ou bien 1RS, 2SR), et A représente un groupe $-(CH_2)_{10}-$,$-(CH_2)_{11}-$ ou $-(CH_2)_4$-(1,4-phénylène)-$(CH_2)_2-$;

   ou bien NU représente un groupe (1RS, 2RS)-5-hydroxy-2-p-hydroxyphénylindane-1-yle ou (1RS, 2RS)-5-hydroxy-2-p-hydroxyphényl-2-méthylindane-1-yle et A représente un groupe $-(CH_2)_{10}-$, $-CH_2)_{11}-$ ou $-(CH_2)_4$-(1,4-phénylène)-$(CH_2)_2-$; et dans laquelle XR^1 représente un groupe $-CONR^1R^2$ dans lequel R^2 représente l'hydrogène ou un groupe méthyle et R^1 représente un groupe n-butyle, 1H,1H-heptafluorobutyle, n-pentyle ou n-hexyle, ou bien XR^1 représente un groupe $-SR^1$, SOR^1 ou $-SO_2R^1$ dans lequel R^1 représente un groupe n-pentyle, n-hexyle, 4,4,5,5,5-pentafluoropentyle ou 1H,1H,2H,2H,3H,3H-heptafluorohexyle.

2. Produit suivant la revendication 1, dans lequel l'oestrogène est l'oestradiol, le benzoate d'oestradiol, le valérate d'oestradiol ou l'undécanoate d'oestradiol et l'anti-oestrogène pur est le 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]oestra-1,3,5(10)-triène-3,17β-diol.

3. Procédé de préparation d'un produit comprenant un oestrogène et un anti-oestrogène pur sous forme d'une préparation mixte pour une utilisation de manière simultanée, séquentielle ou distincte dans une thérapie sélective par oestrogènes de troubles périménopausiques ou postménopausiques, suivant l'une quelconque des revendications 1 et 2, procédé qui comprend l'association dudit oestrogène et dudit anti-oestrogène pur.

4. Composition pharmaceutique comprenant un produit suivant l'une quelconque des revendications 1 et 2, en association avec un dilant ou support pharmaceutiquement acceptable.

5. Utilisation d'un produit suivant l'une quelconque des revendications 1 et 2 pour la production d'une préparation mixte pour une utilisation de manière simultanée, séquentielle ou distincte dans une thérapie sélective par oestrogènes de troubles périménopausiques ou postménopausiques.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé de préparation d'un produit comprenant un oestrogène et un anti-oestrogène pur sous forme d'une préparation mixte pour une utilisation simultanée, séquentielle ou distincte dans une thérapie sélective par oestrogènes de troubles périménopausiques ou postménopausiques, l'anti-oestrogène pur étant choisi entre

le N-n-butyl-N-méthyl-, N-1H,1H-heptafluorobutylN-méthyl- ou N,N-(3-méthylpentaméthylène)-11-(3,17β-dihydroxyoestra-1,3,5(10)-triène-7α-yl)undécanamide ;

le N-n-butyl- ou N-1H,1H-heptafluorobutyl-3-p-[4-(3,17β-dihydroxyoestra-1,3,5(10)-triène-7α-yl)-butyl]phénylpropionamide ;

le 7α-(10-p-chlorophénylthiodécyl)-, 7α-(10-p-chlorophénylsulfinyldécyl)-, 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]-, 7α[10-(4,4,4-trifluorobutylsulfinyl)décyl]- ou 7α-[10-(p-chlorobenzylsulfinyl)-décyl]-oestra-1,3,5(10)-triène-3,17β-diol ;

le7α-(9-n-heptylsulfinylnonyl)oestra-1,3,5(10)-triène-3,17β-diol ; et

un composé de formule :

$$NU\text{-}A\text{-}X\text{-}R^1$$

dans laquelle NU représente un groupe 6-hydroxy-2-p-hydroxyphénylnapht-1-yl et A représente un groupe $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ ou $-(CH_2)_5-$(1,4-phénylène)$-(CH_2)_2-$;

ou NU représente un groupe 1,2,3,4,-tétrahydro-6-hydroxy-2-p-hydroxyphénylnapht-1-yle (isomère 1RS, 2RS, ou 1RS, 2SR), ou un groupe 1,2,3,4-tétrahydro-6-hydroxy-2-p-hydroxyphényl-2-méthylnapht-1-yle (isomère 1RS, 2RS ou bien 1RS, 2SR), et A représente un groupe $-(CH_2)_{10}-$, $-(CH_2)_{11}-$ ou $-(CH_2)_4-$(1,4-phénylène)$-(CH_2)_2-$;

ou bien NU représente un groupe (1RS, 2RS)-5-hydroxy-2-p-hydroxyphénylindane-1-yle ou (1RS, 2RS)-5-hydroxy-2-p-hydroxyphényl-2-méthylindane-1-yle et A représente un groupe $-(CH_2)_{10}-$, $-CH_2)_{11}-$ ou $-(CH_2)_4-$(1,4-phénylène)$-(CH_2)_2$;

et dans laquelle XR^1 représente un groupe $-CONR^1R^2$ dans lequel R^2 représente l'hydrogène ou un groupe méthyle et R^1 représente un groupe n-butyle, 1H,1H-heptafluorobutyle, n-pentyle ou n-hexyle, ou bien XR^1 représente un groupe $-SR^1$, $-SOR^1$ ou $-SO_2R^1$ dans lequel R^1 représente un groupe n-pentyle, n-hexyle, 4,4,5,5,5-pentafluoropentyle ou 1H,1H,2H,2H,3H,3H-heptafluorohexyle,

procédé qui est caractérisé par l'association dudit oestrogène et dudit anti-oestrogène pur.

2. Procédé suivant la revendication 1, dans lequel l'oestrogène est l'oestradiol, le benzoate d'oestradiol, le valérate d'oestradiol ou l'undécanoate d'oestradiol et l'anti-oestrogène pur est le 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]oestra-1,3,5(10)-triène-3,17β-diol.

3. Procédé de production d'une composition pharmaceutique, qui comprend le mélange d'un produit tel que défini dans l'une quelconque des revendications 1 et 2, avec un dilant ou support pharmaceutiquement acceptable.

Abstract

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ICI 182,780, a new antioestrogen with clinical potential

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Abstract

Previous studies in this laboratory identified a series of 7α-alkylamide analogues of 17β-oestradiol which are pure antioestrogens. Among this initial lead series of compounds, exemplified by ICI 164,384, none was of sufficient *in vivo* potency to merit serious consideration as a candidate for clinical evaluation. Further structure-activity studies identified a new compound, ICI 182,780, 7α-[9-(4,4,5,5,5-pentafluoro-pentylsulphinyl)nonyl] oestra-1,3,5(10)-triene-3,17β-diol, with significantly increased antioestrogenic potency. The antiuterotrophic potency of ICI 182,780 is more than 10-fold greater than that of ICI 164,384. ICI 182,780 has no oestrogen-like trophic activity and, like ICI 164,384 is peripherally selective in its antioestrogenic effects. The increased *in vivo* potency of ICI 182,780 was also reflected, in part, by intrinsic activity at the oestrogen receptor and in the growth inhibitory potency of ICI 182,780 in MCF-7 human breast cancer cells. ICI 182,780 was a more effective inhibitor of MCF-7 growth than 4'-hydroxytamoxifen, producing an 80% reduction of cell number under conditions where 4'-hydroxytamoxifen achieved a maximum of 50% inhibition. Sustained antioestrogenic effects of ICI 182,780, following a single parenteral dose of ICI 182,780 in oil suspension, were apparent in both rats and pigtail monkeys. *In vivo*, the antitumour activity of ICI 182,780 was demonstrated with xenografts of MCF-7 and Br10 human breast cancers in athymic mice where, over a 1 month period, a single injection of ICI 182,780 in oil suspension achieved effects comparable with those of daily tamoxifen treatment. Thus, ICI 182,780 provides the opportunity to evaluate clinically the potential therapeutic benefits of complete blockade of oestrogen effects in endocrine-responsive human breast cancer.

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(54) **FORMULATION**

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(52) **U.S. Cl.** **514/182**

(57) **ABSTRACT**

The invention relates to a novel sustained release pharmaceutical formulation adapted for administration by injection containing the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol, more particularly to a formulation adapted for administration by injection containing the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol in solution in a ricinoleate vehicle which additionally comprises at least one alcohol and a non-aqueous ester solvent which is miscible in the ricinoleate vehicle.

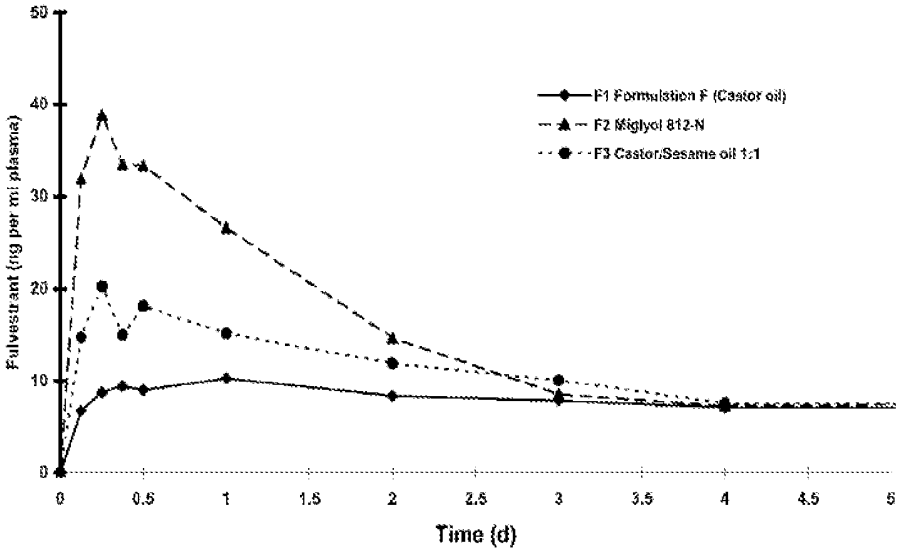
F1 Formulation F (Castor oil)
F2 Miglyol 812-N
F3 Castor/Sesame oil 1:1
Fulvestrant (ng per ml plasma)
Time (d)
0
10
20
30
40
50
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

Figure 1

FLOW DIAGRAM OF MANUFACTURING

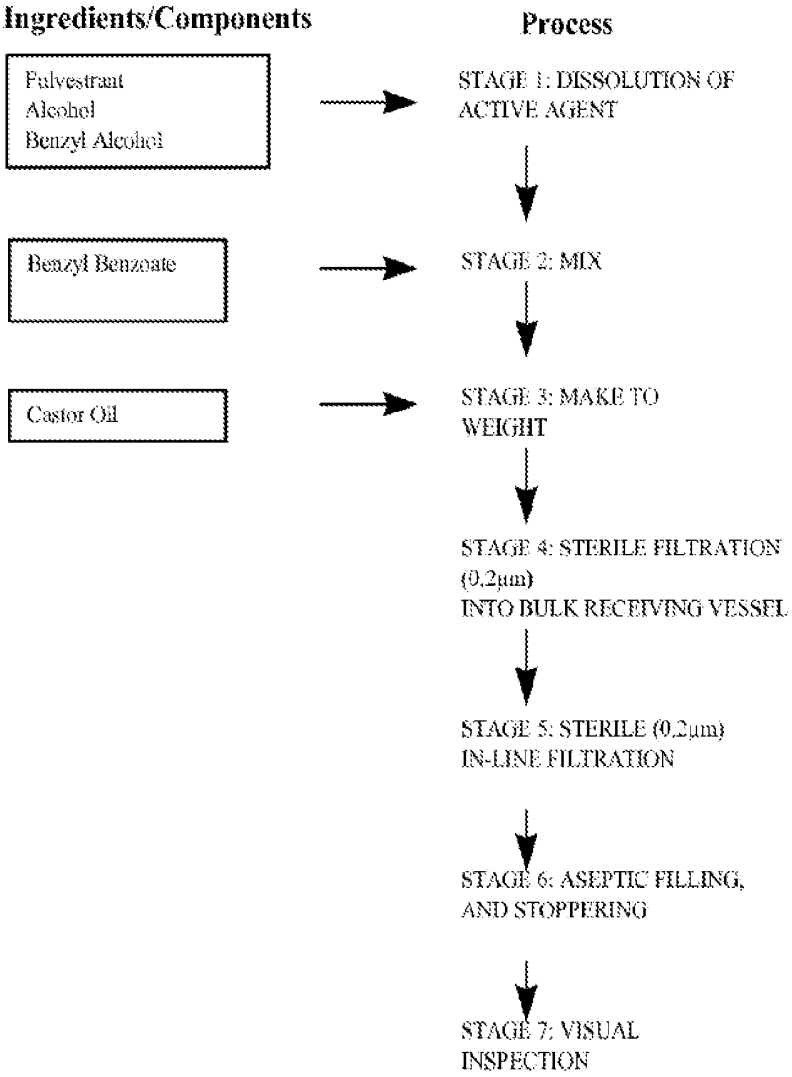

Figure 2

FORMULATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation Application of copending U.S. patent application Ser. No. 10/872,784, filed Jun. 22, 2004, which claims benefit of U.S. patent application Ser. No. 09/756,291, filed Jan. 9, 2001 which claims the benefit of Great Britain Application No. 0008837.7 filed Apr. 12, 2000 and Great Britain Application No. 0000313.7, filed Jan. 10, 2000, all of which are incorporated herein by reference in their entireties.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The invention relates to a novel sustained release pharmaceutical formulation adapted for administration by injection containing the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3, 17β-diol.

[0004] 2. Description of the Related Art

[0005] Oestrogen deprivation is fundamental to the treatment of many benign and malignant diseases of the breast and reproductive tract. In premenopausal women, this is achieved by the ablation of ovarian function through surgical, radiotherapeutic, or medical means, and, in postmenopausal women, by the use of aromatase inhibitors.

[0006] An alternative approach to oestrogen withdrawal is to antagonise oestrogens with antioestrogens. These are drugs that bind to and compete for oestrogen receptors (ER) present in the nuclei of oestrogen-responsive tissue. Conventional nonsteroidal antioestrogens, such as tamoxifen, compete efficiently for ER binding but their effectiveness is often limited by the partial agonism they display, which results in an incomplete blockade of oestrogen-mediated activity (Farr and Jordan 1984, May and Westley 1987).

[0007] The potential for nonsteroidal antioestrogens to display agonistic properties prompted the search for novel compounds that would bind ER with high affinity without activating any of the normal transcriptional hormone responses and consequent manifestations of oestrogens. Such molecules would be "pure" antioestrogens, clearly distinguished from tamoxifen-like ligands and capable of eliciting complete ablation of the trophic effects of oestrogens. Such compounds are referred to as Estrogen Receptor-Downregulators (E.R.D.). The rationale for the design and testing of novel, pure antioestrogens has been described in: Bowler et al 1989, Wakeling 1990a, 1990b, 1990c. Wakeling and Bowler 1987, 1988.

[0008] Steroidal analogues of oestradiol, with an alkylsulphinyl side chain in the 7α position, provided the first examples of compounds devoid of oestrogenic activity (Bowler et al 1989). One of these, 7α-[9-(4,4,5,5,5-pentafluoropentyl sulphinyl)nonyl]oestra-1,3,5-(10)triene-3, 17β-diol was selected for intensive study on the basis of its pure oestrogen antagonist activity and significantly increased antioestrogenic potency over other available antioestrogens. In vitro findings and early clinical experience with 7α[9-(4, 4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3-5(10)-triene-3,17β-diol have promoted interest in the development of the drug as a therapeutic agent for oestrogen-dependent indications such as breast cancer and certain benign gynaecological conditions.

[0009] 7α-[9-(4,4,5,5,5-Pentafluoropentylsulphinyl) nonyl]oestra-1,3-5(10)-triene-3,17β-diol, or ICI 182,780, has been allocated the international non-proprietary name fulvestrant, which is used hereinafter. When referring to fulvestrant we include pharmaceutically-acceptable salts thereof and any possible solvates of either thereof.

[0010] Fulvestrant binds to ER with an affinity similar to that of oestradiol and completely blocks the growth stimulatory action of oestradiol on human breast cancer cells in vitro; it is more potent and more effective than tamoxifen in this respect. Fulvestrant blocks completely the uterotrophic action of oestradiol in rats, mice and monkeys, and also blocks the uterotrophic activity of tamoxifen.

[0011] Because fulvestrant has none of the oestrogen-like stimulatory activity that is characteristic of clinically available antioestrogens such as tamoxifen or toremifene, it may offer improved therapeutic activity characterised by more rapid, complete, or longer-lasting tumour regression; a lower incidence or rate of development of resistance to treatment; and a reduction of tumour invasiveness.

[0012] In intact adult rats, fulvestrant achieves maximum regression of the uterus at a dose which does not adversely affect bone density or lead to increased gonadotrophin secretion. If also true in humans, these findings could be of extreme importance clinically. Reduced bone density limits the duration of oestrogen-ablative treatment for endometriosis. Fulvestrant does not block hypothalamic ER. Oestrogen ablation also causes or exacerbates hot flushes and other menopausal symptoms; fulvestrant will not cause such effects because it does not cross the blood-brain barrier.

[0013] European Patent Application No. 0 138 504 discloses that certain steroid derivatives are effective antioestrogenic agents. The disclosure includes information relating to the preparation of the steroid derivatives. In particular there is the disclosure within Example 35 of the compound 7α-[9-(4, 4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol, which compound is specifically named in claim **4**. It is also disclosed that the compounds of that invention may be provided for use in the form of a pharmaceutical composition comprising a steroid derivative of the invention together with a pharmaceutically-acceptable diluent or carrier. It is stated therein that the composition can be in a form suitable for oral or parenteral administration.

[0014] Fulvestrant shows, along with other steroidal based compounds, certain physical properties which make formulation of these compounds difficult. Fulvestrant is a particularly lipophilic molecule, even when compared with other steroidal compounds, and its aqueous solubility is extremely low at around 10 $ngml^{-1}$ (this is an estimate from a water/solvent mixture solute since measurements this low could not be achieved in a water only solute).

[0015] Currently there are a number of sustained release injectable steroidal formulations which have been commercialised. Commonly these formulations use oil as a solvent and wherein additional excipients may be present. Below in Table 1 are described a few commercialised sustained release injectable formulations.

[0016] In the formulations within Table 1 a number of different oils are used to solubilise the compound and additional excipients such as benzyl benzoate, benzyl alcohol and ethanol have been used. Volumes of oil needed to solubilise the steroid active ingredient are low. Extended release is achievable for periods from 1 to 8 weeks.

TABLE 1

OIL BASED LONG-ACTING INTRAMUSCULAR INJECTIONS

PRODUCT NAME	STEROID	DOSE	TYPE	COMP'.	SOURCE	OIL	BzBz
SUSTANON 100	Testosterone proprionate Testosterone phenylproprionate Testosterone isocaproate Testosterone decanoate	30 mg 60 mg 60 mg 100 mg	Androgen	Organon	ABPI Data Sheet Comp. 1999	Arachis	
PROLUTON DEPOT	Hydroxy progesterone hexanoate	250 $mgml^{-1}$	Progestogen	Schering HC	ABPI Data Sheet Comp. 1999	Castor	up to 46%
TOCOGESTAN	Hydroxy progesterone enantate Progesterone α-Tocopherol	200 mg 50 mg 250 mg	Progestogen	Theramax	Dict. Vidal 1999	Ethyl oleate	*40%
TROPHOBOLENE	Estrapronicate Nandrolone undecanoate Hydroxyprogesterone heptanoate	1.3 mg 50 mg 80 mg	Mixed	Theramax	Dict. Vidal 1997	Olive	45%
NORISTERAT	Norethisterone oenanthoate	200 mg	Contraceptive	Schering HC	ABPI Data Sheet Comp. 1999	Castor	YES
BENZO-GYNOESTRYL	Estradiol hexahydrobenzoate	5 mg	Estradiol	Roussel	Dict. Vidal 1998	Arachis	
PROGESTERONE-RETARD	Hydroxy progesterone caproate	250 $mgml^{-1}$	Progestogen	Pharlon	Dict. Vidal 1999	Castor	YES
GRAVIBINAN	Estradiol 17-β-valerate Hydroxyprogesterone caproate	5 $mgml^{-1}$ 250 $mgml^{-1}$	Mixed	Schering HC	Dict. Vidal 1995	Castor	YES
PARABOLAN	Trenbolone	76 mg	Androgen	Negma	Dict. Vidal 1997	Arachis	
DELESTROGEN	Estradiol valerate	20 $mgml^{-1}$ 40 $mgml^{-1}$	Estradiol	BMS	J. Pharm. Sci (1964) 53(8) 891	Castor	78% 58%
DELALUTIN	17-Hydroxy progesterone	250 $mgml^{-1}$	Progestrogen	DMS	J. Pharm. Sci. (1964) 53(8) 891	Castor	YES

PRODUCT NAME	STEROID	BzOH	EtOH	DOSE	DOSING
SUSTANON 100	Testosterone proprionate Testosterone phenylproprionate Testosterone isocaproate Testosterone decanoate	0.1 ml		1 ml	3 weeks
PROLUTON DEPOT	Hydroxy progesterone hexanoate			1 or 2 ml	1 week
TOCOGESTAN	Hydroxy progesterone enantate Progesterone α-Tocopherol			2 ml	<1 week
TROPHOBOLENE	Estrapronicate Nandrolone undecanoate Hydroxyprogesterone heptanoate			1 ml	15 to 30 days
NORISTERAT	Norethisterone oenanthoate			1 ml	8 weeks
BENZO-GYNOESTRYL	Estradiol hexahydrobenzoate			1 ml	1 week
PROGESTERONE-RETARD	Hydroxy progesterone caproate			1 or 2 ml	1 week
GRAVIBINAN	Estradiol 17-β-valerate Hydroxyprogesterone caproate			1 or 2 ml	1-2 weeks
PARABOLAN	Trenbolone	75 mg	45 mg	1.5 ml	2 weeks
DELESTROGEN	Estradiol valerate	20% 40%	2% 2%		
DELALUTIN	17-Hydroxy progesterone	YES	up to 2%		

BzBz = benzylbenzoate
BzOH = benzylalcohol
EtOH = ethanol
Dict. Vidal = Dictionnaire Vidal
% are w/v and
*approximate as measured directly from a single sample

[text missing or illegible when filed]described which comprises 50 mg of fulvestrant, 400 mg of benzyl alcohol and sufficient castor oil to bring the solution to a volume of 1 ml.

Manufacture at a commercial scale of a formulation as described in U.S. Pat. No. 5,183,814 will be complicated by the high alcohol concentration. Therefore, there is a need to

lower the alcohol concentration in fulvestrant formulations whilst preventing precipitation of fulvestrant from the formulation.

SUMMARY OF THE INVENTION

[0017] The invention relates to a novel sustained release pharmaceutical formulation adapted for administration by injection containing the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3, 17β-diol, more particularly to a formulation adapted for administration by injection containing the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5 (10)-triene-3,17β-diol in solution in a ricinoleate vehicle which additionally comprises at least one alcohol and a non-aqueous ester solvent which is miscible in the ricinoleate vehicle.

BRIEF DESCRIPTION OF THE DRAWING

[0018] FIG. **1** shows the release profile in vivo of the four formulations from the second part of Table 4 below, and shows the effect of the fixed oil component on fulvestrant plasma profile over five days following intramuscular administration in rabbits.

[0019] FIG. **2** shows a process flow diagram associated with the Formulation Example.

DETAILED DESCRIPTION OF THE INVENTION

[0020] Table 2 shows the solubility of fulvestrant in a number of different solvents.

TABLE 2

SOLUBILITY OF FULVESTRANT

SOLVENT	SOLUBILITY ($mgml^{-1}$ at 25° C.)
Water	0.001
Arachis oil	0.45
Sesame oil	0.58
Castor oil	20
Miglyol 810	3.06
Miglyol 812	2.72
Ethyl oleate	1.25
Benzyl benzoate	6.15
Isopropyl myristate	0.80
Span 85 (surfactant)	3.79
Ethanol	>200
Benzyl Alcohol	>200

[0021] As can be seen fulvestrant is significantly more soluble in castor oil than any of the other oils tested. The greater solvating ability of castor oil for steroidal compounds is known and is attributed to the high number of hydroxy groups of ricinoleic acid, which is the major constituent of the fatty acids within the triglycerides present in castor oil—see (Riffkin et. al. J. Pharm. Sci., (1964), 53, 891).

[0022] However, even when using the best oil based solvent, castor oil, we have found that it is not possible to dissolve fulvestrant in an oil based solvent alone so as to achieve a high enough concentration to dose a patient in a low volume injection and achieve a therapeutically significant release rate. To achieve a therapeutically significant release rate the amount of fulvestrant needed would require the formulation volume to be large, at least 10 ml. This requires the doctor to inject an excessively large volume of formulation to administer a dose significantly high enough for human therapy.

[0023] Currently guidelines recommend that no more than 5 mls of liquid is injected intramuscularly in a single injection. Pharmacologically active doses required for a 1 month long acting depot formulation of fulvestrant is around 250 mg. Therefore, when dissolved in just castor oil, fulvestrant would need to be administered in at least 10 ml of castor oil.

[0024] The addition of organic solvents in which fulvestrant is freely soluble, and which are miscible with castor oil, may be used, such as an alcohol. With the addition of high concentrations of an alcohol concentrations of >50 $mgml^{-1}$ of fulvestrant in a castor oil formulation is achievable, thereby giving an injection volumes of <5 ml—see Table 3 below. We have surprisingly found that the introduction of a non-aqueous ester solvent which is miscible in the castor oil and an alcohol surprisingly eases the solubilisation of fulvestrant into a concentration of at least 50 $mgml^{-1}$—see Table 3 below. The finding is surprising since the solubility of fulvestrant in non-aqueous ester solvents—see Table 2 above—is significantly lower than the solubility of fulvestrant in an alcohol. The solubility of fulvestrant is also lower in non-aqueous ester solvents than is the solubility of fulvestrant in castor oil.

[0025] Therefore, we present as a feature of the invention a pharmaceutical formulation comprising fulvestrant (preferably fulvestrant is present at 3-10% w/v, 4-9% w/v, 4-8% w/v, 4-7% w/v, 4-6% w/v and most preferably at about 5% w/v) in a ricinoleate vehicle, a pharmaceutically acceptable non-aqueous ester solvent, and a pharmaceutically acceptable alcohol wherein the formulation is adapted for intramuscular administration and attaining a therapeutically significant blood plasma fulvestrant concentration for at least 2 weeks.

[0026] Another feature of the invention is a pharmaceutical formulation comprising fulvestrant in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration for at least 2 weeks.

[0027] Further features of the invention include a pharmaceutical formulation adapted for intra-muscular injection comprising fulvestrant, 30% or less weight of a pharmaceutically-acceptable alcohol per volume of formulation, at least 1% weight of a pharmaceutically-acceptable non-aqueous ester solvent miscible in a ricinoleate vehicle per volume of formulation and a sufficient amount of a ricinoleate vehicle so as to prepare a formulation which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration for at least 2 weeks.

[0028] Further features of the invention include a pharmaceutical formulation adapted for intra-muscular injection comprising fulvestrant; 35% (preferably 30% and ideally 25%) or less weight of a pharmaceutically-acceptable alcohol per volume of formulation, at least 1% (preferably at least 5% or ideally 10%) weight of a pharmaceutically-acceptable non-aqueous ester solvent miscible within a ricinoleate vehicle per volume of formulation and a sufficient amount of a ricinoleate vehicle so as to prepare a formulation of at least 45 $mgml^{-1}$ of fulvestrant.

[0029] For the avoidance of any doubt when using the term % weight per volume of formulation for the constituents of the formulation we mean that within a unit volume of the formulation a certain percentage of the constituent by weight

will be present, for example a 1% weight per volume formulation will contain within a 100 ml volume of formulation 1 g of the constituent. By way of further illustration

% of x by weight per volume of formulation	weight of x in 1 ml of formulation
30%	300 mg
20%	200 mg
10%	100 mg
5%	50 mg
1%	10 mg

[0030] Preferred pharmaceutical formulations of the invention are as described above wherein:

[0031] 1. The total volume of the formulation is 6 ml, or less, and the concentration of fulvestrant is at least 45 $mgml^{-1}$.

[0032] 2. The total amount of fulvestrant in the formulation is 250 mg, or more, and the total volume of the formulation is 6 ml, or less.

[0033] 3. The total amount of fulvestrant in the formulation is 250 mg and the total volume of the formulation is 5-5.25 ml.

[0034] It is appreciated that in the formulation an excess of formulation may be included to allow the attendant physician or care giver to be able to deliver the required dose. Therefore, when a 5 ml dose is required it would be appreciated that an excess of up to 0.25 ml, preferably up to 0.15 ml will also be present in the formulation. Typically the formulation will be presented in a vial or a prefilled syringe, preferably a prefilled syringe, containing a unit dosage of the formulation as described herein, these being further features of the invention.

[0035] Preferred concentrations of a pharmaceutically-acceptable alcohol present in any of the above formulations are; at least 3% w/v, at least 5% w/v, at least 7% w/v, at least 10% w/v, at least 11% w/v, at least 12% w/v, at least 13% w/v, at least 14% w/v, at least 15% w/v and, preferably, at least 16% w/v. Preferred maximal concentrations of pharmaceutically-acceptable alcohol present in the formulation are ;28% w/v or less, 22% w/v or less and 20% w/v or less. Preferred ranges of pharmaceutically-acceptable alcohol present in any of the above formulations are selected from any minimum or maximum value described above and 3-35% w/v, 4-35% w/v, 5-35% w/v, 5-32% w/v, 7-32% w/v, 10-30% w/v, 12-28% w/v, 15-25% w/v, 17-23% w/v, 18-22% w/v and ideally 19-21% w/v.

[0036] The pharmaceutically-acceptable alcohol may consist of one alcohol or a mixture of two or more alcohols. preferably a mixture of two alcohols. Preferred pharmaceutically-acceptable alcohols for parenteral administration are ethanol, benzyl alcohol or a mixture of both ethanol and benzyl alcohol, preferably the ethanol and benzyl alcohol are present in the formulation in the same w/v amounts. Preferably the formulation alcohol contains 10% w/v ethanol and 10% w/v benzyl alcohol.

[0037] The pharmaceutically-acceptable non-aqueous ester solvent may consist of one or a mixture of two or more pharmaceutically-acceptable non-aqueous ester solvents, preferably just one. A preferred pharmaceutically-acceptable non-aqueous ester solvent for parenteral administration is selected from benzyl benzoate, ethyl oleate, isopropyl myristate, isopropyl palmitate or a mixture of any thereof.

[0038] The ricinoleate vehicle should preferably be present in the formulation in a proportion of at least 30% weight per volume of the formulation, ideally at least 40% or at least 50% weight per volume of formulation.

[0039] It will be understood by the skilled person that the pharmaceutically-acceptable alcohol will be of a quality such that it will meet pharmacopoeial standards (such as are described in the US, British, European and Japanese pharmacopoeias) and as such will contain some water and possibly other organic solvents, for example ethanol in the US Pharmacopeia contains not less than 94.9% by volume and not more than 96.0% by volume of ethanol when measured at 15.56° C. Dehydrated alcohol in the US Pharmacopeia contains not less than 99.5% ethanol by volume when measured at 15.56° C.

[0040] Preferred concentrations of the pharmaceutically-acceptable non-aqueous ester solvent present in any of the above formulations are; at least 5% w/v, at least 8% w/v, at least 10% w/v, at least 11% w/v, at least 12% w/v, at least 13% w/v, at least 15% w/v, at least 16% w/v, at least 17% w/v, at least 18% w/v, at least 19% w/v and at least 20% w/v. Preferred maximal concentrations of the pharmaceutically-acceptable non-aqueous ester solvent are; 60% w/v or less, 50% w/v or less, 45% w/v or less, 40% w/v or less, 35% w/v or less, 30% w/v or less and 25% w/v or less. A preferred concentration is 15% w/v. Preferred ranges of pharmaceutically-acceptable non-aqueous ester solvent present in any of the above formulations are selected from any minimum or maximum value described above and preferably are; 5-60% w/v, 7-55% w/v, 8-50% w/v, 10-50% w/v, 10-45% w/v, 10-40% w/v, 10-35% w/v, 10-30% w/v, 10-25% w/v, 12-25% w/v, 12-22% w/v, 12-20% w/v, 12-18% w/v, 13-17% w/v and ideally 14-16% w/v. Preferably the ester solvent is benzyl benzoate, most preferably at about 15% w/v.

[0041] It will be understood by the skilled person that the pharmaceutically-acceptable non-aqueous ester solvent will be of a quality that it will meet pharmacopoeial standards (such as described in the US, British, European and Japanese pharmacopoeias).

[0042] Preferred combinations of pharmaceutically-acceptable alcohol and pharmaceutically-acceptable non-aqueous ester solvent in the formulation are set out below:

Pharmaceutically-acceptable alcohol(% w/v)	Pharmaceutically-acceptable non-aqueous ester (% w/v)
10-30	5-60, 7-55, 8-50, 10-50, 10-45, 10-40, 10-35, 10-30, 10-25, 12-25, 12-22, 12-20, 12-18, 13-17 and ideally 14-16.
17-23	5-60, 7-55, 8-50, 10-50, 10-45, 10-40, 10-35, 10-30, 10-25, 12-25, 12-22, 12-20, 12-18, 13-17 and ideally 14-16.
3-35, 4-35, 5-35, 5-32, 7-32, 10-30, 12-28, 15-25, 17-23, 18-22 and ideally 19-	10-35
3-35, 4-35, 5-35, 5-32, 7-32, 10-30, 12-28, 15-25, 17-23, 18-22 and ideally 19- 21.	12-18
ethanol and benzyl alcohol, most preferably each at about 10%	benzyl benzoate, most preferably at about 15%

[0043] By the use of the term ricinoleate vehicle we mean an oil which has as a proportion (at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% w/v) of its composition as triglycerides of ricinoleic acid. The ricinoleate vehicle may be a synthetic oil or conveniently is castor oil, ideally of pharmacopoeial standards, as described above.

[0044] We have surprisingly found that the above formulations of the invention provide, after intra-muscular injection, satisfactory release of fulvestrant over an extended period of time.

[0045] This finding is indeed surprising for the following reasons.

[0046] 1. Previously tested by the applicants have been intra-muscular injections of fulvestrant in the form of an aqueous suspension. We have found extensive local tissue irritation at the injection site as well as a poor release profile. It is believed that the tissue irritation/inflammation was due to the presence of fulvestrant in the form of solid particles. The release profile appeared to be determined by the extent of inflammation/irritation present at the injection site and this was variable and difficult to control. Also the fulvestrant release rate was not sufficiently high to be clinically significant.

[0047] 2. Our findings from studies using ^{14}C labelled benzyl alcohol show that it dissipates rapidly from the injection site and is removed from the body within 24 hours of administration.

[0048] It would be expected that ethanol will dissipate at least as quickly, if not more rapidly, from the injection site.

[0049] It is known that benzyl benzoate is metabolised by conjugation to glycine to form hippuric acid by the human liver and excreted into the urine—Martindale: The Extra Pharmacopoeia 32nd edition page 1103, and, therefore, it is unlikely that benzyl benzoate, when used, is present at the injection site during the whole of the extended release period.

[0050] We have found that despite the rapid elimination of the additional solubilising excipients, i.e. the alcohol and pharmaceutically-acceptable non-aqueous ester solvent, from the formulation vehicle and the site of injection after injection of the formulation, extended release at therapeutically significant levels of fulvestrant over an extended period can still achieved by the formulation of the invention.

[0051] By use of the term "therapeutically significant levels" we mean that blood plasma concentrations of at least 2.5 $ngml^{-1}$, ideally at least 3 $ngml^{-1}$, at least 8.5 $ngml^{-1}$, and up to 12 $ngml^{-1}$ of fulvestrant are achieved in the patient. Preferably blood plasma levels should be less than 15 $ngml^{-1}$.

[0052] By use of the term "extended release" we mean at least two weeks, at least three weeks, and, preferably at least four weeks of continuous release of fulvestrant is achieved. In a preferred feature extended release is achieved for 36 days. Preferably extended release of fulvestrant is for at least 2-5 weeks and more preferably for the following periods (weeks) 2.5-5, 2.5-4, 3-4, 3.5-4 and most preferably for at least about 4 weeks.

[0053] It will be understood that the attendant physician may wish to administer the intramuscular injection as a divided dose, i.e. a 5 ml formulation is sequentially administered in two separate injections of 2.5 ml, this is a further feature of the invention

[0054] Simply solubilising fulvestrant in an oil based liquid formulation is not predictive of a good release profile or lack of precipitation of drug after injection at the injection site.

[0055] Table 3 shows the solubility of fulvestrant in a castor oil vehicle additionally containing alcohols ethanol and benzyl alcohol with or without benzyl benzoate. The results clearly show the positive effect of benzyl benzoate on fulvestrant solubility in castor oil, despite fulvestrant having a lower solubility in benzyl benzoate than in either alcohol or castor oil.

TABLE 3

Table 3 - EFFECT OF BENZYL BENZOATE ON FULVESTRANT SOLUBILITY IN CASTOR OIL AT 25° C.

	% w/v							
Ethanol (96%)	5	5	10	10	10	10	15	15
Benzyl Alcohol	5	5	5	5	10	10	15	15
Benzyl Benzoate		15		15		15		15
Castor Oil	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100
Fulvestrant Solubility [$mgml^{-1}$]	27	36	46	54	45	65	76	102

[0056] The following Table 4 shows the solubility of fulvestrant in a range of oil based formulations which contain the same amounts of alcohol and benzyl benzoate but in which the oil is changed. The data also shows solubility of fulvestrant after removal of the alcohols.

TABLE 4

Solubility comparisons of fulvestrant in oil based formulations with and without alcohols

	Fulvestrant Solubility mg ml^{-1} @ 25° C.	
Formulation [a]	Complete vehicle	Vehicle minus alcohols
Castor oil based	81.2	12.6
Miglyol 812-N based	86.8	1.7
Sesame seed/Castor oil (1:1) based	70.1	4.4
Sesame seed oil based	45.7	0.7
Arachis oil based	40.2	<0.2

[a] Complete Vehicle Formulations comprised ethanol [96%](10%), benzyl alcohol (10%) and benzyl benzoate (15%) made to volume with the stated oil. Excess fulvestrant was added to each solvent mixture and solubility determined.

Effect of Formulation on Precipitation of Fulvestrant at the Injection Site

[0057]

	Days						
Formulation [a]	2	3	4	7	10	30	51
Formulation F1 castor oil based	0	0	0	0	0	0	0
Formulation F2 Miglyol 812-N based	++ [b]	+++	+++	+++	+++	++	0
Formulation F3 sesame seed oil/ castor oil based	+ [c]	++	++	+++	++	+	+

0, +, ++, +++ = Degree of precipitation (None detected, Mild, Moderate, Severe)

[a] Formulations comprised fulvestrant (5%), ethanol [96%] (10%), benzyl alcohol (10%) and benzyl benzoate (15%) made to volume with the stated oil.

[b] Mainly large needle shaped crystals

[c] Small needles and/or sheafs of crystals

[0058] Precipitation of fulvestrant and the release profile was determined with the above formulations in an in vivo rabbit study.

[0059] FIG. 1 shows the release profile in vivo of the four formulations from the second part of Table 4 and shows the effect of the fixed oil component on fulvestrant plasma profile over five days following intramuscular administration in rabbits (data normalised to 50 mg per 3 kg; mean given; number of animals per timepoint=8, plasma samples assayed for fulvestrant content using lc-ms/ms detection following solvent extraction). As can be seen the castor oil formulation showed a particularly even release profile with no evidence of precipitation of fulvestrant at the injection site.

[0060] Therefore we present as a further feature of the invention an extended release pharmaceutical formulation adapted for intramuscular injection comprising fulvestrant; 35% (preferably 30% or ideally 25%) or less weight of a pharmaceutically-acceptable alcohol per volume of formulation, at least 1% (preferably at least 5% or ideally 10%) weight of a pharmaceutically-acceptable non-aqueous ester solvent miscible in a ricinoleate vehicle per volume of formulation and sufficient amount of a ricinoleate vehicle, taking into account the addition of any further optional pharmaceutically-acceptable excipients, so as to prepare a formulation of at least 45 $mgml^{-1}$ of fulvestrant.

[0061] A further feature of the invention is a pharmaceutical formulation adapted for intramuscular injection, as defined above, for use in medical therapy.

[0062] A further feature of the invention is a method of treating a benign or malignant diseases of the breast or reproductive tract, preferably treating breast cancer, by administration to a human in need of such treatment by intramuscular injection an extended release ricinoleate vehicle based pharmaceutical formulation comprising at least 45 $mgml^{-1}$ of fulvestrant; 35% (preferably 30% or ideally 25%) or less weight of a pharmaceutically-acceptable alcohol per volume of formulation, at least 1% (preferably at least 5% or ideally 10%) weight of a pharmaceutically-acceptable non-aqueous ester solvent miscible in a ricinoleate vehicle per volume of formulation.

[0063] Preferably 5 ml of the intramuscular injection is administered.

[0064] A further feature of the invention is use of fulvestrant in the preparation of a pharmaceutical formulation as describe hereinabove, for the treatment of a benign or malignant disease of the breast or reproductive tract, preferably treating breast cancer.

[0065] Additional excipients commonly used in the formulation field including, for example, an antioxidant preservative, a colorant or a surfactant may be used. A preferred optional excipient is a surfactant.

[0066] As described above fulvestrant is useful in the treatment of oestrogen-dependent indications such as breast cancer and gynaecological conditions, such as endometriosis.

[0067] In addition to fulvestrant another similar type of molecule is currently under clinical investigation. SH-646 (11β-fluoro-7α-(14,14,15,15,15-pentafluoro-6-methyl-10-thia-6-azapentadecyl)estra-1,3,5(10)-triene-3,17β-diol) is also putatively a compound with the same mode of action as fulvestrant and has a very similar chemical structure. It is believed that the compound will also share with fulvestrant similar physical properties and therefore the current invention will also have application with this compound.

[0068] A further feature of the invention is a pharmaceutical formulation adapted for intra-muscular injection comprising 11β-fluoro-7α-(14,14,15,15,15-pentafluoro-6-methyl-10-thia-6-azapentadecyl)estra-1,3,5(10)-triene-3,17β-diol; 35% or less weight of a pharmaceutically-acceptable alcohol per volume of formulation, at least 1% weight of a pharmaceutically-acceptable non-aqueous ester solvent miscible within a ricinoleate vehicle per volume of formulation and a sufficient amount of a ricinoleate vehicle so as to prepare a formulation of at least 45 $mgml^{-1}$ of 11β-fluoro-7α-(14,14,15,15,15-pentafluoro-6-methyl -10-thia-6-azapentadecyl)estra-1,3,5(10)-triene-3,17β-diol.

[0069] Further features of the invention are those as described above but in which SH-646 is substituted for fulvestrant.

FORMULATION EXAMPLE

[0070] Fulvestrant is mixed with alcohol and benzyl alcohol, stirring until completely dissolved. Benzyl benzoate is added and the solution is made to final weight with castor oil and stirred, (for convenience weight is used rather than volume by using the weight to volume ratio). The bulk solution is overlaid with Nitrogen. The solution is sterilised by filtration using one or two filters of 0.2 μm porosity. The sterile filtrate is kept under a nitrogen overlay as it is filled under aseptic conditions into washed and depyrogenised, sterile primary containers, for example vials or pre-filled syringes. An overage is included in the primary pack to facilitate removal of the dose volume. The primary packs are overlaid with sterile nitrogen, before aseptically sealing.

See also process flow diagram of FIG. 2.

[0071] Quantities of each component of the formulation is chosen according to the required formulation specification, examples are described above. For example quantities are added of each component to prepare a formulation which contains

[0072] 10% weight per volume of benzyl alcohol

[0073] 10% weight per volume of ethanol

[0074] 15% weight per volume of benzyl benzoate

[0075] 250 mg of fulvestrant for each 5 ml of finished formulation

[0076] and the remaining amount as castor oil

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1. A pharmaceutical formulation adapted for intra-muscular injection comprising fulvestrant, 30% or less weight of a pharmaceutically-acceptable alcohol per volume of formulation, at least 1% weight of a pharmaceutically-acceptable non-aqueous ester solvent miscible in a ricinoleate vehicle per volume of formulation and a sufficient amount of a ricinoleate vehicle so as to prepare a formulation which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration of at least 2.5 $ngml^{-1}$ for at least 2 weeks.

2. A pharmaceutical formulation as claimed in claim **1** wherein the blood plasma fulvestrant concentration is attained for at least 4 weeks.

3. A pharmaceutical formulation as claimed in claim **1** wherein the blood plasma fulvestrant concentration is attained for 2 to 5 weeks.

4. A pharmaceutical formulation adapted for intra-muscular injection comprising fulvestrant, 30% or less weight of a pharmaceutically-acceptable alcohol per volume of formulation, at least 1% weight of a pharmaceutically-acceptable non-aqueous ester solvent miscible in a ricinoleate vehicle per volume of formulation and a sufficient amount of a ricinoleate vehicle so as to prepare a formulation of at least 45 $mgml^{-1}$ of fulvestrant.

5. A pharmaceutical formulation as claimed in claim **1** or claim **4** which contains 25% w/v or less of a pharmaceutically-acceptable alcohol.

6. A pharmaceutical formulation as claimed in claim **5** which contains 20% w/v or less of a pharmaceutically-acceptable alcohol.

7. A pharmaceutical formulation as claimed in claim **1** or claim **4** which contains 60% w/v or less of a pharmaceutically-acceptable non-aqueous ester solvent.

8. A pharmaceutical formulation as claimed in claim **7** which contains 50% w/v or less of a pharmaceutically-acceptable non-aqueous ester solvent.

9. A pharmaceutical formulation as claimed in claim **7** which contains 45% w/v or less of a pharmaceutically-acceptable non-aqueous ester solvent.

10. A pharmaceutical formulation as claimed in claim **7** which contains 40% w/v or less of a pharmaceutically-acceptable non-aqueous ester solvent.

11. A pharmaceutical formulation as claimed in claim **7** which contains 35% w/v or less of a pharmaceutically-acceptable non-aqueous ester solvent.

12. A pharmaceutical formulation as claimed in claim **7** which contains 30% w/v or less of a pharmaceutically-acceptable non-aqueous ester solvent.

13. A pharmaceutical formulation as claimed in claim **7** which contains 25% w/v or less of a pharmaceutically-acceptable non-aqueous ester solvent.

14. A pharmaceutical formulation as claimed in claim **1** or claim **4** wherein the pharmaceutically-acceptable alcohol is a mixture of ethanol and benzyl alcohol.

15. A pharmaceutical formulation as claimed in claim **1** or claim **4** wherein the pharmaceutically-acceptable non-aqueous ester solvent is selected from benzyl benzoate, ethyl oleate, isopropyl myristate, isopropyl palmitate or a mixture of any thereof.

16. A pharmaceutical formulation as claimed in claim **1** or claim **4** wherein the pharmaceutically-acceptable non-aqueous ester solvent is benzyl benzoate.

17. A pharmaceutical formulation as claimed in claim **1** or claim **4** wherein the total volume of the formulation is 6 ml, or less, and the concentration of fulvestrant is at least 45 $mgml^{-1}$.

18. A pharmaceutical formulation as claimed in claim **1** or claim **4** wherein the total amount of fulvestrant in the formulation is 250 mg. or more, and the total volume of the formulation is 6 ml, or less.

19. A pharmaceutical formulation as claimed in claim **1** or claim **4** wherein the total amount of fulvestrant in the formulation is 250 mg and the total volume of the formulation is 5 to 5.25 ml.

20. A pharmaceutical formulation as claimed in claim **1** or claim **4** wherein the pharmaceutically-acceptable alcohol is a mixture of 10% weight of ethanol per volume of formulation, 10% weight of benzyl alcohol per volume of formulation and 15% weight of benzyl benzoate per volume of formulation and the ricinoleate vehicle is castor oil.

21. A method of treating a benign or malignant diseases of the breast or reproductive tract by administration to a human in need of such treatment by intramuscular a pharmaceutical formulation as claimed in claim **1** or claim **4**.

22. A method as claimed in claim **21** for treating breast cancer.

23. A syringe or vial containing a pharmaceutical formulation as defined in claim **20**.

* * * * *

definite bearing on the usefulness of any column packing prepared. The performances of the seven supports mentioned previously were examined under the same operating conditions. The supports that can be used for lightly loaded packings are: glass beads, Gas Chrom-P, and Chromosorb W-HMDS. The other four supports cannot be used for lightly loaded column packing since their interaction with the antihistamines causes excessive peak tailing.

The hydrogen flame detector used in conjunction with the 0.010-in. stainless capillary column would not respond to compounds with boiling points above 330°. This limitation prevented evaluation of this column for the analysis of these antihistamines.

The 100-ft. 0.065-in. copper open tubular column was coated with XF-1150 and evaluated using the above group of antihistamines. The Sr^{90} ionization detector was used with a column flow of 36 ml./minute. The retention times obtained were comparable to the 6-ft.-XF-1150 packed column, but the peak base widths were considerably wider. Because of this increase in base width, the 0.065-in. column was less efficient than the 6-ft. packed column.

A 250-ft. 0.065-in. column wound on a $1^1/_4$-in. diameter mandrel has been reported to be more efficient than a packed column (15). There are two possible reasons why efficiency was less than previously reported: (*a*) the column was shorter (100 ft.), and (*b*) the winding configuration was markedly different. The column was wound on a $1^1/_4 \times {}^1/_4$-in. bar which resulted in a definite flattening of the tube around the edge of the bar.

CONCLUSIONS

The antihistamines investigated, except for meclizine, can be separated, identified, and concentration estimated using the Carbowax 20M, PDEAS, and XF-1150 columns described. The PDEAS column is the most efficient of the three for the analysis of antihistamines.

The usefulness of the 0.010-in. capillary and the 0.065-in. open tubular columns cannot be properly evaluated until the mentioned limitations are removed.

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Castor Oil as a Vehicle for Parenteral Administration of Steroid Hormones

By C. RIFFKIN, R. HUBER, and C. H. KEYSSER

Steroid hormones may be administered parenterally in high concentrations as oil solutions. In this form they exhibit a prolonged action and reduce the number of injections required. To accommodate the demand for increasingly greater concentrations of hormones in solution, castor oil in combination with other suitable oil-miscible solvents, has been found to fulfill a need. The development of several formulations together with the results of animal testing, as well as clinical trials in humans, attest to the acceptability of this oil for the purposes intended.

FIXED OILS are included in the "United States Pharmacopeia XVI" as nonaqueous vehicles for injection and are characterized as being of vegetable origin, essentially odorless, and without suggestion of rancidity. They must also comply with certain measurable physical limits specified for the saponification, acid, and iodine values.

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After subcutaneous injection, Deanesly and Parkes (1) observed the persistence of olive oil and castor oil in animal tissue. Comparing other oils Brown, *et al.* (2), reported that sesame and corn oils were superior to cottonseed and peanut oils because they were less irritating, less antigenic, more quickly released from tissue, and possessed superior physical properties.

More recently the use of steroid hormone medication has expanded considerably. Due to limited water solubility, hormones have been administered as aqueous suspensions or solutions in oil. It has been claimed that the latter provided the slow release preferred in cyclical

TABLE I.—ANALYSIS OF COMMERCIAL OILS AND COMPARISON TO U.S.P. XVI SPECIFICATIONS

Oil	Lot No.	ml. 0.02 *N* NaOH Equiv. to Free Fatty Acid in 10-Gm. Sample	Sapon. Value	Iodine Value
Castor Oil	U.S.P. specs.	35.0[a]	179–185	83–88
	23946	14.0	183.3	84.8
	25589	4.6	179.8	87.0
	23463	7.9	182.7	84.5
	33742	9.2	180.4	84.2
Sesame Oil	U.S.P. specs.	3.0	188–195	103–116
	23549A	0.5	189.6	106.9
	26953	1.4	194.0	111.8
	33646	0.75	189.6	104.7
	29981	0.45	191.7	108.2
Cottonseed Oil	U.S.P. specs.	2.0	190–198	109–116
	49684	...	195.9	111.8
	44441	...	196.3	113.1
Corn Oil	U.S.P. specs.	2.0	187–193	102–128
	52148	1.0	194.5	119.1
	36716	1.2	191.4	124.4
	33436	1.2	189.3	125.0
	33715	1.0	189.3	123.0
Peanut Oil	U.S.P. specs.	2.0	185–195	84–100
	22160	1.2	192.0	94.4
	20993	1.4	191.7	93.2
	33622	0.8	193.1	87.8
	26147	1.2	190.4	93.9

[a] The U.S.P. specifies that the titration of free fatty acids in oral grade castor oil shall not exceed 7 ml. of 0.1 *N* NaOH which is equal to 35.0 ml. of 0.02 *N* NaOH.

TABLE II.—SOLUBILITY OF STEROIDS IN U.S.P. OILS AT 25°

Steroid	mg./ml. Castor Oil	mg./ml. Sesame Oil	mg./ml. Peanut Oil
17-Hydroxyprogesterone caproate	55.6	23.4	27.9
Testosterone	38.6	5.4	8.1
Estradiol valerate	60.6	16.1	18.8
Progesterone	52.0	22.9	23.5

therapy (3). Using withdrawal bleeding in human females as the criterion, Master, *et al.* (4), compared the duration of action of an aqueous suspension of progesterone with an oil solution, and confirmed the superiority of the latter. The prolongation of activity was generally related to storage in the fatty depots of the body (5).

In 1952 Junkmann (6) determined that a testosterone ester dissolved in sesame oil prolonged the androgenic effects in castrated rats. Davis and Wied (7) demonstrated that prolonged activity was also obtained in humans when oil solutions of a progesterone derivative were injected. There was still a limiting factor, however, in that only a relatively small amount of hormone could be dissolved in the traditional oils. To increase the solvent power of the oil it was necessary to add compatible and nonirritating cosolvents. Such additions consisted of benzyl benzoate, benzyl alcohol, ethyl lactate, ethyl oleate, etc. The U.S.P. recognized the need for such "other vehicles," with the restrictions that they must be safe in the volume of injection administered, and that they should not interfere with the therapeutic efficacy of the preparation or its testing.

Demand for increased hormone concentrations per dose, furthered the search for an acceptable oil with greater solubilizing power *per se*. Boschann (8) in 1954, observed that 17-hydroxyprogesterone caproate in a castor oil–ethyl lactate vehicle was well tolerated. In addition, private communications from clinicians in West Germany[1] reported good tolerance to Proluton-Depot containing a castor oil–benzyl benzoate vehicle. Since then other hormones have been used as solutions in ricinoleic acid esters, as well as in castor oil (9–11). Accordingly, an investigation was undertaken into the suitability of castor oil as a vehicle for parenteral administration of steroid hormones.

METHODS AND RESULTS

Representative samples of U.S.P. oils obtained from commercial sources were tested in accordance with the official method for free fatty acid content, saponification, and iodine values. The results are listed in Table I along with the U.S.P. XVI specifications for these oils.

Solubility of selected steroids in various oils was determined in the following manner. An excess of steroid was stirred for 4 hours at room temperature (25°) in the test oil, after which the undissolved solids were removed by filtration, and the clear solution assayed for steroid content. Table II shows the results obtained.

An attempt was made to reduce the free fatty acids in castor oil by treatment with alumina and anhy-

[1] Dr. Napp, Universitats-Krankenhaus, Hamburg; Dr. Pots, Humboldt-Universitat-Charite Frauenklinik, Berlin; Dr. Prill, Universitats-Frauenklinik, Wurzburg; and Dr. Rauscher, Universitats-Frauenklinik, Vienna.

TABLE III.—ABSORPTION OF OIL FROM ANIMAL MUSCLE[a]

Days after Injection	Oil	ml. 0.02 N NaOH Equiv.	Residual Oil in Muscle (estd.)
1–3	Castor oil (aged)	50	1 day —50% 3 days—20%
1–3	Castor oil U.S.P.	13	1 day —30% 3 days—10%
1–3	Sesame oil U.S.P.	1.4	1 day —30% 3 days—30%
7–60	All oils	...	Declining 10 to 2%

[a] 1 ml. injected into back muscle of rabbit.

drous sodium sulfate. Three grams of dried, powdered, amorphous aluminum oxide (Merck No. 1097) and 6 Gm. of anhydrous sodium sulfate, reagent grade, were suspended in 120 ml. of oil and heated at 80° under a blanket of nitrogen for 1.5 hours. After allowing the oil to cool to room temperature, the solids were filtered off and the acids titrated in the usual manner. A significant reduction in free fatty acid was not obtained.

The absorption characteristics of oils with varying fatty acid content were examined and compared on a biological basis. Aged castor oil with a high free fatty acid content was compared to fresh U.S.P. castor oil with a low acid content and U.S.P. sesame oil by injecting 1 ml. of oil into the back muscles of rabbits, approximately 2 in. from the iliac crest. A rotational pattern of injection was used and the oil samples were stained to aid visibility in the tissues. The animals were sacrificed and the muscles excised and examined grossly. The results were averaged and appear in Table III.

The test disclosed that oil migrated or was carried to the fascia, and very small amounts remained for 60 days. Localized degeneration produced by the high acid value castor oil was essentially healed in 7 days, and the low acid value castor oil appeared to be no more irritating than sesame oil.[3]

In a specific test for irritation 0.25 ml. of the above oil samples were also injected into the *vastus lateralis* muscles of rabbits. After 2 days the animals were sacrificed and the injected muscles examined grossly for evidence of irritation. It was found that the castor oil containing a high level of free fatty acid produced a lesion size measuring approximately 121 mm.[3]. The lesion itself was characterized mainly by degeneration of local tissue without necrosis. Castor oil with low free fatty acid and sesame oil, on the other hand, produced no measurable lesion at the injection site.

Combinations of benzyl alcohol and benzyl benzoate with both castor oil and sesame oil were also injected into the *vastus lateralis* muscles of rabbits and Table IV lists the lesion sizes produced.

Solutions which were formulated for clinical trials in humans were prepared by dissolving the steroid hormones in appropriate vehicles at 60° under nitrogen. The solutions were then filtered through a coarse sintered-glass filter with the aid of nitrogen pressure, filled into vials, and sterilized by autoclaving for 2 hours at 121° (15 lb. steam pressure). The products were then submitted for assay, safety, and animal muscle irritation testing prior to release for clinical investigation.

[3] Due to the apparent increase in free fatty acids with aging, subsequent work utilized fresh oils which required for neutralization less than 3 ml. of 0.1 N NaOH (15 ml. of 0.2 N NaOH) per 10 Gm. of sample.

DISCUSSION

Throughout the investigation it was desirable to have a reference oil to serve as a basis for comparison. Since sesame oil is universally accepted as a parenteral oil vehicle, it was chosen as the "standard" vegetable oil to be compared to castor oil, with and without other cosolvents. The physical, chemical, and biological properties of sesame oil are well documented and require no comments here.

Chemically, castor oil consists of the triglycerides of ricinoleic acid, together with small quantities of glycerides of other acids. The quantitative composition is given by Eckey (12) as follows: ricinoleic acid 87%, oleic acid 7.4%, linoleic acid 3.1%, dihydroxyricinoleic acid 0.6%, and miscellaneous acids 2.4%. Two grades are commonly recognized in this country—U.S. No. 1 which is cold pressed oil, and U.S. No. 3 which is oil extracted from the pressed cake. Only the former is used for medicinal purposes.

The high viscosity of castor oil compared to other vegetable oils is undoubtedly related to hydrogen bonding and it is probably the hydroxy groups which contribute to the greater polarity and superior solvent power of the oil. As indicated in Table I, the saponification and iodine values of commercial castor oil appear to be slightly lower than the U.S.P. XVI limits for oils used for injection. On the other hand, the content of free fatty acids even in fresh oil, varies considerably and exceeds the traditional limits for injectable oils. The significance of this is somewhat obscure, although "Remington's Practice of Pharmacy, 12th edition," page 387, states "a low free fatty acid content is essential since it indicates a fresh and pure product and not one that is likely to have become old and heavily contaminated with bacterial products."

Despite better solubility of steroids in castor oil, other cosolvents were necessary to dissolve the

TABLE IV.—LOCAL IRRITATION PRODUCED IN RABBIT MUSCLE BY INJECTION OF VARIOUS OIL VEHICLES[a]

Identification	Composition	Lesion size, mm.[3]
SHY-47-2	Sesame oil 98% Benzyl alcohol 2%	61
SHY-47-4	Castor oil 98% Benzyl alcohol 2%	Too small to measure
SHY-47-3	Sesame oil 95% Benzyl alcohol 5%	506
SHY-47-5	Castor oil 95% Benzyl alcohol 5%	106
SHY-14-2	Sesame oil 65% Benzyl benzoate 35%	291
SHY-14-5	Castor oil 65% Benzyl benzoate 35%	184
SHY-47-6	Sesame oil 63% Benzyl benzoate 35% Benzyl alcohol 2%	207
SHY-47-7	Castor oil 63% Benzyl benzoate 35% Benzyl alcohol 2%	262
SHY-14-3	Sesame oil 50% Benzyl benzoate 50%	291
SHY-14-6	Castor oil 50% Benzyl benzoate 50%	158

[a] A 0.25-ml. quantity of the oil vehicle was injected into the *vastus lateralis* muscle of the rabbit. Two days later the muscle was excised and the lesion size measured in mm.[3].

increasingly higher concentrations required by therapeutic regimens. Often these materials contributed additional advantages. For example, the addition of benzyl alcohol or benzyl benzoate to castor oil resulted in a lower and more favorable viscosity, making it easier to inject. Also, benzyl alcohol was an effective preservative and local anesthetic.

The nature of the irritative response depended on the particular hormone, its concentration in the formulations, and/or the composition of the vehicle. Although rabbit muscles are more sensitive than human muscles, they were selected primarily because local changes in the muscle were observed easily. It was not always possible, however, to correlate muscle irritation in animals to that of humans.

A numerical assignment to lesion size was used solely as a convenience for grading response. The numbers alone do not adequately describe the nature of the response, however. More completely it is characterized by the amount of hemorrhage and edema and the incidence, degree, and extent of local degeneration produced by the injection. A slight, reversible irritative response may cover a large area and a severe irreversible one may be comparatively small. A decrease in the size of the degenerated area indicates a reversible condition. The presence of necrosis, which is the most damaging situation, means that the cellular structure was destroyed and repair must take place. The debris must be removed and the original cellular mass in the area replaced with fibrous connective tissue. The extent of this fibrosis or formation of scar tissue gives an index of the amount of irreversible damage. Fortunately necrosis was not encountered, indicating the lack of permanent muscle damage. Since these changes take time, final assessment of the effects of an injection in the muscle frequently required observation for 7 days or longer.

It is unfortunate that pain cannot be measured by any known method of animal testing. The animal usually does not respond unless the painful stimulus is marked. Furthermore, the pain caused by injection into human muscle is not usually proportionate to the irritation produced either in animal muscle or in human muscle. Realizing that these limitations are inherent in animal test methods, it remained for final acceptability to be determined in man.

When it was discovered that 17-hydroxyprogesterone caproate possessed high progestational activity, potencies of the order of 65 mg./ml. were used. By increasing the dose, additional prolongation of action was obtained, and eventually concentrations of the order of 250 mg./ml. were required. Such a solution in sesame oil produced acceptable animal muscle tolerance, but the pain and local reaction in humans was so great as to prohibit the adoption of the formulation as a commercial product (see Table V, Lot Pr. 142-53/15-10).[3] Solutions were also prepared using castor oil as the vehicle, and Table V lists the formulations tested and the results obtained. Information obtained from the clinical trials (14–21) attested to the acceptability and safety of the adopted formulations.

Inherent in the development of an acceptable formulation of 17-hydroxyprogesterone caproate was the required development of a suitable assay method. This was accomplished by Roberts and Florey (13) using paper-strip chromatography.

Since estrogens are more potent than progestogens and require less per dose, an acceptable formulation of estradiol valerate was easier to prepare. Besides use in estrogen therapy, estradiol valerate has found utility in the treatment of carcinoma, and for that purpose high dosages were required. Concentrations were increased from 10 to 40 mg./ml. and

[3] Reactions in excess of 5–6% were considered unacceptable.

Table V.—Evaluation of 250 mg./ml. 17-Hydroxyprogesterone Caproate Solutions in Various Oil Vehicles

Vehicle Composition	Animal Muscle Lesion Size, mm.[2a]	Lot Number and Remarks on Clinical Testing
Sesame oil 50% Benzyl benzoate 50%	1049	Pr.142-53/15-7—238 injections, 20.6% reactions, rejected
Castor oil 58% Benzyl benzoate 40% Benzyl alcohol 2%	691	Pr.142-53/15-8—270 injections, 23.2% reactions, rejected
Sesame oil 60% Benzyl benzoate 35% Benzyl alcohol 5%	697	Pr.142-53/15-10—189 injections, 10.7% reactions, rejected
Castor oil 54% Benzyl benzoate 46%	258	Pr.142-53/15-11—503 injections, 4.2% reactions, accepted
Castor oil 52% Benzyl benzoate 46% Benzyl alcohol 2%	633	Pr.142-53/15-13—924 injections, 1.3% reactions, accepted

[a] Injection of 0.25 ml. into *vastus lateralis* muscle of rabbits and lesion size determined 2 days after injection.

Table VI.—Evaluation of Estradiol Valerate in Various Oil Vehicles

Composition	Animal Muscle Lesion Size, mm.[2a]	Lot Number and Remarks
20 mg./ml. in Castor oil 78%, Benzyl benzoate 20%, Benzyl alcohol 2%	197	Es.31-53/15-B—Commercially available
30 mg./ml. in Sesame oil 60%, Benzyl benzoate 40%	306	DEK-98-2—Not tested clinically; dosage increased to 40 mg./ml.
30 mg./ml. in Castor oil 80%, Benzyl benzoate 20%	194	Es.31-53-V—Not tested clinically; dosage increased to 40 mg./ml.
40 mg./ml. in Sesame oil 65%, Benzyl benzoate 30%, Benzyl alcohol 5%	803	SHX-94-4—Too irritating; not tested clinically
40 mg./ml. in Sesame oil 58%, Benzyl benzoate 40%, Benzyl alcohol 2%	496	Es.31-53-8—201 injections, 23.2% reactions, rejected
40 mg./ml. in Castor oil 58%, Benzyl benzoate 40%, Benzyl alcohol 2%	250	Es.31-53-A—826 injections, 2.67% reactions (all mild), accepted

[a] Injection of 0.25 ml. into *vastus lateralis* muscle of rabbits and lesion size determined 2 days after injection.

again formulations containing castor oil in the vehicle proved to be less irritating than similar preparations containing sesame oil. Physically and chemically both oil solutions were stable. Based on acceptable preliminary data, formulations such as those listed in Table VI were prepared and tested. Acceptability in humans was confirmed by clinicians and described in the literature (22, 23) and in case reports.[4]

SUMMARY

1. The development and testing of parenteral steroid hormone formulations has been described, using castor oil as a vehicle.

2. After ascertaining stability and animal muscle irritation, selected formulations were evaluated in humans. They exhibited a prolonged action, were effective and well tolerated.

3. Examples of commercially available products are the estrogen, estradiol valerate[5] at 20 mg./ml. and 40 mg./ml., and the progestogen, 17-hydroxyprogesterone caproate[6] at 250 mg./ml.

[4] Case reports: estradiol valerate, 20 mg./ml., in castor oil 78%, benzyl benzoate 20%, benzyl alcohol 2%—90 injections in 46 patients. Two mild local reactions. Estradiol valerate 40 mg./ml. in castor oil 58%, benzyl benzoate 40%, benzyl alcohol 2%—51 patients. Number of injections not completely tabulated. One report is in press.

[5] Marketed as Delestrogen by E. R. Squibb & Sons, New York, N. Y.

[6] Marketed as Delalutin by E. R. Squibb & Sons, New York, N. Y.

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Isolation of Marrubiin, a Sterol, and a Sesquiterpene from *Marrubium vulgare*

By HAROLD J. NICHOLAS*

A simple column chromatographic method for isolating the bicyclic diterpene marrubiin from acetone and ethanol extracts of *Marrubium vulgare* L. is described. An unsaturated sterol of the stigmastanol series, present in esterified form, and a sesquiterpene ($C_{15}H_{22}O_2$) have been isolated from the extracts.

IN PREPARATION for radioactive tracer work on the biosynthesis of marrubiin it was necessary to examine extracts of the plant for associated terpenoid substances. A convenient column chromatographic method was therefore devised for separating relatively pure marrubiin from crude acetone extracts. Two new terpenoid substances were detected in the extracts.

EXPERIMENTAL

Materials and Methods.—Ground *M. vulgare* L. was obtained from the Wunderlich-Diez Corp., Hasbrouck Heights, N. J.[1] This material was exhaustively extracted with hot acetone or hot ethanol. Either solution on removal of solvent by distillation (the last stages *in vacuo*) yielded black, viscous material which was used for further examination. Melting points were determined on a Fisher-Johns melting point apparatus. Optical rotations (in $CHCl_3$) and C—H analyses were determined by Drs. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England. An infrared spectrum of the unidentified diterpene was determined on a Perkin-Elmer spectrophotometer by the KBr disk method.[2] An infrared spectrum of the sterol was determined in chloroform solution in a 0.1-mm. sealed cell, compensated with $CHCl_3$, on a Beckman IR-4 recording infrared spectrophotometer,[3] and by the KBr disk method. The

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[1] This firm has given assurance that the material investigated was *M. vulgare* or white horehound, not *Ballota hirsuta* (black horehound).

[2] We are indebted to the Department of Pathology, University of Kansas, for this determination.

[3] Determined by Sadtler Research Laboratories, Philadelphia, Pa.

REVIEW ARTICLE

Excipients and Their Use in Injectable Products

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ABSTRACT: Formulation of a new drug product with excipients, that have been previously added to an approved injectable product, may save pharmaceutical companies developmental time and cost. The Physicians' Desk Reference (PDR) and Handbook on Injectable Drugs were reviewed, extracting all information on excipients. The information was consolidated into eight tables, categorizing excipients as 1) Solvents and Co-solvents, 2) Solubilizing, Wetting, Suspending, Emulsifying or Thickening agents, 3) Chelating Agents, 4) Antioxidants and Reducing Agents, 5) Antimicrobial Preservatives, 6) Buffers and pH Adjusting Agents, 7) Bulking Agents, Protectants, and Tonicity Adjustors, and 8) Special Additives. Where applicable, tables list frequency of use, concentration, and an example of a commercial product containing the excipient. Excipients which are included in the 1996 FDA 'Inactive Ingredient Guide,' but do not appear in the PDR or Handbook on Injectable Drugs, were included as a separate list.

Introduction

Injectable products require a unique formulation strategy. The formulated product has to be sterile, pyrogen free and, in the case of solutions, free of particulate matter. Preferably, the formulation will be isotonic, and depending on the route of administration (for instance, for intra-spinal or intra-cisternal routes), antioxidants and preservatives may not be allowed. For a given drug, the risk of adverse events is higher if it is administered as an injection versus a non-parenteral route. The requirement for sterility demands that the excipients be able to withstand autoclaving or other sterilization processes. These factors limit the choice of excipients available to the formulators.

Generally, a knowledge of which excipients have been deemed safe by the FDA or are already present in a marketed product provides increased assurance to the formulator that these excipients will probably be safe for their new drug product. However, there is no guarantee that the new drug product will be safe as excipients are combined with other additives and/or with a new drug, creating unforeseen potentiation or synergistic toxic effects. Regulatory bodies may view an excipient previously approved in an injectable dosage form favorably, and will frequently require less safety data. A new additive in a formulated product will always require additional studies adding to the cost and timeline of product development.

The purpose of this paper is to present the various excipients that have been included in the formulation of injectable products marketed in the USA. This information is not readily available. A literature search indicates that the last paper dealing with this was published in 1980 (1). Products approved outside the US are not covered in this review. Also, sterile dosage forms not administered parenterally, such as solutions for irrigation, ophthalmic or otic drops, and ointments were excluded.

Methodology

Physicians' Desk Reference published in 1994 & 1996 (2, 3), and Handbook on Injectable Drugs (4) were used as the primary source of information. Entries on all injectable drugs were summarized in an Excel worksheet. Each product was classified by Manufacturer, Trade name, Drug name, Route of Administration, SVP/LVP, pH of Product, Solvent Used, Solubilizing/Suspending Agent, Preservative, Antioxidant, Chelator and Other Formulation Additives.

The resulting Excel sheet had information on more than 700 products. This information was condensed into easy-to-read tables. Each table has been categorized based on the primary function of excipient in the formulation. For example, citrates are classified as buffers and not as chelating agents, and ascorbates are categorized as antioxidants, although they can serve as buffers. This classification system was based on our experience in formulation development and on the published literature. Such simplification avoids duplication of entries and provides the audience with easy-to-read tables.

Some duplication was unavoidable. Tables VII and VIII contain some excipients which may have also been listed in the first six tables. Whenever the reference specifically designated a specific function to an ingredient it was re-listed in Tables VII and VIII. For example, glycine can be used as a buffer or as a stabilizing (protecting) agent. Therefore, glycine is listed in Tables VI and VII. Methyl paraben is a preservative (Table V) but also has a special function in Adriamycin RDF® formulation (Table VIII).

The concentration of excipients is listed as percentages weight by volume (w/v) or volume by volume (v/v). If the product was listed as lyophilized or powder, these percent-

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TABLE I
Solvents and Co-solvents

Excipient	Frequency	Range	Example
Benzyl Benzoate	2	20% v/v	Depo-Testosterone® (Upjohn) 20% v/v
Cottonseed Oil	1	73.6% w/v	Depo-Testosterone® (Upjohn) 73.6% w/v
N,N Dimethylacetamide	1	6% w/v	Vumon® (Bristol Myers) 6% w/v
Ethanol	24	0.6–80%	Prograf® (Fujisawa) 80% v/v
Glycerin (Glycerol)	9	1.6–70% w/v	Multitest CMI® (Connaught) 70% w/v
Peanut oil	1	*	Bal la Oil® (Becton Dickinson)
Polyethylene glycol			
PEG	4	0.15–50%	Secobarbital sodium (Wyeth-Ayerst) 50%
PEG 300	2	50–65%	VePesid® (Bristol Myers) 65% w/v
PEG 400	2	*	Ativan® (Wyeth-Ayerst)
PEG 3350	5	0.3–3%	Depo-Medrol® (Upjohn) 2.95% w/v
Poppyseed oil	1	1%	Ethiodol® (Savage) 1%
Propylene Glycol	25	0.2–75.2%	Terramycin Solution (Roerig) 75.2%
Safflower oil	2	5–10%	Liposyn II® (Abbott) 10%
Sesame oil	6	*	Solganal Inj.® (Schering)
Soybean oil	4	5–20% w/v	Intralipid® (Clintec) 20%
Vegetable oil	2	*	Viriion IM Inj.® (Star Pharmaceuticals)

* No data available.

ages were derived based on the reconstitution volume commonly used. The tables list the range of concentration used, typical or most common concentration employed, and examples of products containing the excipient, specifically those which use extremely low or high concentrations.

Discussions

Table I list solvents and co-solvents used in parenteral products. Water for injection is the most common solvent but may be combined or substituted with a co-solvent to improve the solubility or stability of drugs. Oils like safflower and soybean are used in total parenteral nutrition products where they serve as a fat source and as carriers for fat-soluble vitamins. Ethanol and propylene glycol are used, either alone or in combination with other solvents, in more than 50% of parenteral co-solvent systems. It is surprising to see propylene glycol used more often than polyethylene glycols (PEGs) in spite of its higher myotoxicity and hemolyzing effects (5, 6). Probably, the presence or generation of peroxides in PEGs is a major limitation.

Table II includes a broad category of excipients whose function in formulation could be—(1) Viscosity imparting or suspending agents like carboxy methyl cellulose, sodium carboxy methyl cellulose, sorbitol, acacia, Povidone, hydrolyzed gelatin; (2) Solubilizing, wetting or emulsifying agents like Cremophore EL, sodium desoxycholate, Polysorbate 20 or 80, PEG 40 castor oil, PEG 60 castor oil, sodium dodecyl sulfate, lecithin or egg yolk phospholipid; (3) Aluminum monostearate which is added to fixed oil to form viscous or gel-like suspending medium. Polysorbate 80 is the most common and versatile solubilizing, wetting and emulsifying agent.

Only a limited number of chelating agents are used in parenteral products (Table III). They serve to complex heavy

TABLE II
Solubilizing, Wetting, Suspending, Emulsifying or Thickening Agents

Excipient	Frequency	Range	Example
Acacia	2	7%	Tuberculin Old Test® (Lederle) 7%
Aluminum monostearate	1	2%	Solganal Inj.® (Schering) 2%
Carboxy methyl cellulose	4	1%	Bicillin® (Wyeth-Ayerst) 0.55%
Carboxy methyl cellulose, sodium	9	0.1–0.75%	Lupron Depot® (TAP) 0.75% w/v
Cremophore EL*	3	50–65% w/v	Sandimmune® (Sandoz) 65% w/v
Desoxycholate sodium	1	0.4% w/v	Fungizone® (Bristol Myers) 0.41% w/v
Egg yolk phospholipid	3	1.2%	Intralipid® (Clintec) 1.2%
Gelatin, Hydrolzyed	1	16% w/v	Cortone® (Merck) 16% w/v
Lecithin	7	0.4–1.2% w/v	Diprivan® (Zeneca) 1.2% w/v
Polyoxyethylated fatty acid	1	7% w/v	AquaMephyton® (Merck) 7% w/v
Polysorbate 80 (Tween 80)	31	0.01–12%	Cordarone X I.v.®. (Wyeth-Ayerst) 10%
Polysorbate 20 (Tween 20)	5	0.01–0.4%	Calcijex® (Abbott) 0.4% w/v
PEG 40 castor oil**	1	11.5% v/v	Monistat® (Janssen) 11.5% v/v
PEG 60 castor oil***	1	20% w/v	Prograf® (Fujisawa) 20% w/v
Povidone (Polyvinyl pyrrolidone)	6	0.5–0.6% w/v	Bicillin® (Wyeth-Ayerst) 0.6% w/v
Sodium dodecyl sulfate (Na lauryl sulfate)	1	0.018% w/v	Proleukin® (Cetus) 0.018% w/v
Sorbitol	3	25–50%	Aristrospan® (Fujisawa) 50% v/v

* Cremophor EL: Etocas 35, polyethoxylated castor oil, polyoxyethylene 35 castor oil.
** PEG 40 castor oil; polyoxyl 40 castor oil, castor oil POE-40, Croduret 40, polyoxyethylene 40 castor oil, Protachem CA-40.
*** PEG 60 hydrogenated castor oil; Cremophor RH 60, hydrogenated castor oil POE-60, Protachem CAH-60.

TABLE III
Chelating Agents

Excipient	Frequency	Range	Example
Calcium disodium EDTA*	9	0.01–0.1%	Wydase® (Wyeth-Ayerst) 0.1% w/v
Disodium EDTA	34	0.01–0.1%	Calcijex® (Abbott) 0.11% w/v
Sodium EDTA	1	0.20%	Folvite® (Lederle) 0.2%
DTPA**	1	0.04%	Magnevist® (Berlex) 0.04%

* EDTA = Ethlenediaminetetraacetic acid.
** DTPA = Diethylenetriaminepentaacetic acid; Pentetic acid.

metals and therefore can improve the efficacy of antioxidants or preservatives. In our opinion, calcium EDTA has an advantage over tetrasodium salt by not contributing sodium and not chelating calcium from the blood.

An antioxidant as a class is defined as those compounds that can act as reducing agents or may serve as free radical scavengers. Table IV summarizes the antioxidants, their frequency of use, concentration range and examples of products containing them. Sulfite, bisulfite, and metabisulfite constitute the majority of antioxidants used in parenteral products despite several reports of incompatibilities and toxicity (7, 8). Butylated hydroxy anisole, butylated hydroxy toluene and propyl gallate are primarily used in semi/non-aqueous vehicles because of their low aqueous solubility. Ascorbic acid/sodium ascorbate may serve as an antioxidant, buffer, and chelating agent in the same formulation.

Benzyl alcohol was the most common antimicrobial preservative present in parenteral formulations (Table V). This is consistent with other surveys (9). Parabens are the next most common preservatives. Thirty-nine products had a combination of methyl and propyl parabens; eleven had only methyl, and one had only propyl paraben. Thimerosal was surprisingly common, especially in vaccines, even though some individuals have sensitivity to mercurics. Chlorocresol is purported to be a good preservative for parenterals, but our survey did not find any examples of commercial products containing chlorocresol.

Table VI lists buffers and chemicals used to adjust the pH of formulations. Phosphate, citrate, and acetate are the most common buffers used in parenteral products. Mono and diethanolamine are added to adjust pH and form corresponding salts. Hydrogen bromide, sulfuric acid, benzene sulfonic acid and methane sulfonic acids are added to drugs which are bromide (Scopolamine HBr, Hyoscine HBr, UDL), sulfate (Nebcin, Tobramycin sulfate, Lilly), besylate

TABLE IV
Antioxidants and Reducing Agents

Excipient	Frequency	Range	Example
Acetone sodium bisulfite	4	0.2–0.4% w/v	Novocaine® (Sanofi-Winthrop) 0.4% w/v
Ascorbate (sodium/acid)	7	0.1–4.8% w/v	Vibramycin® (Roerig) 4.8% w/v
Bisulfite sodium	28	0.02–0.66% w/v	Amikin® (Bristol Myers) 0.66% w/v
Butylated hydroxy anisole (BHA)	3	0.00028–0.03% w/v	Aquasol® (Astra) 0.03%
Butylated hydroxy toluene (BHT)	3	0.00116–0.03% w/v	Aquasol® (Astra) 0.03%
Cystein/Cysteinate HCl	2	0.07–0.10% w/v	Acthar Gel® (Rhone-Poulanc) 0.1% w/v
Dithionite sodium (Na hydrosulfite, Na sulfoxylate)	1	0.10%	Numorphan® (DuPont) 0.10%
Gentisic acid	1	0.02% w/v	OctreoScan® (Mallinckrodt)
Gentisic acid ethanolamine	1	2%	M.V.I. 12® (Astra) 2%
Glutamate monosodium	2	0.1% w/v	Varivas® (Merck) 0.1% w/v
Formaldehyde sulfoxylate sodium	9	0.075–0.5% w/v	Terramycin Solution (Roerig) 0.5% w/v
Metabisulfite potassium	1	0.10%	Vasoxyl® (Glaxo-Wellcome) 0.10%
Metabisulfite sodium	29	0.02–1% w/v	Intropin® (DuPont) 1% w/v
Monothioglycerol (Thioglycerol)	6	0.1–1%	Terramycin Solution (Roerig) 1%
Propyl gallate	2	0.02%	Navane® (Roerig)
Sulfite sodium	7	0.05–0.2% w/v	Enion® (Ohmeda) 0.2% w/v
Thioglycolate sodium	1	0.66% w/v	Sus-Phrine® (Forest) 0.66% w/v

TABLE V
Antimicrobial Preservatives

Excipient	Frequency	Range	Example
Benzalkonium chloride	1	0.02% w/v	Celestone Soluspan® (Schering) 0.02% w/v
Benzethonium chloride	4	0.01%	Benadryl® (Parke-Davis) 0.01% w/v
Benzyl alcohol	74	0.75–5%	Dimenhydrinate® (Steris) 5%
Chlorobutanol	17	0.25–0.5%	Codine phosphate (Wyeth-Ayerst) 0.5%
m-Cresol	3	0.1–0.3%	Humatrope® (Lilly) 0.30%
Myristyl gamma-picolinium chloride	2	0.0195–0.169% w/v	Depo-Provera® (Upjohn) 0.169% w/v
Paraben methyl	50	0.05–0.18%	Inapsine® (Janssen) 0.18% w/v
Paraben propyl	40	0.01–0.1%	Xylocaine w/Epinephrine (Astra) 0.1% w/v
Phenol	48	0.2–0.5%	Calcimar® (Rhone Poulanc) 0.5% w/v
2-Phenoxyethanol	3	0.50%	Havrix® (SmithKline Beecham) 0.50% w/v
Phenyl mercuric nitrate	3	0.001%	Antivenin® (Wyeth-Ayerst) 0.001%
Thimerosal	46	0.003–0.01%	Atgam® (Upjohn) 0.01%

TABLE VI
Buffers and pH Adjusting Agents

Excipient	Example
Acetate	
Sodium	Miacalcin Injection® (Sandoz)
Acetic acid	Miacalcin Injection® (Sandoz)
Glacial acetic acid	Brevibloc Injection® (Ohmeda)
Ammonium	Bumex Injection® (Roche)
Ammonium hydroxide	Triostat Injection® (SmithKline Beecham)
Benzene sulfonic acid	Tracrium Injection® (Glaxo-Wellcome)
Benzoate Sodium/acid	Valium Injection® (Roche)
Bicarbonate Sodium	Cefotan Injection® (Zeneca)
Carbonate Sodium	HypoRho-D® (Bayer)
Citrate	
Acid	DTIC-Dome® (Bayer)
Sodium	Ceredase® (Genzyme)
Disodium	Cerezyme® (Genzyme)
Trisodium	Cerezyme® (Genzyme)
Diethanolamine	Bactrim IV® (Roche)
Glucono delta lactone	Quinidine® (Lilly)
Glycine	Hep-B Gammagee® (Merck)
Hydrochloric acid	Amicar® (Immunex)
Hydrogen bromide	Scopolamine (UDL)
Lactate acid/Sodium	Fentenyl citrate & Droperidol (Astra)
Lysine	Eminase Injection® (Roberts)
Maleic acid	Librium Injection® (Roche)
Methanesulfonic acid	DHE-45 Injection® (Sandoz)
Monoethanolamine	Terramycin Solution (Roerig)
Phosphate	
Acid (phosphoric)	Humegon® (Organon)
Monobasic potassium	Zantac Injection® (Glaxo-Wellcome)
Monobasic sodium*	Pregnyl® (Organon)
Dibasic sodium**	Prolastin® (Bayer)
Tribasic sodium	Synthroid® (Knoll)/
Sodium hydroxide	Optiray® (Mallinckrodt)
Sulfuric acid	Nebcin® (Lilly)
Tartrate acid/sodium	Methergine Injection® (Sandoz)
Tromethamine	Optiray® (Mallinckrodt)

* Sodium biphosphate, Sodium dihydrogen phosphate or Na dihydrogen orthophosphate.

** Sodium phosphate, Disodium hydrogen phosphate.

(Tracrium Inj., Atracurium besylate) or mesylate (DHE 45 Injection, Dihydroergotamine mesylate) salts. Glucono delta lactone is used to adjust the pH of Quinidine gluconate (Lilly). Benzoate buffer, at a concentration of 5%, is used in Valium Injection. Citrates are common buffers that can have a dual role as chelating agents. Lysine and glycine are amino acids which function as buffers and stabilize protein and peptide formulations. These amino acids are also used as lyo-additives and may prevent cold denaturation. Lactate and tartrate are occasionally used as buffer systems.

Table VII lists additives which are used to modify osmolality, and as bulking or lyo-cryo protective agents. Dextrose and sodium chloride are used to adjust tonicity in the majority of formulations. Some amino acids, glycine, alanine, histidine, imidazole, arginine, asparagine, aspartic acid, are used as bulking agents for lyophilization and may serve as stabilizers for proteins or peptides and as buffers. Monosaccharides (dextrose, glucose, lactose), disaccharide (sucrose), polyhydric alcohols (inositol, mannitol, sorbitol), glycol (PEG 3350), Povidone (polyvinylpyrrolidone), and proteins (albumin, gelatin) are commonly used as lyo-additives.

TABLE VII
Bulking Agents, Protectants, and Tonicity Adjustors

Excipient	Example
Alanine	Thrombate III® (Bayer)
Albumin	Bioclate® (Arco)
Albumin human	Botox® (Allergan)
Amino acids	Havrix® (SmithKline Beecham)
L-Arginine	Activase® (Genentech)
Asparagine	Tice BCG® (Oganon)
L-Aspartic acid	Pepcid® (Merck)
Calcium chloride	Phenergan Injection® (Wyeth-Ayerst)
Citric acid	Sensorcaine-MPF® (Astra)
Dextrose	Betaseron® (Berlex)
Gelatin hydrolyzed	Acthar® (Rhone-Poulanc Rorer)
Glucose	Iveegam® (immuno-US)
Glycerin	Tice BCG® (Oganon)
Glycine	Atgam Injection® (Upjohn)
Histidine	Antihemophilic Factor, human (Am. Red Cross)
Imidazole	Helixate® (Armour)
Inositol	OctreoScan® (Mallinckrodt)
Lactose	Caverject® (Upjohn)
Magnesium chloride	Terramycin Solution® (Roerig)
Magnesium sulfate	Tice BCG® (Oganon)
Mannitol	Elspar® (Merck)
Polyethylene glycol 3350	Bioclate® (Arco)
Polysorbate 80	Helixate® (Armour)
Potassium chloride	Varivax® (Merck)
Povidone	Alkeran® (Glaxo-Wellcome)
Sodium chloride	WinRho SD® (Univax)
Sodium succinate	Actimmune® (Genentech)
Sodium sulfate	Depo-Provera® (Upjohn)
Sorbitol	Panhematin® (Abbott)
Sucrose	Prolastin® (Bayer)

Special Additives

These additives have been included in pharmaceutical formulation to serve specific functions (Table VIII). Below is a summary of the special additives along with their intended use—

(1) Calcium gluconate injection (American Regent) is a saturated solution of 10% w/v; calcium d-saccharate tetrahydrate 0.46% w/v is added to prevent crystallization during temperature fluctuations.

(2) Cipro IV® (Ciprofloxacin, Bayer) contains lactic acid as a solubilizing agent for the antibiotic.

(3) Premarin Injection® (Conjugated Estrogens, Wyeth-Ayerst Labs) is a lyophilized product that contains simethicone to prevent formation of foam during reconstitution.

(4) Dexamethasone acetate (Dalalone DP, Forest, Decadron-LA, Merck, Dalalone DP Injection, UAD Labs) and Dexamethasone Na phosphate (Merck) are available as suspension or solution. These dexamethasone formulations contain creatine or creatinine as an additive.

(5) Adriamycin RDF® (Doxorubicin hydrochloride, Pharmacia) contains methyl paraben, 0.2 mg/mL, to increase dissolution (10).

(6) Ergotrate maleate (Ergonovine maleate, Lilly) contains 0.1% ethyl lactate as a solubilizing agent.

(7) Estradurin Injection® (Polyestradiol phosphate, Wyeth-Ayerst Labs) uses Niacinamide (12.5 mg/ml)

TABLE VIII
Special Additives

Excipient	Example
Acetyl tryptophanate	Human Albumin (American Red Cross)
Aluminum hydroxide	Recombinant HB® (Merck)
Aluminum phosphate	Tetanus Toxoid Adsorbed® (Lederle)
Aluminum potassium sulfate	TD Adsorbed Adult® (Connaught)
E-Aminocaproic acid	Eminase® (Roberts)
Calcium d-saccharate	Calcium Gluconate (American Regent)
Caprylate sodium	Human Albumin (American Red Cross)
8-Chlorotheophylline	Dimenhydrinate (Steris)
Creatine	Dalalone DP® (Forest)
Creatinine	Hydrocortone Phosphate (Merck)
Diatrizoic acid	Conray (Mallinckrodt)
Gamma Cyclodextrin	Cardiotec (Squibb)
Ethyl lactate	Ergotrate maleate® (Lilly)
Ethylenediamine	Aminophylline® (Abbott)
L-Glutamate sodium	Kabikinase® (Pharmacia)
Iron ammonium citrate	Tice BCG® (Oganon)
Lactic acid	Cipro IV® (Bayer)
D,L-Lactic and Glycolic acid copolymer	Zoladex® (Zeneca)
Maltose	Gamimune® (Bayer)
Meglumine	Magnevist® (Berlex)
Niacinamide	Estradurin® (Wyeth-Ayerst)
Paraben methyl	Adriamycin RDF® (Pharmacia)
Protamine	Insulatard NPH® (Novo Nordisk)
Simethicone	Premarin Injection® (Wyeth-Ayerst)
Sodium saccharin	Compazine Injection® (Smith-Kline Beecham)
Tri-n-butyl phosphate	Venoglobulin® (Apha Therapeutic)
von Willebrand factor	Bioclate® (Arco)
Zinc	Lente Insulin® (Novo Nordisk)

TABLE IX
List of Excipient from 1996 FDA 'Inactive Ingredient Guide'

Ammonium sulfate	Pentetate (DTPA) calcium trisodium
Benzyl chloride	Poloxamer 165
Butyl paraben	PEG 4000
Caldiamide sodium	PEG 600
Calteridol calcium	Polyglactin
Castor oil	Polylactide
Cellulose (microcrystalline)	Polyoxyethlene fatty acid esters
Cholesterol	Polyoxyethylene sorbitan monosterate
Deoxycholic acid	Polyoxyl 35 Castor oil
Diatrizoic acid	Polysorbate 40
Dicyclohexyl carbodiimide	Polysorbate 85
Diethyl amine	Potassium hydroxide
Dimyristoyl lecithin	Potassium phosphate, dibasic
Dimyristoyl phosphatidyl-glycerol	Sodium bisulfate
Disofenin	Sodium chlorate
Docusate sodium	Sodium hypochloride
Edamine	Sodium iodide
Exametazime	Sodium pyrophosphate
Gluceptate sodium	Sodium thiosulfate, anhydrous
Gluceptate calcium	Sodium trimetaphosphate
Glucuronic acid	Sorbitan monopalmitate
Guanidine HCl	Stannous chloride
Iofetamine HCl	Stannous fluoride
Lactobionic acid	Stannous tartrate
Lecithin hydrogenated soy	Starch
Lidofenin	Succimer
Medrofenin	Succinic acid
Medronate disodium	Sulfurous acid
Medronic acid	Tetrakis (1-isocyano-2-methoxy-2,methyl-propante) copper (I) Te
Methyl boronic acid	Thiazoximic acid
Methyl cellulose	Trithiazoximic acid
Methylene blue	Urea
N-(carbamoyl-methoxy polyethylene-glycol 2000)-1,2-distearoyl	Zinc acetate
N-2-hydroxyethyl piperazine N'-2' ethane sulphonic acid	Zinc chloride
Nioxime	Zinc oxide
Nitric acid	2-ethyl hexanoic acid
Oxyquinoline	PEG vegetable oil

as a solubilizing agent. Hydeltrasol® (Merck) also contains niacinamide.

(8) Aluminum in the form of aluminum hydroxide, aluminum phosphate or aluminum potassium sulfate is used as adjuvant in various vaccine formulations to elicit an increased immunogenic response.

(9) Zoladex® (Goserelin acetate, Zeneca) is administered subcutaneously as microspheres. These spheres are made of D,L-lactic and glycolic acid copolymer. Lupron Depot Injection® (TAP) are lyophilized microspheres of gelatin and glycolic-lactic acid for intramuscular injection.

(10) Gamma cyclodextrin is used as a stabilizer in Cardiotec® at a concentration of 50 mg/mL.

(11) Sodium caprylate (sodium octoate) has antifungal properties, but it is also used to improve the stability of albumin solution against effects of heat. Albumin solution can be heat pasteurized by heating at 60°C for 10 hours in the presence of sodium caprylate. Acetyl tryptophanate sodium is also added to albumin formulations.

(12) Meglumine (N-methylglucamine) is used as an excipient and to form in-situ salt. For example, diatrizoic acid, an X-ray contrast agent, is more stable when autoclaved as meglumine salt than as sodium salt (11). Meglumine is also added to Magnevist®, a magnetic resonance contrast agent, formulation.

(13) Surprisingly, sodium saccharine is used in Stelazine® and Compazine® formulations; our guess is that it serves as a stabilizer and tonicity adjuster.

(14) Tri-n-butyl phosphate is present as an excipient in human immune globulin solution (Venoglobulin®). Its exact function in the formulation is not known, but it may serve as a scavenging agent.

(15) von Willebrand factor is used to stabilize recombinant antihemophilic factor (Bioclate®).

(16) Maltose serves as a tonicity adjuster and stabilizer in immune globulin formulation (Gamimune N®).

(17) Epsilon amino caproic acid (6-amino hexanoic acid) is used as a stabilizer in anistreplase (Eminase injection®).

(18) Zinc and protamine have been added to insulin to form complexes and control the duration of action.

Recently, FDA has published 'Inactive Ingredient Guide' which lists all the excipients in alphabetical order. Each ingredient is followed by the route of administration (for example, iv, oral) and, in some cases, the range of concentration used in the approved drug product. However, this list does not provide the name of commercial product(s) corresponding to each excipient. Table IX is a summary of all the excipients which are included in the 'Inactive Ingredient Guide,' but do not appear in PDR or Handbook on Injectable Drugs.

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Pharmacokinetics of probenecid in sheep

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Six Merino ewes were given 1 g (27 g/kg) probenecid by the intravenous (i.v.), intramuscular (i.m.) and subcutaneous (s.c.) routes. After i.v. injection, the biological half-life was 1.55 h and apparent volume of distribution at the steady state (Vd_{ss}) 0.18 l/kg. Body clearance (Cl_B) and renal clearance (Cl_R) were 0.12 l/h/kg and 0.03 l/h/kg, respectively. Approximately 28% of unchanged probenecid was excreted in urine. Plasma probenecid concentrations after i.v., i.m. and s.c. injections were 133, 37, and 31 μg/ml, respectively, at 15 min; 76, 36, and 34 μg/ml at 1 h; and 43, 23 and 34 μg/ml at 2 h. The average bioavailability of probenecid given by i.m. and s.c. injection was 46% and 34%, respectively. However, after 2 h, probenecid plasma concentrations remained higher when it was given subcutaneously than when it was given intramuscularly.

Urine output was correlated positively ($P < 0.05$) with k_{el} and Cl_B. Urine pH increased significantly ($P < 0.01$) for the first 2 h, and then steadily declined over the subsequent 6 h. The results suggested that probenecid in sheep was rapidly eliminated because it was rapidly excreted in the normal but alkaline urine. Subcutaneous administration of probenecid in animals may be a useful alternative to oral or i.v. administration.

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INTRODUCTION

Probenecid, also used as a uricosuric agent in man, has mainly been used to prolong the biological half-life and to alter the distribution characteristics of some penicillins and third-generation cephalosporins (Gibaldi & Schartz, 1968; Welling *et al.*, 1979). The third-generation cephalosporins have a relatively short plasma half-life, and this has impaired their use in veterinary medicine (Guerrini *et al.*, 1983; Kalager *et al.*, 1982). The pharmacokinetics of probenecid must be well known if this drug is to be used to improve the disposition of the third-generation cephalosporins. Since the oral administration of probenecid to ruminants is not practical, the pharmacokinetics of this drug had to be determined after parenteral administration.

Urinary pH and urine flow rate are known to affect the elimination of probenecid, and these parameters should be measured in conjunction with the plasma concentration of this drug (Melethil & Conway, 1976; Dayton *et al.*, 1963). The purpose of this study was to determine the pharmacokinetics of probenecid after i.v., i.m. and s.c. administration to sheep. The effects of urine flow rate and urinary pH on the pharmacokinetics of probenecid were also investigated.

MATERIALS AND METHODS

Animals

Six adult Merino ewes (average body weight 36.8 ± 4.8 kg) were acclimatized for two weeks before the study. Each sheep was kept in an individual metabolism cage with water and lucerne chaff freely available (ambient temperature 25 ± 2°C; ambient humidity 55 ± 10%). A jugular vein was cannulated and the urinary bladder was catheterized for the collection of blood and urine samples, respectively.

Drug administration

Each sheep was given 1 g of probenecid (Merck Sharp and Dohme, Granville South, NSW) as a solution in phosphate buffer (pH 7.4) (Martindale, 1982). The drug was given by rapid i.v., deep i.m. (gluteal muscle) or s.c. (pre-scapular region) injection in three separate experiments each separated by a 2-week rest period.

Specimen collection

Blood samples were collected from the jugular vein via an indwelling cannula. The patency of cannula was maintained by injecting a 1/1000 sodium heparin solution into cannulae before each sampling. The initial portion of each blood sample was discarded. Urine was collected in plastic vials via indwelling catheters using a method described previously (Guerrini *et al.*, 1983). After the drug was given by i.v., i.m. and s.c. administration, blood samples (3–5 ml) were collected at 5, 10, 15, 20, 40 minutes and 1, 2, 3, 4, 5, 6, 8, 12, 14, and 18 h. Urine (total volume) was collected at 0, 30 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 16 h after dosing.

Analytical methods

Plasma and urine concentrations of probenecid were measured by high-pressure liquid chromatography (HPLC) with u.v. detection (Model 440 absorbance detector, Waters Associates, Milford, MA). Plasma and urine samples (250 µl each) were vortexed with acetonitrile (250 and 1500 µl, respectively) and centrifuged for 3 minutes to remove protein material. Twenty µl of the supernatant was then injected onto the HPLC column (RP-8, 10 µm, Brownlee Lab., Santa Clara, CA). The mobile phase (65% sodium dihydrogen phosphate buffer [0.02 M, pH 5] 30% methanol and 5% acetonitrile) was pumped (Model M-45, Waters Associates) through the column at a flow rate of 1.5 ml/min. Probenecid was quantitated at 254 nm with a retention time of 3 min. Peak heights were used to calculate plasma probenecid concentrations and all determinations were performed in duplicate. Standard curves were prepared in the range 0.2–400 µg/ml for plasma, and 2–2000 µg/ml for urine.

Renal function tests

The glomerular filteration rate and effective renal plasma flow were estimated using ^{125}I-labeled sodium iothalamate and sodium *p*-aminohippurate (PAH) as previously described (Filippich, 1982; Guerrini *et al.*, 1983).

Statistical analysis

The individual animal plasma and urine data, and the averaged plasma and urine data, were fitted with appropriate pharmacokinetic models using the digital computer program NONLIN (Metzler *et al.*, 1974) modified to operate on a minicomputer (Bourne & Wright, 1981). Mean data points with standard deviation values greater than the mean values were not included for analysis. These points were those measured 2.5 h after i.v., 4.0 h after i.m. and 8.0 h after s.c. administration. Initial estimates obtained for the pharmacokinetic parameters, apparent volume of the central compartment (V_C), elimination rate constant (k_{el}), urinary excretion rate constant (k_e), rate constant for diffusion into tissue (k_{12}) and out of tissue (k_{21}) were subjected to a weighted iterative least-squares analysis using NONLIN. In the case of i.m. and s.c. data, V_C/F (where F was the bioavailability expressed as a fraction of 1) was

used as an initial parameter. The harmonic mean plasma half-life value was calculated as 0.693/beta. Renal clearance (Cl_R) was calculated as $k_e.V_C$, and body clearance (Cl_B) as $k_{el}.V_C$. The apparent volume of distribution at the steady state ($V_{d_{ss}}$) was calculated as $(k_{12} + k_{21}).V_C$ divided by k_{21}. The area under the plasma concentration *vs* time curve (AUC) was calculated by the trapezoidal rule. Linear regression was used to estimate the degree of correlation between urine volumes voided and k_e, terminal plasma half-life, or Cl_B. A paired *t*-test was used to test the significance of the difference between mean pH values and mean urine flow rate values.

RESULTS

Averaged plasma concentrations of probenecid and averaged cumulative amounts of probenecid excreted into urine measured in six sheep given probenecid by the i.v. route are shown in Fig. 1. Similar data determined in the same sheep given probenecid by the i.m. and s.c. routes are presented in Figs 2 and 3, respectively.

After i.v. injection of the drug, plasma probenecid concentrations were 133 ± 18 μg/ml at 15 minutes, 76 ± 26 μg/ml at 1 h, and 42.6 ± 32.8 μg/ml at 2 h. The plasma and urine data were subsequently fitted by a two-compartment pharmacokinetic model and the pharmacokinetic parameters obtained with NONLIN are shown in Table I. Urine volume voided over 12 h correlated significantly ($P < 0.05$) with the excretion rate constant, the renal clearance, and the biological half-life (negative correlation) of probenecid.

After i.m. administration, the average plasma probenecid concentration was 37.4 ± 26.8 μg/ml at 15 minutes, 35.5 ± 29.0 μg/ml at 1 h, and 22.7 ± 18.6 μg/ml at 2 h. The average AUC value 118 ± 69 μg.h/ml. The individual and average data were fitted by a two-compartment pharmacokinetic model (NONLIN) with an added first-order absorption step (k_a) and are shown in Table II. The values of the distribution rate constants obtained in the analysis of the i.v. data were used in the i.m. analysis as fixed constants. The bioavailability of the i.m. dosage form was calculated from the ratio of V_C (i.v.) to V_C/F (i.m.) and the ratio of the AUC values corrected for k_{el}. Calculated by these two methods, the bioavailability was 48 ± 36% and 43 ± 33%, respectively, giving an average value of 46%. The average total amount of unchanged probenecid found in the urine was 39.5 ± 4.1% of the dose. The

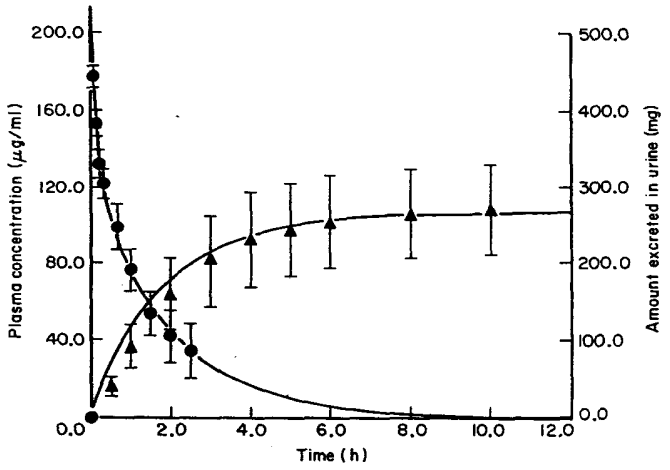

FIG. 1. Linear plot of average plasma concentration (●) and cumulative amount of drug excreted into urine (▲) *vs* time in six sheep after i.v. administration of 1 g (27 mg/kg) of probenecid. The points are the experimentally determined average values (with ± 1 SE shown as vertical bars) and the solid line was calculated using the 'best fit' values (as calculated with NONLIN).

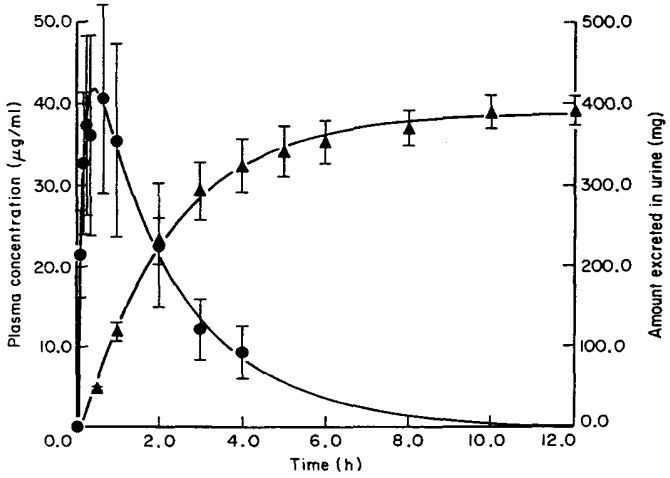

FIG. 2. Linear plot of average plasma concentration (●) and cumulative amount of drug excreted into urine (▲) *vs* time in six sheep after i.m. adminstration of 1 g (27 mg/kg) of probenecid. The points are the experimentally determined average values (with ± SE shown as vertical bars) and the solid line was calculated using the 'best fit' values (as calculated with NONLIN). Standard errors for values taken at 0.08, 0.17, 0.25 and 0.33 h were 13.1, 21.2, 26.7 and 30.1, respectively.

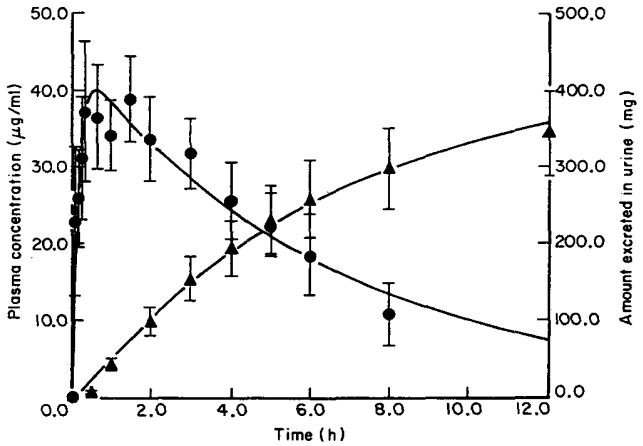

FIG. 3. Linear plot of average plasma concentration (●) and cumulative amount of drug excreted into urine (▲) *vs* time in six sheep after s.c. administration of 1 g (27 mg/kg) of probenecid. The points are the experimentally determined average values (with ± 1 SE shown as vertical bars) and the solid line was calculated using the 'best fit' values (as calculated with NONLIN).

averaged parameter values were used to calculate the solid lines in Fig. 2.

After s.c. administration, the average plasma probenecid concentration was 31.2 ± 19.7 μg/ml at 15 minutes, 34.3 ± 11.8 μg/ml at 1 h, and 33.7 ± 13.7 μg/ml at 2 h. Plasma probenecid concentration remained higher ($P < 0.05$) after 2 h by s.c. injection than by i.m. administration. The individual and average plasma and urine data were fitted by a two-compartment pharmacokinetic model with an added first-order absorption step as described for the i.m. data. The pharmacokinetic parameters obtained by this method are

TABLE I. Pharmacokinetic parameters measured after i.v. administration of 1 g probenecid to each of six sheep

Values	Sheep No. 1 (37 kg)	No. 2 (32 kg)	No. 3 (41 kg)	No. 4 (34 kg)	No. 5 (33 kg)	No. 6 (44 kg)	$\bar{x}$ ± SD (37 ± 5)	Average
k_e (h^{-1})	0.072	0.385	0.399	0.295	0.060	0.485	0.373 ± 0.180	0.215
k_{misc} (h^{-1})	0.479	0.958	1.240	1.025	0.343	0.442	0.748 ± 0.372	0.586
k_{el} (h^{-1})	0.55	1.34	1.64	1.32	0.40	0.93	1.03 ± 0.49	0.80
k_{21} (h^{-1})	3.95	1.79	5.94	0.84	2.57	2.41	2.92 ± 1.80	1.57
k_{21} (h^{-1})	10.2	3.07	9.10	6.51	4.34	5.26	6.42 ± 2.78	3.26
V_C (l/kg)	0.10	0.15	0.08	0.15	0.15	0.10	0.12 ± 0.03	0.13
$V_{d_{ss}}$ (l/kg)	0.18	0.23	0.14	0.17	0.23	0.14	0.18 ± 0.04	0.19
$t_{1/2}$* (h)	1.76	0.22	0.73	0.61	2.80	1.14	1.55†	1.36
AUC (μg.h/ml)	437	141	178	148	466	221	265 ± 147	241
Cl_B (l/h/kg)	0.06	0.20	0.13	0.20	0.06	0.09	0.12 ± 0.006	0.10
Cl_R (l/h/kg)	0.01	0.06	0.04	0.06	0.02	0.03	0.03 ± 0.002	0.03
GFR (ml/min/kg)	1.97	2.89	2.20	1.74	2.21	2.10	2.19 ± 0.39	
RPF (ml/min/kg)	12.4	17.4	13.4	10.5	13.1	11.0	13.0 ± 2.84	
Unchanged drug in urine (% of dose)	16	31	28	24	16	54	28 ± 14	
Urine voided at 12 h (ml)	106	308	428	627	136	584	348 ± 100	

*Terminal half-life.
†Harmonic mean calculated as 0.693/β.

shown in Table III. The bioavailability of the s.c. dosage form was 35 ± 12% and 32 ± 14%, respectively (calculated by the two methods described above), giving an average value of 34% of the adminstered dose. The average total amount of unchanged drug found in urine was 36.3 ± 13.6% of the dose. The average values were used to calculate the solid lines in Fig. 3.

Urine flow rate after i.v., i.m. and s.c. administration of probenecid is shown in Table IV. Average urinary pH values measured after IM administration are also shown on Table 4. The pH values increased significantly from 8.08 ± 0.37 at 0 minutes to 8.53 ± 0.24 ($P < 0.01$) at 2 h, and then steadily declined to 7.51 ± 1.19 (not significantly different to the zero time value, $P > 0.05$) over the subsequent 6 h. Increased urine output was correlated ($P < 0.05$) with increased k_e ($r = 0.55$ for 18 pairs) and increased Cl_B ($r = 0.46$ for 18 pairs). The glomerular filtration rate (GFR) and renal plasma flow rate (RPF) for individual sheep are shown in Table I. The average values for GFR and RPF were 2.19 ± 0.39 and 13 ± 2.5 ml/min/kg body weight, respectively.

DISCUSSION

The average plasma half-life or body clearance for probenecid in sheep after i.v., i.m. and s.c. administration in the present study was shorter than that found in man and in other mammalian species. Cunningham *et al.* (1981) reported that the plasma half-life of probenecid in man after doses of 0.5–1.0 g intravenously was 2–6 h, whereas, in the present study, the average biological half-life in sheep was 0.6–2.8 h. The body clearance for probenecid in man (70 kg body weight) was 0.02 l/h/kg (Perel *et al.*, 1971), compared with 0.12 l/h/kg in sheep. The normally

TABLE II. Pharmacokinetic parameters measured after i.m. administration of 1 g probenecid to each of six sheep

Values	Sheep No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	$\bar{x}$ ± SD	Average
k_e (h^{-1})	0.119	0.715	0.151	0.346	0.364	0.396	0.349 ± 0.214	0.282
k_{misc} (h^{-1})	0.150	1.034	0.315	0.607	0.473	0.546	0.519 ± 0.298	0.445
k_{cl} (h^{-1})	0.27	1.74	0.47	0.95	0.84	0.94	0.87 ± 0.51	0.73
Cl_B (l/h/kg)	0.43	0.95	0.77	0.30	0.14	0.13	0.37 ± 0.31	0.25
k_a (h^{-1})	8.75	1.14	10.9	6.51	2.64	2.70	5.45 ± 3.90	3.74
Bioava* (%)	8.3	37.9	16.2	44.2	99.1	82.2	48.0 ± 36.0	42.7
Bioava† (%)	6.5	27.6	14.0	46.8	85.5	77.5	43.0 ± 32.9	37.6
V_C/F (l/kg)	1.61	0.54	0.57	0.31	0.17	0.13	0.56 ± 0.55	0.34
AUC (μg.h/ml)	73.7	41.4	101.0	90.6	222.0	179.0	118.0 ± 68.5	113.0
Cl_R (l/h)	7.1	12.5	3.5	3.7	2.0	2.3	7.2 ± 4.4	3.5
Drug in urine (% of dose)	41	41	33	37	44	41	40 ± 4	
Urine voided at 12 h (ml)	160	632	819	547	582	654	565 ± 220	

*Bioavailability calculated as Auc (i.m.)/AUC (i.v.) × k_{cl} (i.m.)/k_{cl} (i.v.).
†Bioavailability calculated as V_C (i.v.)/V_C (i.m.).

TABLE III. Pharmacokinetic parameters measured after s.c. administration of 1 g of probenecid to each of six sheep

Values	Sheep No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	$\bar{x}$ ± SD	Average
k_e (h^{-1})	0.191	0.116	0.125	0.020	0.145	0.088	0.114 ± 0.057	0.097
k_{misc} (h^{-1})	0.160	0.314	0.118	0.114	0.213	0.122	0.173 ± 0.078	0.127
k_{cl} (h^{-1})	0.35	0.43	0.24	0.13	0.36	0.21	0.29 ± 0.11	0.22
Cl_B (l/h/kg)	0.18	0.12	0.11	0.05	0.13	0.09	0.11 ± 0.04	0.09
k_a (h^{-1})	2.56	9.43	4.87	1.61	2.48	2.29	3.87 ± 2.94	3.87
Bioava* (%)	21.5	51.9	26.7	38.6	43.6	28.8	35.2 ± 11.5	28.8
Bioava† (%)	20.6	54.0	17.6	38.0	40.1	23.0	32.2 ± 14.2	30.7
AUC (μg.h/min)	148	229	322	561	228	282	295 ± 143	249
V_C/F (l/kg)	0.51	0.28	0.46	0.39	0.36	0.44	0.41 ± 0.08	0.42
Cl_R (l/h/kg)	0.33	0.29	0.17	0.05	0.35	0.15	0.22 ± 0.12	0.21
Drug in urine (%) of dose)	52	28	46	14	40	38	36 ± 14	
Urine voided at 12 h (ml)	185	201	555	279	286	394	316 ± 138	

*Bioavailability calculated as [AUC (s.c./AUC i.v.] × [k_{cl} (s.c.)/k_{cl} (s.c.)/k_{cl} (i.v.)].
†Bioavailability calculated as V_C (i.v.)/(V_C/F) (s.c.)].

TABLE IV. Average (± SD) urinary pH and urine volume after i.v., i.m. and probenecid in six Merino Ewes

Time (h)	pH* (units)	Urine volume (ml/min)	Urine volume (ml/min)	Urine volume (ml/min)
		i.v.	i.m.	s.c.
0	8.08			
	(0.37)			
0.5	8.45	0.62	0.93	0.61
	(0.22)	(0.37)	(0.48)	(0.54)
1.0	8.50	0.58	1.20	0.71
	(0.24)	(0.37)	(1.25)	(0.52)
2.0	8.53	0.38†	0.80	1.08
	(0.24)	(0.18)	(0.71)	(1.10)
3.0	8.52	0.53	0.64	0.47
	(0.28)	(0.47)	(0.40)	(0.27)
4.0	8.48	0.46	0.41‡	0.32
	(0.30)	(0.27)	(0.22)	(0.11)
5.0	8.06	0.40	0.34‡	0.33
	(0.37)	(0.28)	(0.16)	(0.12)
6.0	7.76	0.65	0.53	0.26
	(0.82)	(0.71)	(0.36)	(0.09)
8.0	7.51	0.35	0.26†	0.32
	(1.19)	(0.24)	(0.21)	(0.11)

*pH measured during i.m. study.
†Significantly different from the 0.5 h value ($P < 0.05$).
‡Significantly different from the 0.5 h value ($P < 0.01$).

higher urine pH value found in sheep should increase the ionized fraction of probenecid in the renal tubules, thereby decreasing the drug's tubular reabsorption and enhancing its renal excretion (Goodman & Gilman, 1980). The Cl_R value for probenecid in man (based on 70 kg body weight) was approximately 0.001 l/h/kg (Dayton *et al.*, 1963; Perel *et al.*, 1971), whereas in the present experiment the Cl_R value was 0.03 l/h/kg. The higher proportion of the dose excreted as unchanged drug in sheep (25–40%) compared with the value found in man (5–11%) further supports the suggestion that high urinary pH in sheep retards the reabsorption of the drug from the kidneys.

In sheep with higher urine output over 12 h, the biological half-life of probenecid was shorter, and the rate constant for urinary excretion (k_e) was larger than in sheep with slower urinary flow rates. This confirmed previous findings in man and in the dog, and underlines the need to measure urinary pH, urine volume and urinary drug concentrations in pharmacokinetic studies (Cunningham *et al.*, 1981; Goodman & Gilman, 1980).

The present pharmacokinetic analysis indicated that the rate of diffusion of probenecid between the central and peripheral compartment was rapid. This rapid diffusion of probenecid is probably due to its high lipophilicity (Cunningham *et al.*, 1981). The values for V_C and Vd_{ss} and the ratio of k_{12} to k_{21} also suggested that there was extravascular distribution of probenecid in sheep. The Vd_{ss} value found for probenecid in man (0.16 l/kg) (Dayton *et al.*, 1963) compared well with the values found in sheep (0.18 l/kg).

Previous experiments carried out in man and in the dog indicated that probenecid was completely absorbed when given by the oral route (Cunningham *et al.*, 1981). In the present study, probenecid given i.m. or s.c. was incompletely absorbed with only one-half

to one-third of the administered dose reaching the circulation intact. However after 2 h, the s.c. injection provided higher and more prolonged plasma probenecid concentration than did the i.m. administration. Some of the drug may have precipitated at the site of injection and continued to be released from the site of injection after blood collection was terminated. There was no visible discomfort or damage done to the sheep. The subcutaneous administration of 1 g of probenecid in sheep provided similar plasma concentrations to those found in man after oral administration. Subcutaneous administration of drugs avoids muscle damage and is generally less painful; thus, this route is a useful alternative for the administration of probenecid in animals. Precipitated drug could result in tissue residues if the drug is given intramuscularly.

In conclusion, the present results suggest that one of the reasons why probenecid has a short biological half-life in the ruminant is because the urinary pH is normally high. Urine output also appeared to influence the renal elimination of probenecid. The s.c. administration of probenecid in animals is preferred because muscle damage is avoided and it provided useful plasma concentrations.

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(54) Title: PHARMACEUTICAL FORMULATION FOR THE INTRAMUSCULAR ADMINISTRATION OF FULVESTRANT

(57) Abstract: The invention relates to a sustained release pharmaceutical formulation adapted for administration by injection containing the compound fulvestrant, 7 a-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17 ß-diol, at concentration of at least 100mg/ml in solution in a ricinoleate vehicle which additionally comprises at least one alcohol and a non-aqueous ester solvent which is miscible in the ricinoleate vehicle.

PHARMACEUTICAL FORMULATION FOR THE INTRAMUSCULAR ADMINISTRATION OF FULVESTRANT

The invention relates to a sustained release pharmaceutical formulation adapted for administration by injection containing the compound fulvestrant, 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol, at concentration of at least 100mg/ml in solution in a ricinoleate vehicle which additionally comprises at least one alcohol and a non-aqueous ester solvent which is miscible in the ricinoleate vehicle.

Oestrogen deprivation is fundamental to the treatment of many benign and malignant diseases of the breast and reproductive tract. In premenopausal women, this is achieved by the ablation of ovarian function through surgical, radiotherapeutic, or medical means, and, in postmenopausal women, by the use of aromatase inhibitors.

An alternative approach to oestrogen withdrawal is to antagonise oestrogens with antioestrogens. These are drugs that bind to and compete for oestrogen receptors (ER) present in the nuclei of oestrogen-responsive tissue. Conventional nonsteroidal antioestrogens, such as tamoxifen, compete efficiently for ER binding but their effectiveness is often limited by the partial agonism they display, which results in an incomplete blockade of oestrogen-mediated activity (Furr and Jordan, Pharmacology & Therapeutics, 25:127-206, 1984; May and Westley, J Biol Chem 262:15894-15899, 1987).

The potential for nonsteroidal antioestrogens to display agonistic properties prompted the search for novel compounds that would bind ER with high affinity without activating any of the normal transcriptional hormone responses and consequent manifestations of oestrogens. Such molecules would be "pure" antioestrogens, clearly distinguished from tamoxifen-like ligands and capable of eliciting complete ablation of the trophic effects of oestrogens. Such compounds are referred to as Estrogen Receptor-Downregulators (E.R.D.). The rationale for the design and testing of novel, pure antioestrogens has been described in: Bowler et al 1989, Wakeling 1990a, 1990b, 1990c. Wakeling and Bowler 1987, 1988.

Steroidal analogues of oestradiol, with an alkylsulphinyl side chain in the 7α position, provided the first examples of compounds devoid of oestrogenic activity (Bowler et al 1989). One of these, 7α-[9-(4,4,5,5,5-pentafluoropentyl sulphinyl)nonyl]oestra-1,3,5-(10)triene-3,17β-diol was selected for intensive study on the basis of its pure oestrogen antagonist activity and significantly increased antioestrogenic potency over other available antioestrogens. *In vitro* findings and early clinical experience with

7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3-5(10)-triene-3,17β-diol have promoted interest in the development of the drug as a therapeutic agent for oestrogen-dependent indications such as breast cancer and certain benign gynaecological conditions.

7α-[9-(4,4,5,5,5-Pentafluoropentylsulphinyl)nonyl]oestra-1,3-5(10)-triene-3,17β-diol, or ICI 182,780, has been allocated the international non-proprietary name fulvestrant, which is used hereinafter. When referring to fulvestrant we include pharmaceutically-acceptable salts thereof and any possible solvates of either thereof.

Fulvestrant binds to ER with an affinity similar to that of oestradiol and completely blocks the growth stimulatory action of oestradiol on human breast cancer cells *in vitro*; it is more potent and more effective than tamoxifen in this respect. Fulvestrant blocks completely the uterotrophic action of oestradiol in rats, mice and monkeys, and also blocks the uterotrophic activity of tamoxifen.

Because fulvestrant has none of the oestrogen-like stimulatory activity that is characteristic of clinically available antioestrogens such as tamoxifen or toremifene, it may offer improved therapeutic activity characterised by more rapid, complete, or longer-lasting tumour regression; a lower incidence or rate of development of resistance to treatment; and a reduction of tumour invasiveness.

In intact adult rats, fulvestrant achieves maximum regression of the uterus at a dose which does not adversely affect bone density or lead to increased gonadotrophin secretion. If also true in humans, these findings could be of extreme importance clinically. Reduced bone density limits the duration of oestrogen-ablative treatment for endometriosis. Fulvestrant does not block hypothalamic ER. Oestrogen ablation also causes or exacerbates hot flushes and other menopausal symptoms; fulvestrant will not cause such effects because it does not cross the blood-brain barrier.

European Patent Application No. 0 138 504 discloses that certain steroid derivatives are effective antioestrogenic agents. The disclosure includes information relating to the preparation of the steroid derivatives. In particular there is the disclosure within Example 35 of the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol, which compound is specifically named in Claim 4. It is also disclosed that the compounds of that invention may be provided for use in the form of a pharmaceutical composition comprising a steroid derivative of the invention together with a

pharmaceutically-acceptable diluent or carrier. It is stated therein that the composition can be in a form suitable for oral or parenteral administration.

Fulvestrant shows, along with other steroidal based compounds, certain physical properties which make formulation of these compounds difficult. Fulvestrant is a particularly lipophilic molecule, even when compared with other steroidal compounds, and its aqueous solubility is extremely low at around 10 $ngml^{-1}$ (this is an estimate from a water/solvent mixture solute since measurements this low could not be achieved in a water only solute).

Currently there are a number of sustained release injectable steroidal formulations which have been commercialised. Commonly these formulations use oil as a solvent and wherein additional excipients may be present.

In US 5,183,814 Example 3 an oil based injection formulation of fulvestrant is described which comprises 50mg of fulvestrant, 400mg of benzyl alcohol and sufficient castor oil to bring the solution to a volume of 1 ml. Manufacture at a commercial scale of a formulation as described in US 5,183,814 will be complicated by the high alcohol concentration. Therefore, there is a need to lower the alcohol concentration in fulvestrant formulations whilst preventing precipitation of fulvestrant from the formulation.

The Table below shows the solubility of fulvestrant in a number of different solvents.

SOLUBILITY OF FULVESTRANT

SOLVENT	SOLUBILITY ($mgml^{-1}$ at 25°C)
Water	0.001
Arachis oil	0.45
Sesame oil	0.58
Castor oil	20
Miglyol 810	3.06
Miglyol 812	2.72
Ethyl oleate	1.25
Benzyl benzoate	6.15
Isopropyl myristate	0.80
Span 85 (surfactant)	3.79

Ethanol	>200
Benzyl Alcohol	>200

As can be seen fulvestrant is significantly more soluble in castor oil than any of the other oils tested. The greater solvating ability of castor oil for steroidal compounds is known and is attributed to the high number of hydroxy groups of ricinoleic acid, which is the major constituent of the fatty acids within the triglycerides present in castor oil - see (Riffkin et.al. J. Pharm. Sci., (1964), 53, 891).

Our earlier application PCT/GB01/00049, WO 01/51056, describes certain fulvestrant formulations at a most preferred concentration of 50mg/ml. This application disclosed one formulation with a solubility up to 102 mg/ml – see the last formulation in Table 3 thereof with 15 % weight of ethanol per volume of formulation, 15 % weight of benzyl alcohol per volume of formulation, 15 % weight of benzyl benzoate per volume of formulation in a ricinoleate vehicle. However there is a need for further formulations of fulvestrant that contain high concentrations of fulvestrant to facilitate administration thereof at higher doses or less frequent intervals.

According to another aspect of the invention there is provided a pharmaceutical formulation adapted for intramuscular injection comprising 100 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle and provided that the following formulation is excluded: fulvestrant up to 102 mg/ml, 15 % weight of ethanol per volume of formulation vehicle, 15 % weight of benzyl alcohol per volume of formulation vehicle, 15 % weight of benzyl benzoate per volume of formulation vehicle and 30 % or more weight of ricinoleate excipient per volume of formulation vehicle.

A preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 105 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation comprises at least 5 % weight of ethanol per volume of formulation vehicle.

A more preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 110 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

A more preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 115 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

A more preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 120 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

A more preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 130 mg/ml or more of fulvestrant, 15 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

A more preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 140 mg/ml or more of fulvestrant, 15 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 12.5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the

formulation vehicle comprises at least 10 % weight of ethanol per volume of formulation vehicle.

A more preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 150 mg/ml or more of fulvestrant, 15 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 17.5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 10 % weight of ethanol per volume of formulation vehicle.

Another aspect of the invention provides any of the formulations described herein stated as having any minimum ethanol content removed. For example, the formulation described in the paragraph immediately above becomes: a pharmaceutical formulation adapted for intramuscular injection is one comprising 150 mg/ml or more of fulvestrant, 15 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 17.5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle.

According to another aspect of the invention there is provided a pharmaceutical formulation having a solubility for fulvestrant of at least Y mg/ml adapted for intramuscular injection comprising;

100 mg/ml or more of fulvestrant;

5% (w/v) or more castor oil per volume of formulation vehicle;

and at least the following amounts (% weight/volume of formulation vehicle) of ethanol (ETOH), benzyl alcohol (BA), benzyl benzoate (BB) determined by the algorithm:

$Y = -29.77 + 5.44 \times ETOH + 2.38 \times BA + 1.57 \times BB$

wherein x is at least 100, ETOH is at least 5, BA is at least 5 and BB is at least 5.

A preferred pharmaceutical formulation is one wherein Y is selected from the group consisting of 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 170, 180, 190, and 200.

A more preferred pharmaceutical formulation is one wherein Y is selected from the group consisting of 120, 125, 130, 135, 140, 145, 150, 155, 160, 170, 180, 190, and 200.

A more preferred pharmaceutical formulation is one wherein Y is selected from the group consisting of 150, 155, 160, 170, 180, 190 and 200.

A more preferred pharmaceutical formulation is one wherein Y is selected from 150, 155, 160, 170, 180, 190 and 200 and the formulation comprises at least 150mg/ml of fulvestrant.

A more preferred pharmaceutical formulation is one wherein Y is 200 and the formulation comprises at least 200mg/ml of fulvestrant.

According to another aspect of the present invention there is provided a pharmaceutical formulation having a solubility for fulvestrant of at least 100 mg/ml adapted for intramuscular injection comprising;

100 mg/ml or more of fulvestrant;

5% (w/v) or more castor oil per volume of formulation vehicle;

and at least the following amounts (% weight/volume of formulation vehicle) of ethanol (EtOH), benzyl alcohol (BA), benzyl benzoate (BB) determined by the algorithm:

$100 = -29.77 + 5.44 \times ETOH + 2.38 \times BA + 1.57 \times BB$; and

provided that the following formulation is excluded: fulvestrant up to 102 mg/ml, 15 % weight of ethanol per volume of formulation vehicle, 15 % weight of benzyl alcohol per volume of formulation vehicle, 15 % weight of benzyl benzoate per volume of formulation vehicle and 30 % or more weight of castor oil per volume of formulation vehicle.

According to another aspect of the invention there is provided a pharmaceutical formulation comprising fulvestrant at a concentration of at least 100 mg/ml in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months and provided that the following formulation is excluded: fulvestrant up to 102 mg/ml, 15 % weight of ethanol per volume of formulation vehicle, 15 % weight of benzyl alcohol per volume of formulation vehicle, 15 % weight of benzyl benzoate per volume of formulation vehicle and 30 % or more weight of ricinoleate excipient per volume of formulation vehicle.

According to another aspect of the invention there is provided a pharmaceutical formulation comprising fulvestrant in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months.

According to another aspect of the invention there is provided a pharmaceutical formulation comprising fulvestrant at a concentration of at least 100 mg/ml in which the formulation is adapted for intra-muscular injection into a human and which is capable after

injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months.

According to another aspect of the invention there is provided a pharmaceutical formulation comprising fulvestrant at a concentration of at least 150 mg/ml in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months.

According to another aspect of the invention there is provided a pharmaceutical formulation comprising fulvestrant at a concentration of at least 200 mg/ml in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months.

According to another aspect of the invention there is provided a pharmaceutical formulation comprising fulvestrant at a concentration of at least 300 mg/ml in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months.

According to one aspect of the invention there is provided any one of the following pharmaceutical formulations comprising about:

i)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

15% weight per volume of benzyl benzoate

500-555mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

ii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-700mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

iii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500-750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

iv)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-1175mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

v)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500-810 mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

vi)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500 mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

vii)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-630mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

viii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

ix)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

x)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xi)

9% weight per volume of ethanol

19% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

700mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xii)

14% weight per volume of ethanol

19% weight per volume of benzyl alcohol

48% weight per volume of benzyl benzoate

700mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xiii)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

45% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xiv)

9% weight per volume of ethanol

19% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xv)

19% weight per volume of ethanol

19% weight per volume of benzyl alcohol

28% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xvi)

14% weight per volume of ethanol

9% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xvii)

14% weight per volume of ethanol

19% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xviii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

45% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xix)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

45% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xx)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

25% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxi)

10% weight per volume of ethanol

30% weight per volume of benzyl alcohol

25% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxii)

10% weight per volume of ethanol

25% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxiii)

10% weight per volume of ethanol

30% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxiv)

15% weight per volume of ethanol

25% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxv)

15% weight per volume of ethanol

25% weight per volume of benzyl alcohol

25% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil; and

xxvi)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil.

The term "comprising about" in this context means that the numerical value assigned to each component of the formulation may be varied independently to accommodate manufacturing specifications encountered by a skilled person when making up the formulations. Typically this means plus or minus 5%, more preferably plus or minus 4%, more preferably plus or minus 3%, more preferably plus or minus 2%, more preferably plus or minus 1%. In a preferred embodiment, more variation in drug level is allowed compared with other components. For example:

Drug (+/- %)	Other components (+/- %)
5	4, 3, 2 or 1
4	3, 2 or 1
3	2 or 1
2	1

The individual formulations described herein may comprise further excipients commonly used in the formulation field including, for example, an antioxidant preservative, a colorant or a surfactant.

According to another aspect of the invention there is provided any one of the following pharmaceutical formulations:

i)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

15% weight per volume of benzyl benzoate

500-555mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

ii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-700mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

iii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500-750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

iv)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-1175mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

v)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500-810 mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

vi)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500 mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

vii)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-630mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

viii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

ix)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

x)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil.

A preferred pharmaceutical formulation described herein is one wherein the pharmaceutically-acceptable alcohol is a mixture of ethanol and benzyl alcohol.

A preferred pharmaceutical formulation described herein is one wherein the pharmaceutically-acceptable non-aqueous ester solvent is selected from benzyl benzoate, ethyl oleate, isopropyl myristate, isopropyl palmitate or a mixture of any thereof.

A preferred pharmaceutical formulation described herein is one wherein the pharmaceutically-acceptable non-aqueous ester solvent is benzyl benzoate.

A preferred pharmaceutical formulation described herein is one wherein the ricinoleate excipient is castor oil.

According to one aspect of the present invention there is provided a pharmaceutical formulation adapted for intramuscular injection comprising 100 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of pharmaceutical formulation, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of pharmaceutical formulation and 5 % or more weight of ricinoleate excipient per volume of pharmaceutical formulation provided:

a) the pharmaceutical formulation comprises at least 5 % weight of ethanol per volume of pharmaceutical formulation;

b) if the pharmaceutically acceptable alcohol is less than or equal to 13%, then the pharmaceutical formulation must comprise at least 50 % non-aqueous ester solvent; and

c) if the pharmaceutically acceptable alcohol is greater than 20 % but less than or equal to 25 %, then the pharmaceutical formulation must comprise at least 30 % non-aqueous ester solvent;

and also provided that the following pharmaceutical formulation is excluded: fulvestrant up to 102 mg/ml, 15 % weight of ethanol per volume of formulation vehicle, 15 % weight of benzyl alcohol per volume of formulation vehicle, 15 % weight of benzyl benzoate per volume of formulation vehicle and 30 % or more weight of ricinoleate excipient per volume of formulation vehicle.

A preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 100 mg/ml or more of fulvestrant, 20 % or more weight of a pharmaceutically acceptable alcohol per volume of pharmaceutical formulation, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of pharmaceutical formulation and 5 % or more weight of ricinoleate excipient per volume of pharmaceutical formulation provided:

a) the pharmaceutical formulation comprises at least 10 % weight of ethanol per volume of pharmaceutical formulation;

b) if the pharmaceutically acceptable alcohol is 20%, then the pharmaceutical formulation must comprise at least 22.5 % non-aqueous ester solvent; and

c) if the pharmaceutically acceptable alcohol is greater than 20 % but less than or equal to 25 %, then the pharmaceutical formulation must comprise at least 15 % non-aqueous ester solvent;

and also provided that the following pharmaceutical formulation is excluded: fulvestrant up to 102 mg/ml, 15 % weight of ethanol per volume of formulation vehicle, 15 % weight of benzyl

alcohol per volume of formulation vehicle, 15 % weight of benzyl benzoate per volume of formulation vehicle and 30 % or more weight of ricinoleate excipient per volume of formulation vehicle.

A more preferred pharmaceutical formulation adapted for intramuscular injection is one comprising 150 mg/ml or more of fulvestrant, 25 % or more weight of a pharmaceutically acceptable alcohol per volume of pharmaceutical formulation, 30 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of pharmaceutical formulation and 5 % or more weight of ricinoleate excipient per volume of pharmaceutical formulation provided:

a) the pharmaceutical formulation comprises at least 10 % weight of ethanol per volume of pharmaceutical formulation;

b) if the pharmaceutically acceptable alcohol is less than 30 %, then the pharmaceutical formulation must comprise at least 35 % non-aqueous ester solvent.

A particularly preferred pharmaceutical formulation is one which comprises 15% w/v or less of ethanol and in which the solubility of fulvestrant is at least 155mg/ml.

According to another aspect of the invention there is provided a unit dose of a pharmaceutical formulation as described herein wherein the total volume of the formulation is 6ml or less.

According to another aspect of the invention there is provided a pharmaceutical formulation adapted for intramuscular injection, as defined in any preceding claim for use in medical therapy.

According to another aspect of the invention there is provided use of fulvestrant in the preparation of a pharmaceutical formulation, as defined herein for the treatment of a benign or malignant disease of the breast or reproductive tract.

According to another aspect of the invention there is provided use of fulvestrant in the preparation of a pharmaceutical formulation, as defined in any preceding claim for the treatment of a benign or malignant disease of the breast or reproductive tract in a human with dosage intervals of at least 8 weeks.

According to another aspect of the invention there is provided a sterile syringe or vial comprising a pharmaceutical formulation as defined in any preceding claim.

The term "pharmaceutical formulation" as used herein means the combination of drug plus formulation vehicle. The terms "finished formulation" and "finished pharmaceutical formulation" mean the same as "pharmaceutical formulation".

The term "formulation vehicle" as used herein means the combination of all excipients used in the pharmaceutical formulation (and therefore excludes drug per se).

The distinction between pharmaceutical formulation and formulation vehicle is important for the following reason. For example, if the concentration (y % w/v) of an excipient "A" is measured by its concentration in formulation vehicle and then drug is added, the addition of drug will result in a concentration of excipient A that is lower than concentration y in the finished pharmaceutical formulation. To convert a concentration expressed in terms of "formulation vehicle" into a concentration of "finished pharmaceutical formulation" it is necessary to use a displacement value.

The "displacement value" is defined as the number of parts by weight of compound that displaces one part by weight of the formulation vehicle. The displacement value allows determination of the amount of formulation vehicle displaced by the compound. The displacement value is used to calculate the actual composition of the finished formulation in terms of proportions of excipients. The density of the compound affects the amount of formulation vehicle required to make the pharmaceutical formulation to the correct concentration. One part by weight of the compound with a density equal to the formulation vehicle will displace an equivalent volume of the formulation vehicle. A compound with twice the density of the formulation vehicle will displace half the volume. It is therefore necessary to make allowance for the compound in terms of the particular formulation vehicle, using the displacement value.

For the avoidance of any doubt when using the term % weight per volume of formulation for the constituents of the formulation we mean that within a unit volume of the formulation a certain percentage of the constituent by weight will be present, for example a 1% weight per volume formulation will contain within a 100ml volume of formulation 1g of the constituent. By way of further illustration

% of x by weight per volume of formulation	weight of x in 1ml of formulation
30%	300mg
20%	200mg
10%	100mg
5%	50mg
1%	10mg

Where whole numbers are used for % weight per volume of formulation, these refer to rounded numbers where appropriate. For example, 4.6% would be rounded to 5%.

It is appreciated that in the formulation an excess of formulation may be included to allow the attendant physician or care giver to be able to deliver the required dose. Therefore, when a 5ml dose is required it would be appreciated that an excess of up to 0.25ml, preferably up to 0.15ml will also be present in the formulation. Typically the formulation will be presented in a vial or a prefilled syringe, preferably a prefilled syringe, containing a unit dosage of the formulation as described herein, these being further features of the invention.

The pharmaceutically-acceptable alcohol may consist of one alcohol or a mixture of two or more alcohols, preferably a mixture of two alcohols. Preferred pharmaceutically-acceptable alcohols for parenteral administration are ethanol, benzyl alcohol or a mixture of both ethanol and benzyl alcohol.

The pharmaceutically-acceptable non-aqueous ester solvent may consist of one or a mixture of two or more pharmaceutically-acceptable non-aqueous ester solvents, preferably just one. A preferred pharmaceutically-acceptable non-aqueous ester solvent for parenteral administration is selected from benzyl benzoate, ethyl oleate, isopropyl myristate,isopropyl palmitate or a mixture of any thereof.

It will be understood by the skilled person that the pharmaceutically-acceptable alcohol will be of a quality such that it will meet pharmacopoeial standards (such as are described in the US, British, European and Japanese pharmacopoeias) and as such will contain some water and possibly other organic solvents, for example ethanol in the US Pharmacopeia contains not less than 94.9% by volume and not more than 96.0% by volume of ethanol when measured at 15.56°C. Dehydrated alcohol in the US Pharmacopeia contains not less than 99.5% ethanol by volume when measured at 15.56°C.

It will be understood by the skilled person that the pharmaceutically-acceptable non-aqueous ester solvent will be of a quality that it will meet pharmacopoeial standards (such as described in the US, British, European and Japanese pharmacopoeias).

Preferred combinations of pharmaceutically-acceptable alcohol and pharmaceutically-acceptable non-aqueous ester solvent in the formulation are set out below:

By the use of the term ricinoleate excipient we mean an oil which has as a proportion (at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% w/v) of its composition as

triglycerides of ricinoleic acid. The ricinoleate vehicle may be a synthetic oil or conveniently is castor oil, ideally of pharmacopoeial standards, as described above.

We have surprisingly found that the above formulations of the invention provide, after intra-muscular injection, satisfactory release of fulvestrant over an extended period of time.

We have found that despite the rapid elimination of the additional solubilising excipients, i.e. the alcohol and pharmaceutically-acceptable non-aqueous ester solvent, from the formulation vehicle and the site of injection after injection of the formulation, extended release at therapeutically significant levels of fulvestrant over an extended period can still achieved by the formulation of the invention.

By use of the term "extended release" we mean at least 4 weeks, at least 5 weeks, and, preferably at least 8 weeks of continuous release of fulvestrant is achieved. In a preferred feature extended release is achieved for at least 8 weeks or 2 months, more preferably for at least 12 weeks or 3 months.

It will be understood that the attendant physician may wish to administer the intramuscular injection as a divided dose, i.e. a 5ml formulation is sequentially administered in two separate injections of 2.5ml, this is a further feature of the invention

Simply solubilising fulvestrant in an oil based liquid formulation is not predictive of a good release profile.

Preferably 5ml of the intramuscular injection is administered.

Additional excipients commonly used in the formulation field including, for example, an antioxidant preservative, a colorant or a surfactant may be used. A preferred optional excipient is a surfactant, more preferably an antioxidant.

As described above fulvestrant is useful in the treatment of oestrogen-dependent indications such as breast cancer and gynaecological conditions, such as endometriosis.

In addition to fulvestrant another similar type of molecule is currently under clinical investigation. SH-646 (11β-fluoro- 7α-(14,14,15,15,15-pentafluoro-6-methyl-10-thia-6-azapentadecyl)estra-1,3,5(10)-triene-3,17β-diol) is also putatively a compound with the same mode of action as fulvestrant and has a very similar chemical structure. It is believed that the compound will also share with fulvestrant similar physical properties and therefore the current invention will also have application with this compound.

Further features of the invention are those as described above but in which SH-646 is substituted for fulvestrant.

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The invention will now be illustrated by the following non-limiting Examples in which:

Figure 1 shows plasma profiles obtained following IM injection (data normalised for rabbit weight, based on 3.2 kg rabbit) in which the y-axis is conc (ng/ml) and the x-axis is time (days);

Figure 2 shows comparison of plasma profiles in which:

Figure 2A shows plasma profiles from group A in which the y-axis is conc (ng/ml) and the x-axis is time (days); and

Figure 2B shows plasma profiles from group B in which the y-axis is conc (ng/ml) and the x-axis is time (days);

Figure 3 shows plasma profiles from formulations 1, 5 and Control (normalised for rabbit weight, based on 3.2 kg rabbit in which the y-axis is conc (ng/ml) and the x-axis is time (days);

Figure 4 shows muscle residue data from 3 month PK study in which the y-axis is % fulvestrant remaining per injection site and the x-axis is formulation number. Each bar represents one injection site (2 sites per animal).

Figure 5 shows predicted versus actual solubility

Figure 6 shows a confidence interval for predicted solubility

Figure 7 shows plasma profiles obtained following IM injection (data normalised for rabbit weight, based on 3.2 kg rabbit) in which the y-axis is conc (ng/ml) and the x-axis is time (days);

Abbreviations

IM	intramuscular
PK	pharmacokinetic
AUC	area under curve
SD	standard deviation

Reference Example 1

Measurement of Solubility of Fulvestrant in Formulations

1. Materials and Apparatus

Balance

2 mL glass vials with screw caps

Magnetic stirrer bars

Temperature control reaction block with magnetic stirring facility

Positive displacement pipette (PDP) 20 - 25 μL with appropriate microsyringe tips

Polycarbonate ultracentrifuge tubes

Ultracentrifuge

Pipette 0.5 - 200 μL PDP with appropriate microsyringe tips

Pipette 200 μL - 1 mL with appropriate plastic tips

2 mL amber Snap Top glass HPLC vials

1 mm Snap Caps for HPLC vials

HPLC kit with diode array detector

Methanol (MeOH) HPLC Grade

Acetonitrile (ACN) Far UV HPLC Grade

Ultrapure de-ionised water

25 cm H5 ODS 5μ 4.6 mm i.d. HPLC column

Vortex mixer

Ultrasonic bath

20 - 200 μL pipette with appropriate plastic tips

Aluminium weigh pans 5 mL glass volumetric flasks

2. Experimental procedure

2.1 1ml formulation vehicles were made up in triplicate by adding the appropriate volumes of alcohols and benzyl benzoate, and then adding castor oil by weight

2.2 Fulvestrant was then added to excess, until no more drug was seen to visibly dissolve. The weight of fulvestrant added was noted.

2.3 A magnetic stirrer bar was placed in each vial.

2.4 All the samples were overlaid with nitrogen and the vials were capped placed in the reaction block and stirred at a speed of 1000 at a temperature of 4°C

2.5 Using the PDP 20 - 200 µL pipettor, 200 µL aliquots were removed from each vial after 6 days and transferred to ultracentrifuge tubes. These tubes were centrifuged at a speed of 80,000 r.p.m. for 30 minutes at 25°C.

2.6 HPLC eluent was prepared by adding 1400 mL methanol, 450 mL water and 150 mL acetonitrile to a 2 litre plastic-coated solvent bottle.

2.7 990 µL HPLC eluent was added to 60 amber glass HPLC vials using a 1mL Pipette.

2.8 3 x 10 µL of supernatant were removed from each ultracentrifuge tube using the Pipette PDP 0.5-25 µL pipette and added to the vials containing eluent.

2.9 The samples were diluted again 1 in 10. 100µl sample was added to 900µl HPLC eluent

2.10 The amber vials were capped, vortex mixed for 10 seconds, sonicated for 10 minutes and then placed in the HPLC autosampler tray.

3. Calibration preparation

3.1 Approximately 10 mg fulvestrant was accurately weighed into an aluminium weigh pan on the microbalance and placed in a 5 mL glass volumetric flask The actual weight was recorded.

3.2 Approximately 4.5 mL HPLC eluent was added to the flask using a plastic pasteur pipette. The flask was then sonicated for 5 minutes prior to making accurately to volume (to give a spiking solution of approximately 2 $mg.mL^{-1}$).

3.3 0 - 250 µL spiking solution was added to 2 mL amber HPLC vials using the appropriate Pipette and the volume made to 1 mL with HPLC eluent using a 1 mL Pipette as shown in the table below:

Volume Spiking Solution (µL)	Volume HPLC eluent (µL)	Theoretical fulvestrant Concentration (µg.mL^{-1})
0	1000	0
5	995	10
25	975	50
50	950	100
100	900	200
150	850	300
200	800	400
250	750	500

3.4 The HPLC vials were capped, vortexed for 10 seconds and placed on the HPLC autosampler tray.

3.5 A calibration was prepared (as per 3.1 - 3.4) for both batches of ICI 182780.

4. HPLC Conditions

Eluent :	70% MeOH / 22.5% Water / 7.5% ACN
Column :	25 cm 5µ Hypersil ODS 4.6 mm i.d. with guard column
Detection wavelength :	280 nm
Flow rate :	1.2 mL.min^{-1}
Temperature :	Ambient
Injection volume :	50 µL
Retention time :	12 minutes approximately

Example 1

Pharmaceutical Formulations

Fulvestrant is mixed with ethanol and benzyl alcohol, stirring until completely dissolved. Benzyl benzoate is added and the solution is made to final weight with castor oil and stirred, (for convenience weight is used rather than volume by using the weight to volume

ratio). The bulk solution is overlaid with nitrogen. The solution is sterilised by filtration using one or two filters of 0.2μm porosity. The sterile filtrate is kept under a nitrogen overlay as it is filled under aseptic conditions into washed and depyrogenised, sterile primary containers, for example vials or pre-filled syringes. An overage is included in the primary pack to facilitate removal of the dose volume. The primary packs are overlaid with sterile nitrogen, before aseptically sealing. The process flow diagram below depicts the manufacturing process.

Quantities of each component of the formulation is chosen according to the required formulation specification, examples are described above. For example quantities are added of each component to prepare the following formulations:

a)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

15% weight per volume of benzyl benzoate

500mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

b)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

c)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

d)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

e)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

f)

15% weight per volume of ethanol

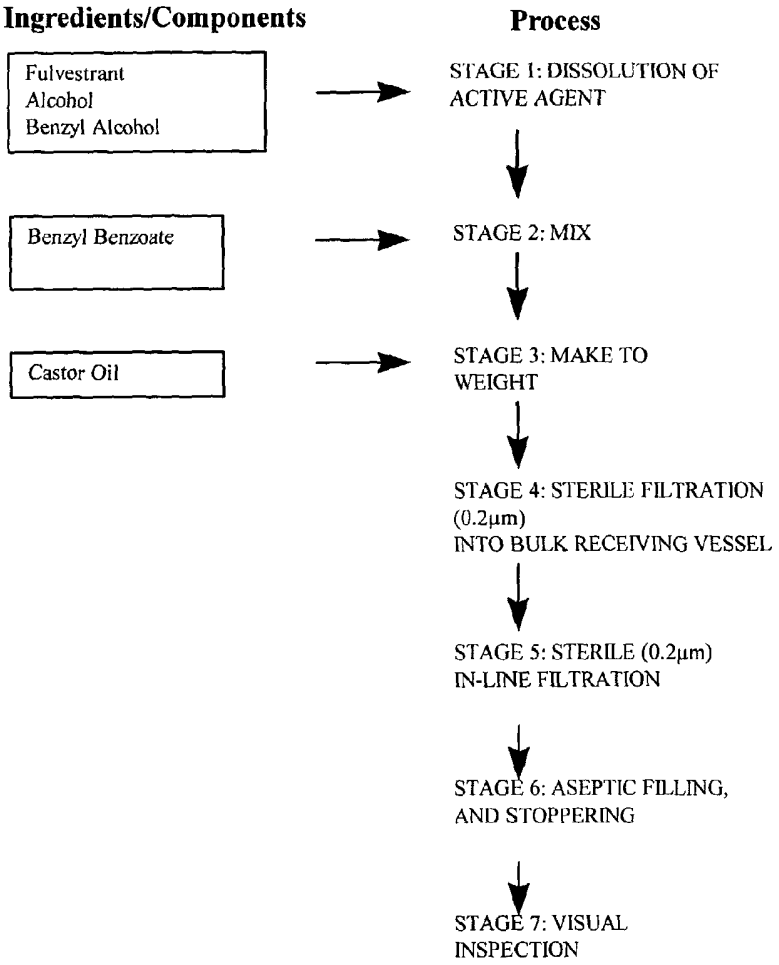
20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil

FLOW DIAGRAM OF MANUFACTURING



Example 2

Stability studies to 4 months for selection of stable 100mg/ml Pharmaceutical formulations

Formulation No.	Fulvestrant	96% EtOH	Benzyl Alcohol	Benzyl Benzoate	Castor oil	Observations (visual)
	(mg/ml)	% w/v	% w/v	% w/v	% w/v	4 months
Sample 1	100	5	10	15	to 100%	Precipitated
Sample 2	100	5	10	30	to 100%	Precipitated
Sample 3	100	7.5	10	15	to 100%	Precipitated
Sample 4	100	7.5	10	30	to 100%	Precipitated
Sample 5	100	10	10	15	to 100%	Precipitated
Sample 6	100	10	10	17.5	to 100%	Precipitated
Sample 7	100	10	10	20	to 100%	Precipitated
Sample 8	100	10	10	22.5	to 100%	Solution
Sample 9	100	10	10	25	to 100%	Solution
Sample 10	100	10	10	27.5	to 100%	Solution
Sample 11	100	10	10	30	to 100%	Solution
Sample 12	100	10	10	40	to 100%	Solution
Sample 13	100	10	10	50	to 100%	Solution
Sample 14	100	10	15	15	to 100%	Solution
Sample 15	100	10	15	30	to 100%	Solution
Sample 16	100	10	15	40	to 100%	Solution
Sample 17	100	10	15	50	to 100%	Solution
Sample 18	100	10	20	15	to 100%	Solution
Sample 19	100	10	20	30	to 100%	Solution
Sample 20	100	10	20	40	to 100%	Solution
Sample 21	100	10	20	50	to 100%	Solution
Sample 22	100	15	10	15	to 100%	Solution
Sample 23	100	15	10	30	to 100%	Solution
Sample 24	100	15	10	40	to 100%	Solution
Sample 25	100	15	10	50	to 100%	Solution
Sample 26	100	20	5	15	to 100%	Solution
Sample 27	100	20	5	30	to 100%	Solution
Sample 28	100	20	10	15	to 100%	Solution
Sample 29	100	20	10	30	to 100%	Solution
Sample 30	100	20	10	40	to 100%	Solution
Sample 31	100	20	10	50	to 100%	Solution
Sample 32	100	15	15	15	to 100%	Solution
Sample 33	100	15	15	30	to 100%	Solution
Sample 34	100	15	15	40	to 100%	Solution
Sample 35	100	15	15	50	to 100%	Solution
Sample 36	100	15	20	15	to 100%	Solution
Sample 37	100	15	20	30	to 100%	Solution
Sample 38	100	15	20	50	to 100%	Solution
Sample 39	100	20	20	15	to 100%	Solution

Sample 40	100	20	20	30	to 100%	Solution
Control Sample	**52.16**	**10**	**10**	**15**	**60**	**Solution**

The Control Sample refers to the following Pharmaceutical formulation: fulvestrant 50mg/ml, ethanol 10% w/v, benzylalcohol 10% w/v, benzyl benzoate 15% w/v and made to volume with castor oil.

Example 3

Pharmaceutical formulations selected for *in vivo* deposition and *in vitro* precipitation studies

The pharmaceutical formulations below were selected from Example 2 for further study.

Formulation	Fulvestrant	95% ETOH	Benzyl Alcohol	Benzyl Benzoate	Castor oil
	% w/v	%w/v	%w/v	%w/v	% w/v
Sample 1	10	10	10	30	to 100%
Sample 2	10	10	10	50	to 100%
Sample 3	10	10	20	15	to 100%
Sample 4	10	10	20	30	to 100%
Sample 5	10	10	20	50	to 100%
Sample 6	10	20	5	15	to 100%
Sample 7	10	20	5	30	to 100%
Sample 8	10	20	20	15	to 100%
Sample 9	10	20	20	30	to 100%
Sample 10	10	15	10	15	to 100%
Sample 11	10	15	10	30	to 100%
Sample 12	10	15	10	50	to 100%
Sample 13	10	15	20	15	to 100%
Sample 14	10	15	20	50	to 100%
Sample 15	10	15	20	30	to 100%
Sample 16	5	10	10	15	to 100%

A matrix of 7 pharmaceutical formulations (samples 3, 4, 5, 9, 12, 14 and 16 – see Example 3 below) was identified for further evaluation from in vitro precipitation and deposition studies.

Sample 16 was a control. The precipitation experiment involved visual inspection of each sample under conditions where evaporation of alcohols led to precipitation of drug.

Example 4

In vivo Studies

Pharmaceutical formulations identified for further *in vivo* evaluation

		Excipients (% w/v)			
No.	Fulvestrant (%w/v)	Ethanol 96%	Benzyl alcohol	Benzyl benzoate	Castor oil
F1	10	10	20	15	45
F2	10	10	20	30	30
F3	10	10	20	50	10
F4	10	20	20	30	20
F5	10	15	10	50	15
F6	10	15	20	50	5
F7	10	15	20	30	25
Control	5	10	10	15	60

(a) **An in vivo pharmacokinetic (PK) study on these 7 pharmaceutical formulations was performed over 3 months duration**; results are shown in Figures 1, 2 and 3.

Analysis of PK results

Plasma levels were more variable than Control over the first 30 days; variability was similar to control thereafter. After 2 months, drug levels were equivalent to Control at 1 month indicating a prolonged period of action over Control. This release profile was surprising because, compared with Control which does not precipitate drug locally, local precipitation at the site of injection of the tested formulations was expected to impair their release profile.

Some differences in profiles were noted over the first 30 days such that they were divided into 2 groups (with Formulation F7 showing intermediate behaviour).

Group A, rapid release early time points (50% Benzyl benzoate and low castor oil ≤ 15%) – see figure 2A

Group B, lower release, flatter profile (≤ 30% Benzyl benzoate and higher castor oil ≥ 20%) – see figure 2B

(b) **Histopathology**

Local tolerance was assessed to IM injection of the 7 pharmaceutical formulations and Control. Lesions were observed over a 51 day period. All pharmaceutical formulations caused tissue reactions greater than Control.

(c) **Plasma levels for 100mg dose at critical time points (ng/ml)**

Form.	Dose	Time point (days)		
	(mg)	28	56	84
1	100	8.7	4.6	3.5
2	100	8.0	3.6	2.6
3	100	9.4	3.5	2.0
4	100	7.7	5.0	3.4
5	100	9.1	4.5	2.6
6	100	9.9	3.3	1.9
7	100	9.9	5.5	3.2
Control	50	**3.3**	**1.7**	

Summary

Duration for 100mg dose = min 2 months.

Duration for 150mg dose = min 3 months.

(d) **Measurement of Fulvestrant solubility in 7 pharmaceutical formulations after 6 days**

Formulation	**Solubility (mg/ml)**
	6days
F1	111
F2	140
F3	175
F4	235
F5	162
F6	212
F7	126

Example 5

Model for Fulvestrant Solubility

Formulation vehicles for Fulvestrant - solubility

Formulation No.	96% ETOH % w/v	Benzyl Alcohol % w/v	Benzyl Benzoate % w/v	Castor oil % w/v	Measured Fulvestrant Solubility (mg/ml)	Predicted solubility (mg/ml)
Sample 1	5	5	0	to 100%	27	9.4
Sample 2	5	5	15	to 100%	36	32.8
Sample 3	10	5	0	to 100%	46	48.5
Sample 4	10	5	15	to 100%	54	36.6
Sample 5	10	10	0	to 100%	45	60.1
Sample 6	10	10	15	to 100%	65	72
Sample 7	15	15	0	to 100%	76	87.6
Sample 8	15	15	15	to 100%	102	111.1
Sample 9	11	22	17	to 100%	111	109.8
Sample 10	11	22	33	to 100%	140	166.1
Sample 11	11	22	56	to 100%	175	135.8
Sample 12	22	22	33	to 100%	235	174.4
Sample 13	17	11	56	to 100%	162	170.6
Sample 14	17	22	56	to 100%	212	200.9
Sample 15	17	22	33	to 100%	126	196.3

A linear regression model was fitted to solubility data from 15 samples using as independent variables the % ethanol, benzyl alcohol and benzyl benzoate levels in the formulations. The following model was obtained which had an R-Squared value of 93.2%:

SOLUBILITY = -29.77 + 5.44 x ETOH + 2.38 x BA + 1.57 x BB

Benzyl alcohol = BA, benzyl benzoate = BB, Ethanol = ETOH.

Solubility measured as mg/ml

See Figures 5 and 6 based on the following data

measured	lower C.L.	predicted	upper C.L.
27	0	9.4	31.1
36	10	32.8	55.7
45	31.6	48.5	65.4
46	17.1	36.6	56.1
54	40.9	60.1	79.2
65	59	72	85
76	64.7	87.6	110.6
102	95.6	111.1	126.7
111	85.4	109.8	134.1
126	149	166.1	183.1

140	114.2	135.8	157.5
162	142.5	174.4	206.3
175	143.4	170.6	197.9
212	179.6	200.9	222.2
235	168.7	196.3	224

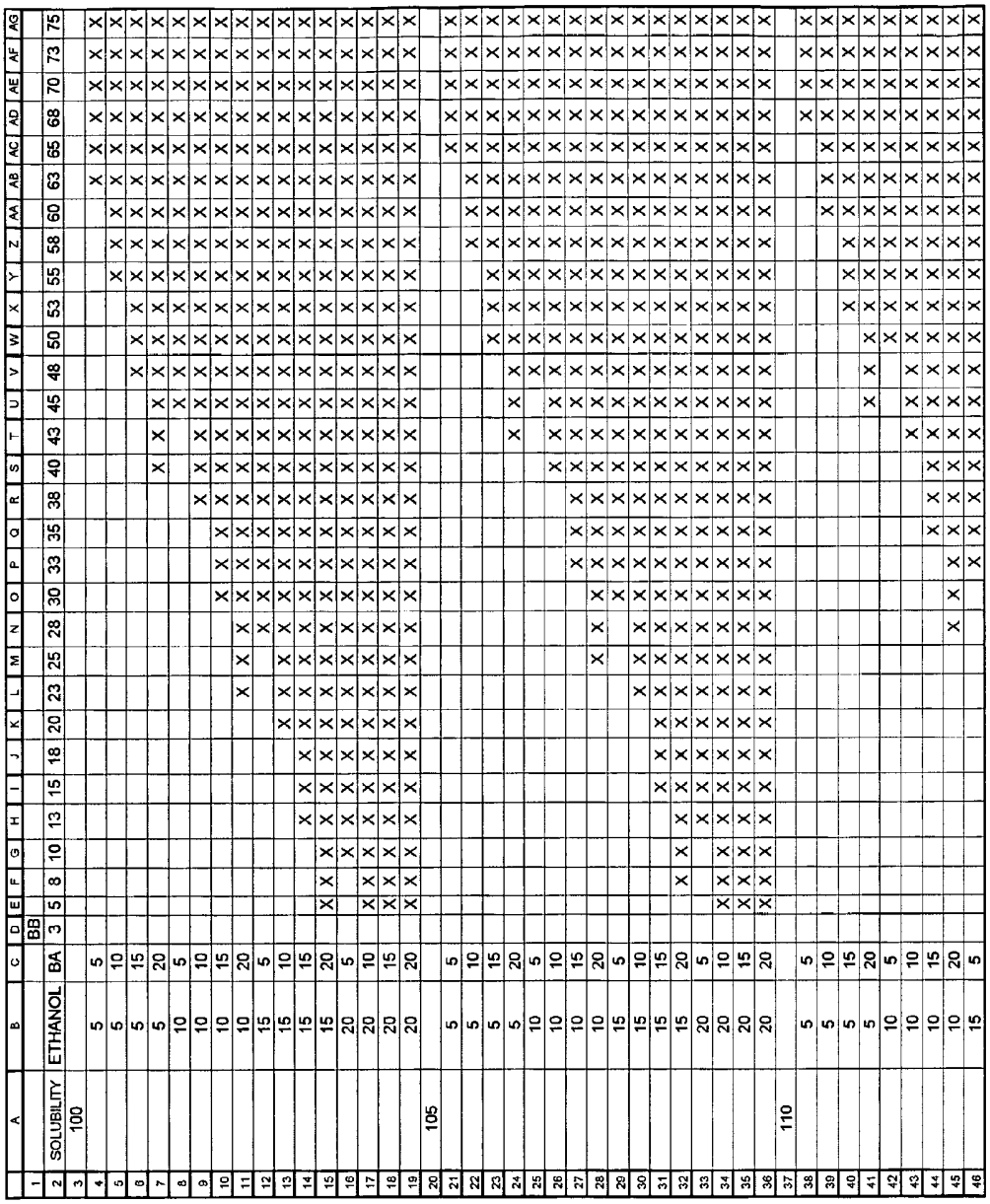
The table below shows predicted solubilities for a matrix of pharmaceutical formulations tested for stability. A good correlation was obtained with visual observations.

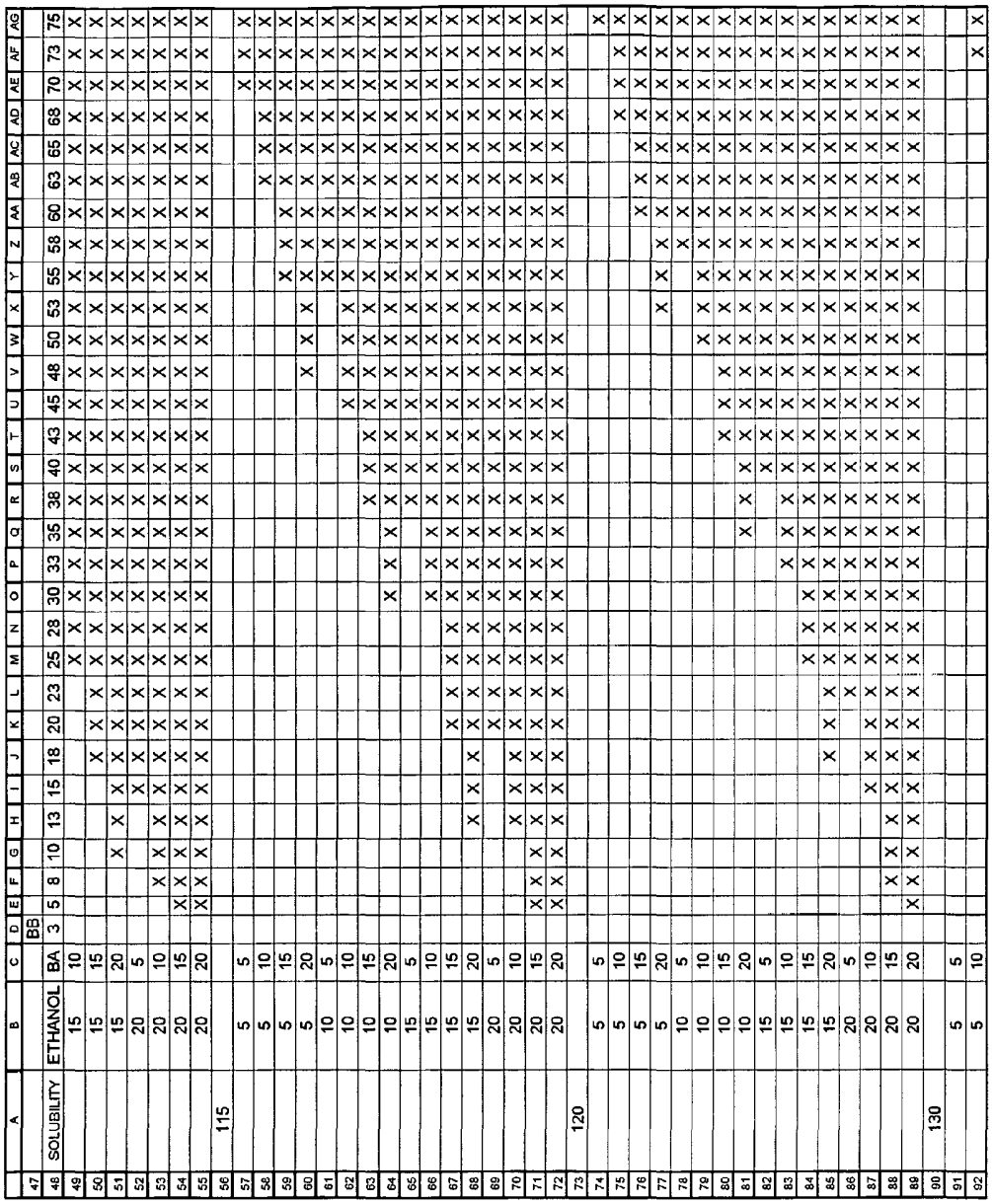
Formulation of Fulvestrant - stability

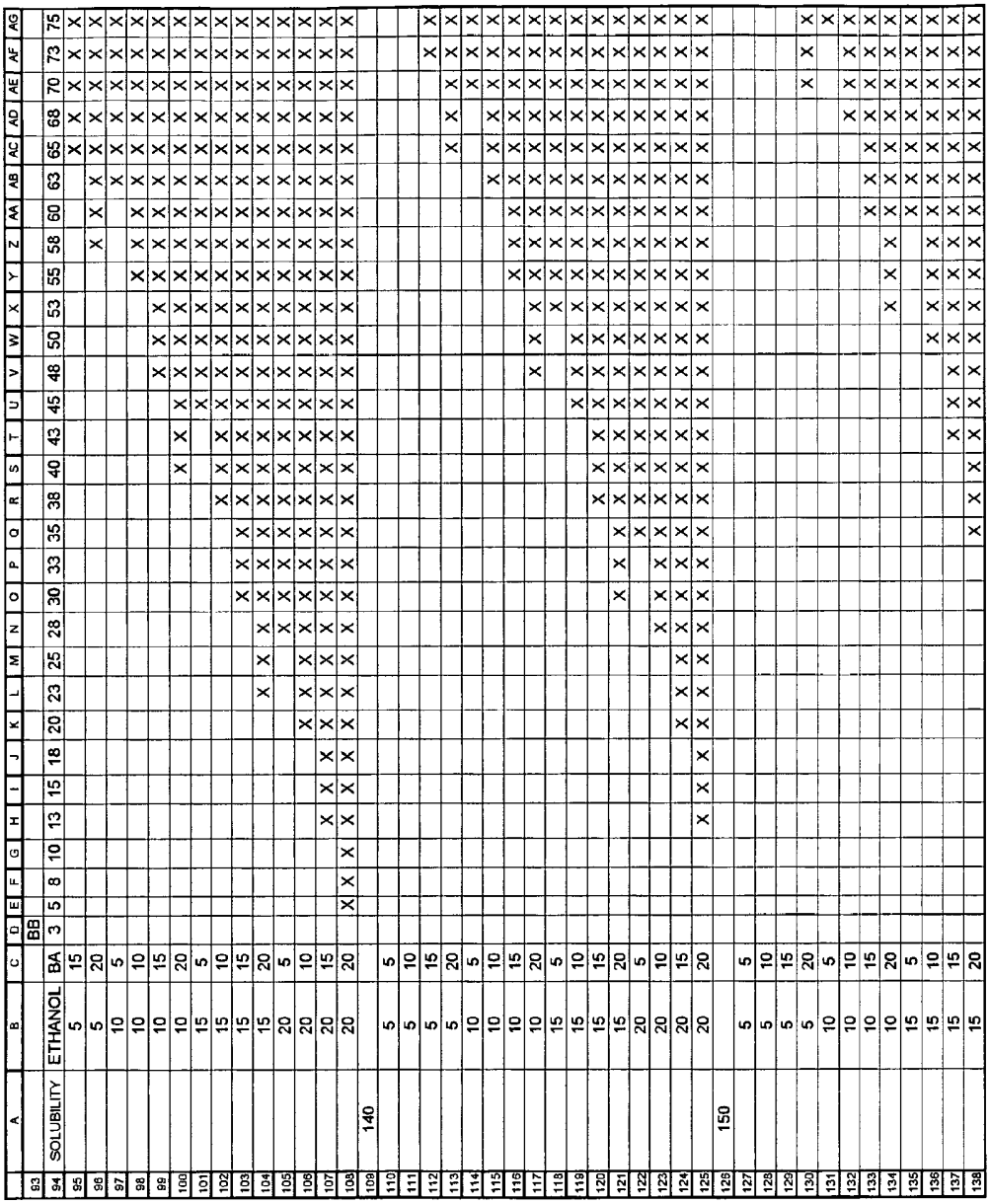
Formulation No.	Fulvestrant (mg/ml)	95% ETOH % w/v	Benzyl Alcohol % w/v	Benzyl Benzoate % w/v	Castor oil % w/v	Observations 4 months	**Predicted solubility (mg/ml)**
Sample 1	100	5	10	15	60	Precipitated	53.1
Sample 2	100	5	10	30	45	Precipitated	79.2
Sample 3	100	7.5	10	15	57.5	Precipitated	68.2
Sample 4	100	7.5	10	30	42.5	Precipitated	94.3
Sample 5	100	10	10	15	55	Precipitated	83.3
Sample 6	100	10	10	17.5	52.5	Precipitated	87.6
Sample 7	100	10	10	20	50	Precipitated	92.0
Sample 8	100	10	10	22.5	47.5	Solution	96.4
Sample 9	100	10	10	25	45	Solution	100.7
Sample 10	100	10	10	27.5	42.5	Solution	105.1
Sample 11	100	10	10	30	40	Solution	109.5
Sample 12	100	10	10	40	30	Solution	126.9
Sample 13	100	10	10	50	20	Solution	144.3
Sample 14	100	10	15	15	50	Solution	96.5
Sample 15	100	10	15	30	35	Solution	122.7
Sample 16	100	10	15	40	25	Solution	140.1
Sample 17	100	10	15	50	15	Solution	157.6
Sample 18	100	10	20	15	45	Solution	109.7
Sample 19	100	10	20	30	30	Solution	135.9
Sample 20	100	10	20	40	20	Solution	153.3
Sample 21	100	10	20	50	10	Solution	170.8
Sample 22	100	15	10	15	50	Solution	113.5
Sample 23	100	15	10	30	35	Solution	139.7
Sample 24	100	15	10	40	25	Solution	157.1
Sample 25	100	15	10	50	15	Solution	174.6
Sample 26	100	20	5	15	50	Solution	130.5
Sample 27	100	20	5	30	35	Solution	156.7
Sample 28	100	20	10	15	45	Solution	143.7
Sample 29	100	20	10	30	30	Solution	169.9
Sample 30	100	20	10	40	20	Solution	187.3
Sample 31	100	20	10	50	10	Solution	204.8
Sample 32	100	15	15	15	45	Solution	126.7
Sample 33	100	15	15	30	30	Solution	152.9
Sample 34	100	15	15	40	20	Solution	170.3
Sample 35	100	15	15	50	10	Solution	187.8
Sample 36	100	15	20	15	40	Solution	140.0
Sample 37	100	15	20	30	25	Solution	166.1
Sample 38	100	15	20	50	5	Solution	201.0

Sample 39	100	20	20	15	35	Solution	170.2
Sample 40	100	20	20	30	20	Solution	196.3
Control Sample [Faslodex]	**52.16**	**10**	**10**	**15**	**60**	**Solution**	72.0

The Tables below show predicted formulations for various solubilities of fulvestrant; where an "X" means in solution. Note that the Tables include some impractical formulations where the sum of components becomes greater than 100%. The principal purpose is to illustrate the wide combinations of ethanol/ benzyl alcohol / benzyl benzoate taught by the invention to achieve different solubilities of fulvestrant.







	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG
139				BB																													
140	SOLUBILITY	ETHANOL	BA	3	5	8	10	13	15	18	20	23	25	28	30	33	35	38	40	43	45	48	50	53	55	58	60	63	65	68	70	73	75
141		20	5																	X	X	X	X	X	X	X	X	X	X	X	X	X	X
142		20	10														X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
143		20	15										X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
144		20	20							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
145	200																																
146		5	5																														
147		5	10																														
148		5	15																														
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154		15	5																														
155		15	10																														
156		15	15																														X
157		15	20																											X	X	X	X
158		20	5																													X	X
159		20	10																										X	X	X	X	X
160		20	15																							X	X	X	X	X	X	X	X
161		20	20																				X	X	X	X	X	X	X	X	X	X	X

Example 6

In-vivo Pharmacokinetic study using compositions at 140mg/ml of fulvestrant

Formulations F3 and F6 as described in Example 4 were modified to contain an increased level of fulvestrant to 140mg/ml. The modified formulations were named F8 and F9 as described below.

Formulation Composition

The compositions of the formulations dosed in the PK study are shown in the table below.

Formulation No.	Fulvestrant (%w/v)	Ethanol 96% (%w/v)	Benzyl alcohol (%w/v)	Benzyl Benzoate (%w/v)	Castor Oil (%w/v)
F8	14	9	19	47	To 100%
F9	14	14	19	48	To 100%

PK Profile

The results are set out in Figure 7. The composition of the Control is the same as described in Example 4. Compositions F8 and F9 gave similar profiles with improved performance in terms of extended release of higher levels fulvestrant compared with Control.

Example 7

Compositions at 150mg/ml fulvestrant

Compositions analogous or similar to F3, F4, F5 and F6 (see Example 4) but comprising 150mg/ml of fulvestrant are prepared as follows.

Formulation No.	Fulvestrant (%w/v)	Ethanol 96% (%w/v)	Benzyl alcohol (%w/v)	Benzyl Benzoate (%w/v)	Castor Oil (%w/v)
F10	15	10	20	50	To 100%
F11	15	20	20	30	To 100%
F12	15	15	10	50	To 100%
F13	15	15	20	45	To 100%
F14	15	9	19	47	To 100%
F15	15	19	19	28	To 100%

F16	15	14	9	47	To 100%
F17	15	14	19	47	To 100%
F18	15	10	20	45	To 100%
F19	15	15	10	45	To 100%
F20	15	20	20	25	To 100%
F21	15	10	30	25	To 100%
F22	15	10	25	30	To 100%
F23	15	10	30	30	To 100%
F24	15	15	25	30	To 100%
F25	15	15	25	25	To 100%
F26	15	15	20	30	To 100%

Claims

1. A pharmaceutical formulation adapted for intramuscular injection comprising 100 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle and provided that the following formulation is excluded: fulvestrant up to 102 mg/ml, 15 % weight of ethanol per volume of formulation vehicle, 15 % weight of benzyl alcohol per volume of formulation vehicle, 15 % weight of benzyl benzoate per volume of formulation vehicle and 30 % or more weight of ricinoleate excipient per volume of formulation vehicle.

2. A pharmaceutical formulation adapted for intramuscular injection according to claim 2 comprising 105 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation comprises at least 5 % weight of ethanol per volume of formulation vehicle.

3. A pharmaceutical formulation adapted for intramuscular injection according to claim 1 comprising 110 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

4. A pharmaceutical formulation adapted for intramuscular injection according to claim 1 comprising 115 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the

formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

5. A pharmaceutical formulation adapted for intramuscular injection according to claim 1 comprising 120 mg/ml or more of fulvestrant, 10 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

6. A pharmaceutical formulation adapted for intramuscular injection according to claim 1 comprising 130 mg/ml or more of fulvestrant, 15 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 5 % weight of ethanol per volume of formulation vehicle.

7. A pharmaceutical formulation adapted for intramuscular injection according to claim 1 comprising 140 mg/ml or more of fulvestrant, 15 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 12.5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the formulation vehicle comprises at least 10 % weight of ethanol per volume of formulation vehicle.

8. A pharmaceutical formulation adapted for intramuscular injection according to claim 1 comprising 150 mg/ml or more of fulvestrant, 15 % or more weight of a pharmaceutically acceptable alcohol per volume of formulation vehicle, 17.5 % or more weight of a pharmaceutically acceptable non-aqueous ester solvent per volume of formulation vehicle and 5 % or more weight of ricinoleate excipient per volume of formulation vehicle provided the

formulation vehicle comprises at least 10 % weight of ethanol per volume of formulation vehicle.

9. A pharmaceutical formulation having a solubility for fulvestrant of at least Y mg/ml adapted for intramuscular injection comprising;
100 mg/ml or more of fulvestrant;
5% (w/v) or more castor oil per volume of formulation vehicle;
and at least the following amounts (% weight/volume of formulation vehicle) of ethanol (ETOH), benzyl alcohol (BA), benzyl benzoate (BB) determined by the algorithm:
$Y = -29.77 + 5.44ETOH + 2.38BA + 1.57BB$
wherein Y is at least 100, ETOH is at least 5, BA is at least 5 and BB is at least 5.

10. A pharmaceutical formulation according to claim 9 wherein Y is selected from the group consisting of 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 170, 180, 190, and 200.

11. A pharmaceutical formulation according to claim 9 wherein Y is selected from the group consisting of 120, 125, 130, 135, 140, 145, 150, 155, 160, 170, 180, 190, and 200.

12. A pharmaceutical formulation according to claim 9 wherein Y is selected from the group consisting of 150, 155, 160, 170, 180, 190 and 200.

13. A pharmaceutical formulation according to claim 9 wherein Y is selected from 150, 155, 160, 170, 180, 190 and 200 and the formulation comprises at least 150mg/ml of fulvestrant.

14. A pharmaceutical formulation according to claim 9 wherein Y is 200 and the formulation comprises at least 200mg/ml of fulvestrant.

15. A pharmaceutical formulation comprising fulvestrant at a concentration of at least 100 mg/ml in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months and provided that the following

formulation is excluded: fulvestrant up to 102 mg/ml, 15 % weight of ethanol per volume of formulation vehicle, 15 % weight of benzyl alcohol per volume of formulation vehicle, 15 % weight of benzyl benzoate per volume of formulation vehicle and 30 % or more weight of ricinoleate excipient per volume of formulation vehicle.

16. A pharmaceutical formulation comprising fulvestrant at a concentration of at least 150 mg/ml in which the formulation is adapted for intra-muscular injection into a human and which is capable after injection of attaining a therapeutically significant blood plasma fulvestrant concentration in a human for at least 2 months.

17. Any one of the following pharmaceutical formulations comprising about:

i)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

15% weight per volume of benzyl benzoate

500-555mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

ii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-700mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

iii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

500-750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

iv)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

500-1175mg of fulvestrant for each 5ml of finished formulation
and the remaining amount as castor oil;

v)

15% weight per volume of ethanol
10% weight per volume of benzyl alcohol
50% weight per volume of benzyl benzoate
500-810 mg of fulvestrant for each 5ml of finished formulation
and the remaining amount as castor oil;

vi)

15% weight per volume of ethanol
20% weight per volume of benzyl alcohol
50% weight per volume of benzyl benzoate
500 mg of fulvestrant for each 5ml of finished formulation
and the remaining amount as castor oil;

vii)

15% weight per volume of ethanol
20% weight per volume of benzyl alcohol
30% weight per volume of benzyl benzoate
500-630mg of fulvestrant for each 5ml of finished formulation
and the remaining amount as castor oil;

viii)

10% weight per volume of ethanol
20% weight per volume of benzyl alcohol
50% weight per volume of benzyl benzoate
750mg of fulvestrant for each 5ml of finished formulation
and the remaining amount as castor oil;

ix)

20% weight per volume of ethanol
20% weight per volume of benzyl alcohol
30% weight per volume of benzyl benzoate
750mg of fulvestrant for each 5ml of finished formulation
and the remaining amount as castor oil;

x)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

50% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xi)

9% weight per volume of ethanol

19% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

700mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xii)

14% weight per volume of ethanol

19% weight per volume of benzyl alcohol

48% weight per volume of benzyl benzoate

700mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xiii)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

45% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xiv)

9% weight per volume of ethanol

19% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xv)

19% weight per volume of ethanol

19% weight per volume of benzyl alcohol

28% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xvi)

14% weight per volume of ethanol

9% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xvii)

14% weight per volume of ethanol

19% weight per volume of benzyl alcohol

47% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xviii)

10% weight per volume of ethanol

20% weight per volume of benzyl alcohol

45% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xix)

15% weight per volume of ethanol

10% weight per volume of benzyl alcohol

45% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xx)

20% weight per volume of ethanol

20% weight per volume of benzyl alcohol

25% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxi)

10% weight per volume of ethanol

30% weight per volume of benzyl alcohol

25% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxii)

10% weight per volume of ethanol

25% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxiii)

10% weight per volume of ethanol

30% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxiv)

15% weight per volume of ethanol

25% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil;

xxv)

15% weight per volume of ethanol

25% weight per volume of benzyl alcohol

25% weight per volume of benzyl benzoate

750mg of fulvestrant for each 5ml of finished formulation

and the remaining amount as castor oil; and

xxvi)

15% weight per volume of ethanol

20% weight per volume of benzyl alcohol

30% weight per volume of benzyl benzoate
750mg of fulvestrant for each 5ml of finished formulation
and the remaining amount as castor oil.

18. A pharmaceutical formulation as claimed in any preceding claim wherein the pharmaceutically-acceptable alcohol is a mixture of ethanol and benzyl alcohol.

19. A pharmaceutical formulation as claimed in any preceding claim wherein the pharmaceutically-acceptable non-aqueous ester solvent is selected from benzyl benzoate, ethyl oleate, isopropyl myristate, isopropyl palmitate or a mixture of any thereof.

20. A pharmaceutical formulation as claimed in any preceding claim wherein the pharmaceutically-acceptable non-aqueous ester solvent is benzyl benzoate.

21. A pharmaceutical formulation as claimed in any preceding claim wherein the ricinoleate excipient is castor oil.

22. A unit dose of a pharmaceutical formulation as claimed in any preceding claim wherein the total volume of the formulation is 6ml or less.

23. A pharmaceutical formulation adapted for intramuscular injection, as defined in any preceding claim for use in medical therapy.

24. Use of fulvestrant in the preparation of a pharmaceutical formulation, as defined in any preceding claim for the treatment of a benign or malignant disease of the breast or reproductive tract.

25. Use of fulvestrant in the preparation of a pharmaceutical formulation, as defined in any preceding claim for the treatment of a benign or malignant disease of the breast or reproductive tract in a human with dosage intervals of at least 8 weeks.

26. A sterile syringe or vial comprising a pharmaceutical formulation as defined in any preceding claim.

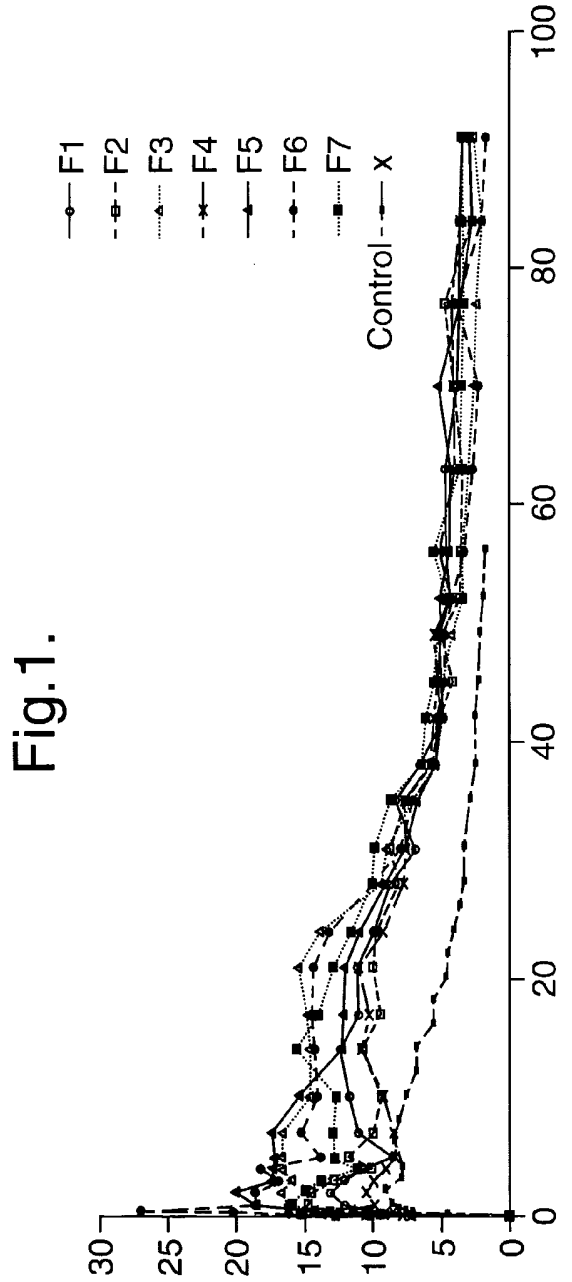
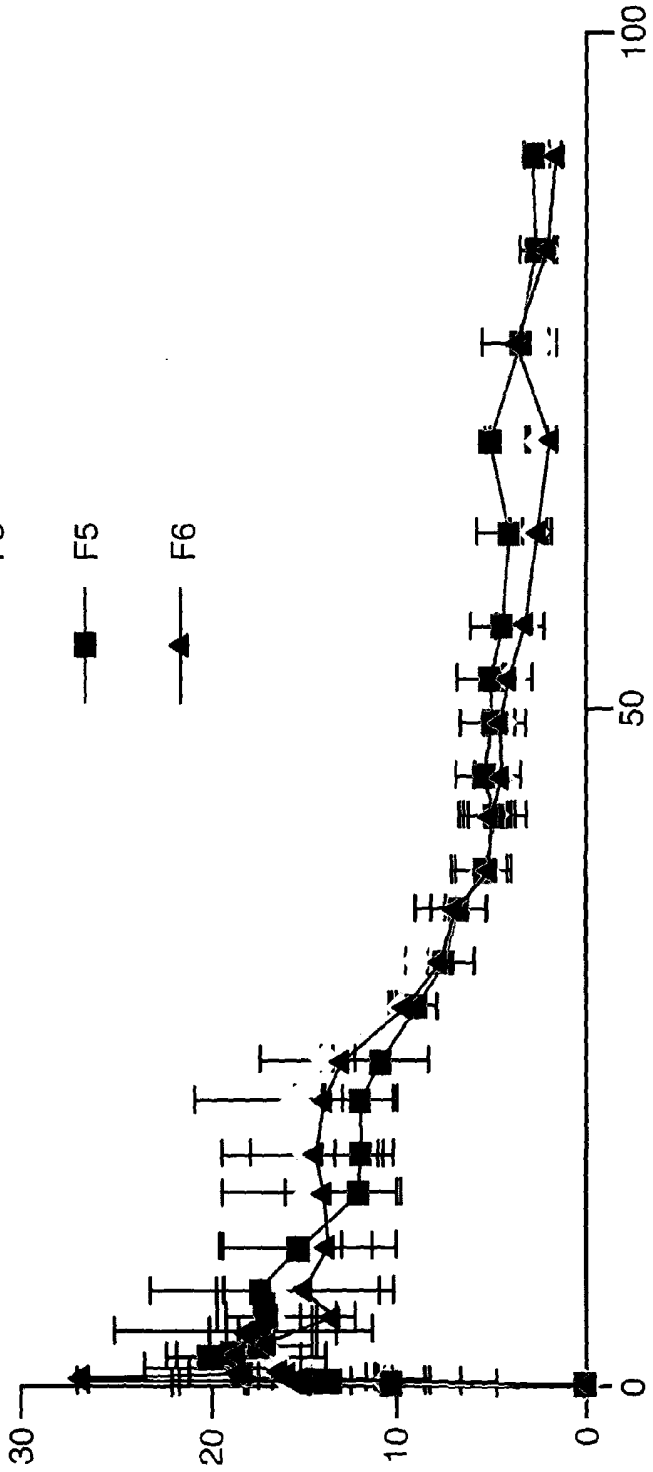


Fig.1.

Fig.2A.



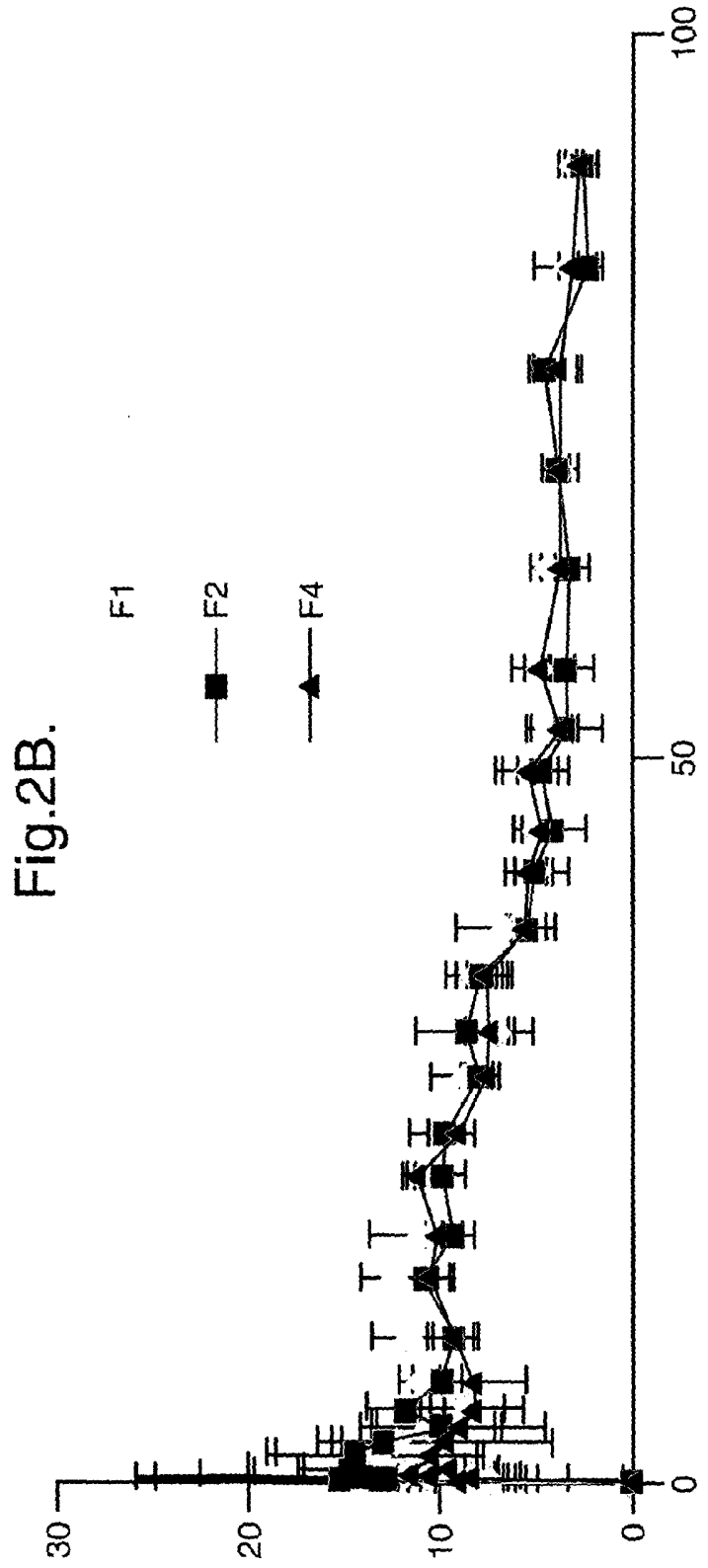
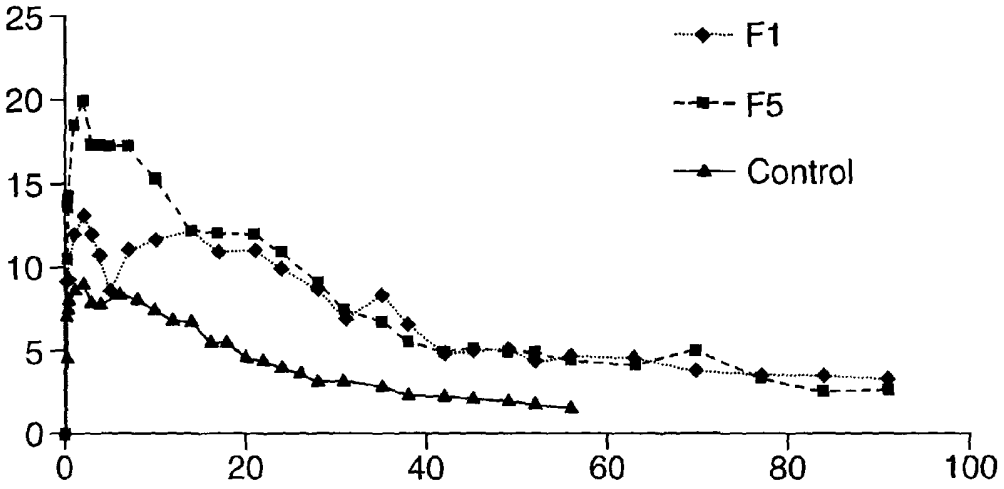


Fig.2B.

Fig.3.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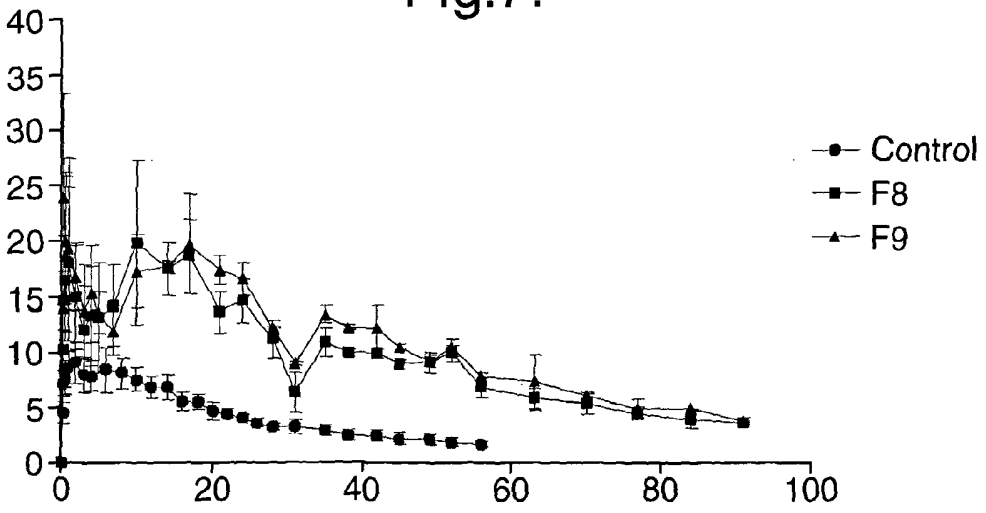
5
0
0
20
40
60
80
100
F1
F5
Control

Fig.7.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Control
F8
F9

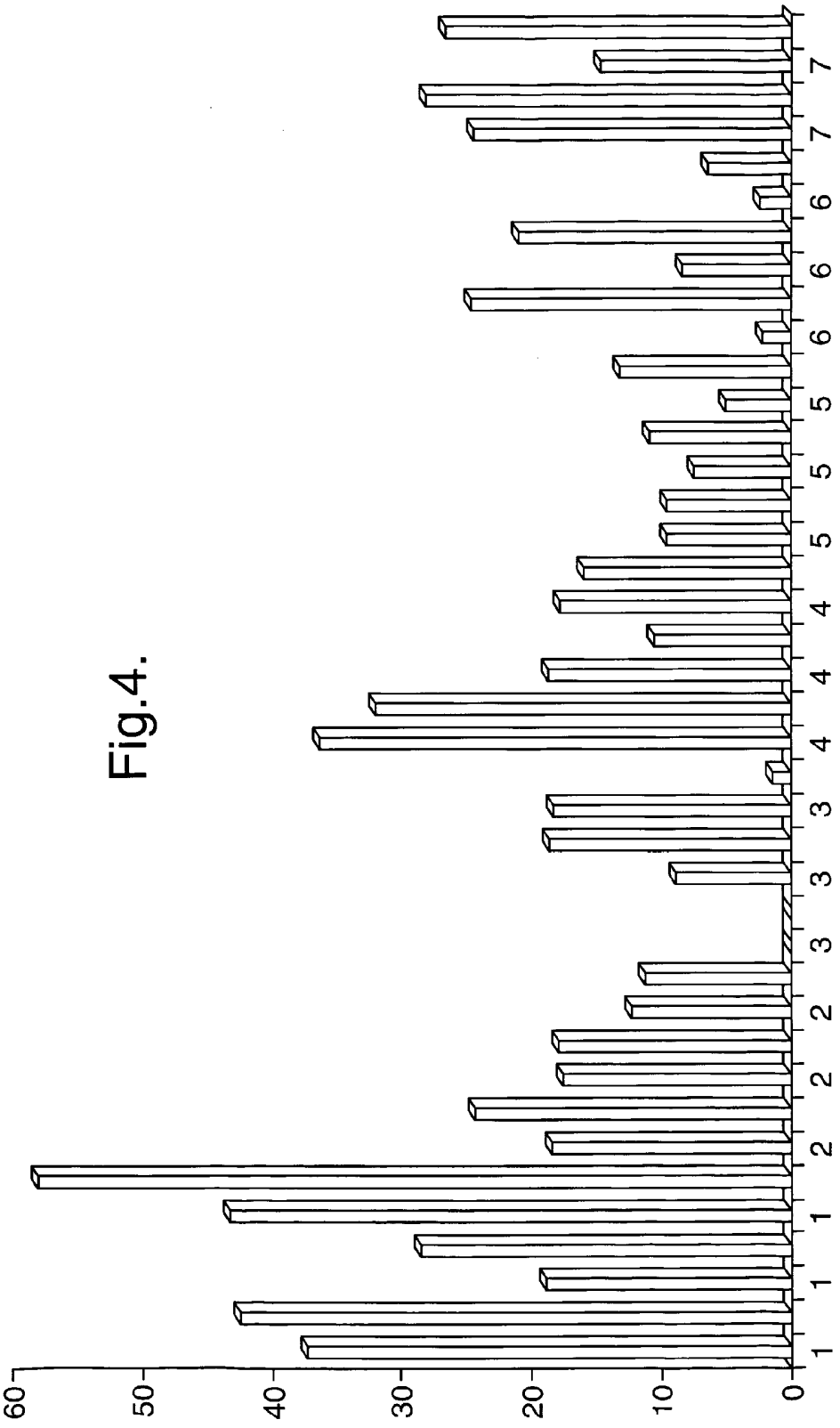
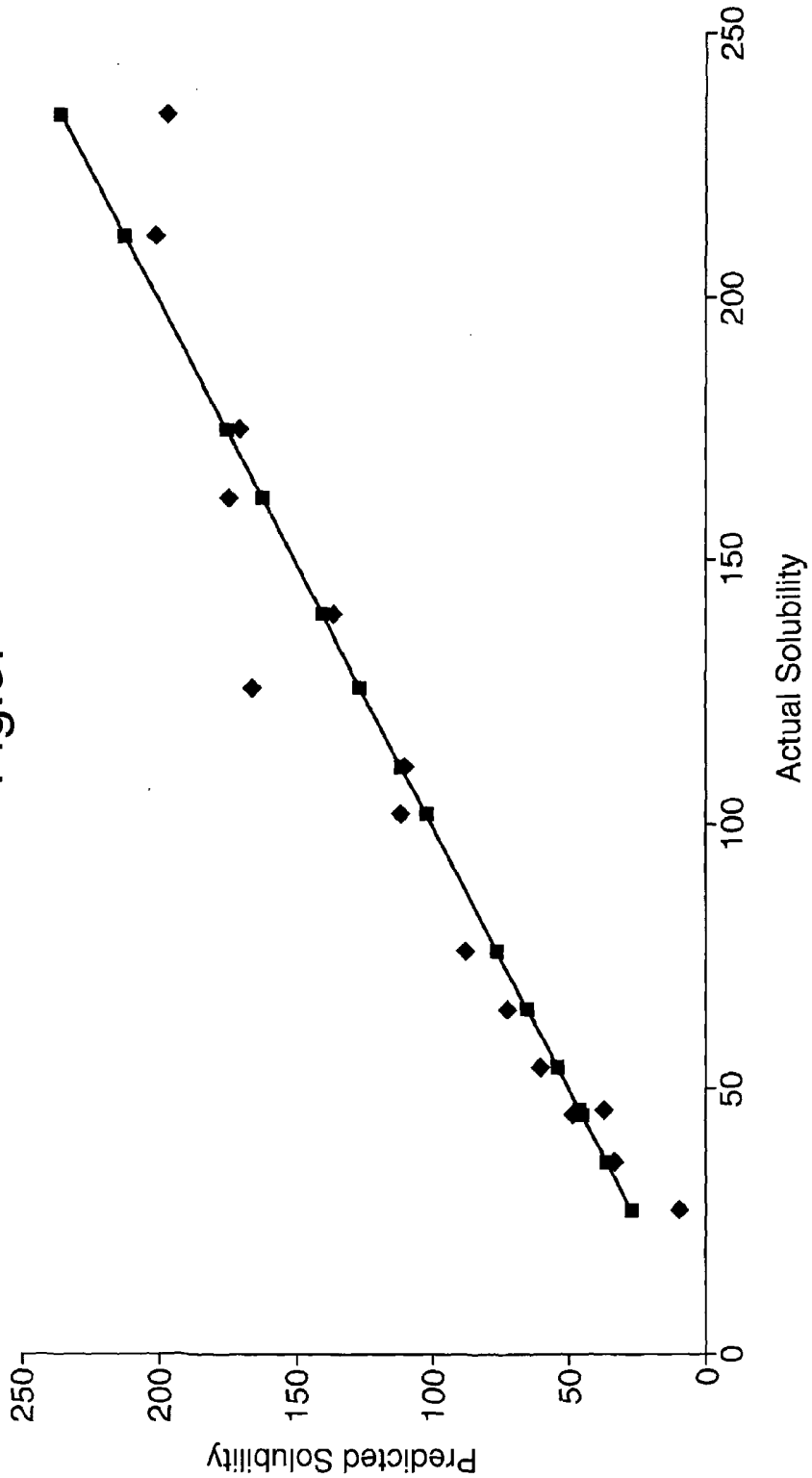


Fig.4.

Fig.5.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50
100
150
200
250
Actual Solubility
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50
100
150
200
250

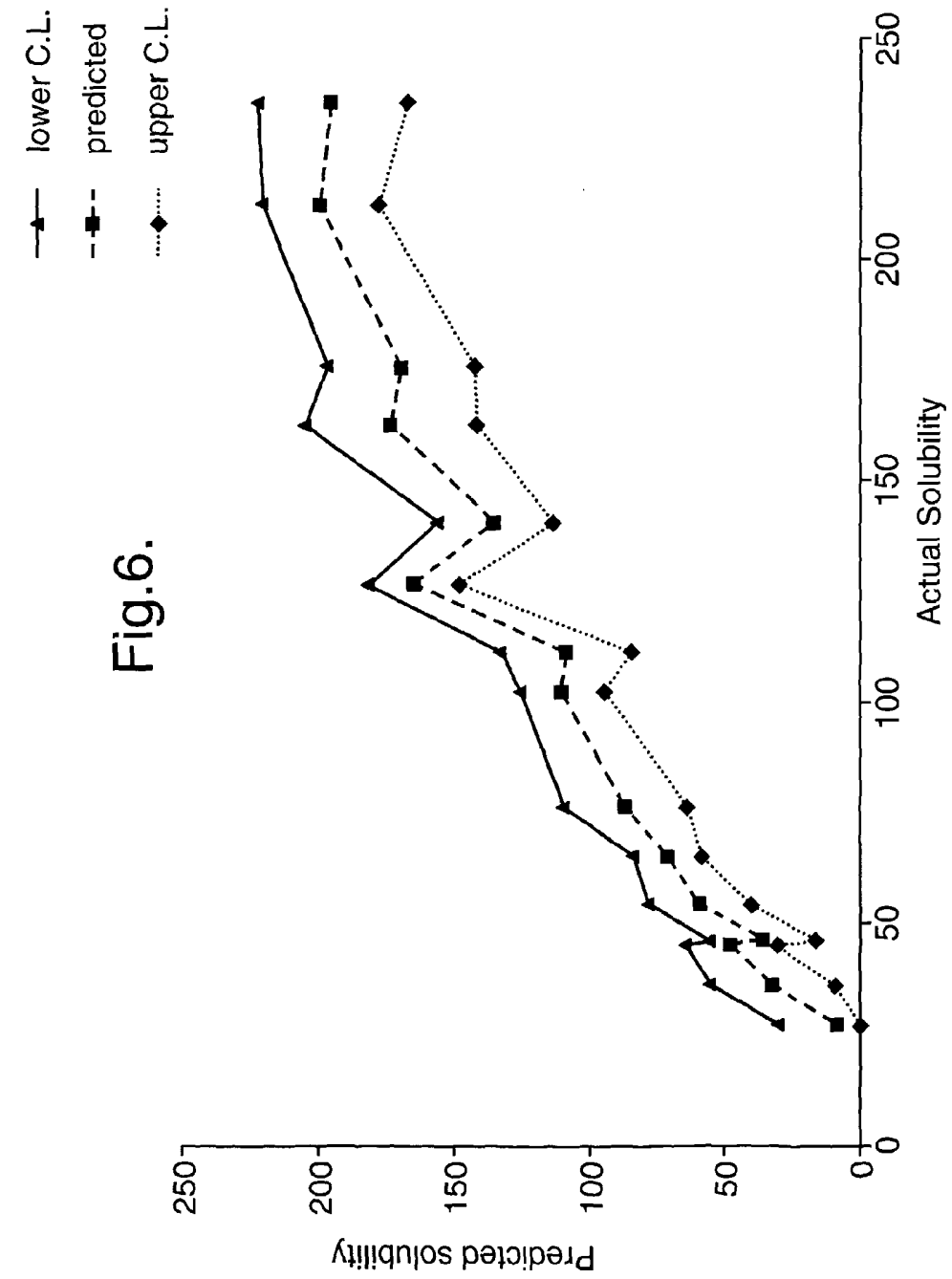
lower C.L.
predicted
upper C.L.
Predicted solubility
Actual Solubility
0
50
100
150
200
250

Fig.6.

INTERNATIONAL SEARCH REPORT

ational Application No
PCT/GB 02/03092

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/14 A61K47/44 A61K47/10 A61K31/565

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)
IPC 7 A61K

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 practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 21440 A (ZENECA) 19 June 1997 (1997-06-19) claims 1,5 examples 1,3,4 page 4, line 15 -page 6, line 30 ---	1-26
Y	DR H. FIEDLER EDITOR: "Lexicon der Hilfstoffe für Pharmazie, Kosmetik und angrenzende Gebiete" 1981 , EDITIO CANTOR AULENDORF , D-7960 AULENDORF XP002221673 page 788 -page 789 ---	1-26
Y	EP 0 346 014 A (IMPERIAL CHEMICAL INDUSTRIES) 13 December 1989 (1989-12-13) page 9; example 3 --- -/--	1-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but 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
20 November 2002	05/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Giacobbe, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOHN C. WATERTON; ET AL.: "A Case of Adenomyosis in a Pigtailed Monkey Diagnosed by Magnetic Resonance Imaging and treated with the Novel Pure Antiestrogen, ICI 182,780" LABORATORY ANIMAL SCIENCE, vol. 43, no. 3, 1993, pages 247-251, XP000998289 page 247, column 2, line 32 - line 35 ---	1-26
P,X	WO 01 51056 A (ASTRAZENECA UK LTD ;EVANS JOHN RAYMOND (GB); GRUNDY ROSALIND URSUL) 19 July 2001 (2001-07-19) page 17, line 5 - line 13 tables 3,4 claims 1-23 ---	1-26
P,X	WO 01 74366 A (THURLIMANN BEAT ;ASTRAZENECA UK LTD (GB); ASTRAZENECA AB (SE)) 11 October 2001 (2001-10-11) page 3, line 20 -page 4, line 8 page 16, paragraph 2.3 - paragraph 2.4 -----	1-26

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/03092

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9721440	A	19-06-1997	AU	1039097 A	03-07-1997
			WO	9721440 A1	19-06-1997
			ZA	9610426 A	23-06-1997
EP 346014	A	13-12-1989	AT	109351 T	15-08-1994
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			CA	1337591 A1	21-11-1995
			DE	68917219 D1	08-09-1994
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			DK	273489 A	07-12-1989
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WO 0151056	A	19-07-2001	AU	2386301 A	24-07-2001
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			EP	1250138 A1	23-10-2002
			FR	2803516 A1	13-07-2001
			WO	0151056 A1	19-07-2001
			GB	2359254 A ,B	22-08-2001
			NL	1017075 C2	25-09-2001
			NL	1017075 A1	11-07-2001
			NO	20023227 A	03-07-2002
			PT	102548 A	31-07-2001
			US	2001020016 A1	06-09-2001
WO 0174366	A	11-10-2001	AU	4437201 A	15-10-2001
			WO	0174366 A1	11-10-2001

PATENT
ATTORNEY DOCKET NO.: **056291-5004-01**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:	)	Confirmation No. **2093**
EVANS et al.	)	
Application No.: **10/872,784**	)	Group Art Unit: **1617**
Filed: **June 22, 2004**	)	Examiner: **Hui, San-Ming R**
FOR: **FORMULATION**	)	

DECLARATION UNDER 35 U.S.C. § 1.132
OF PAUL RICHARD GELLERT

PAUL RICHARD GELLERT of AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK declares:

1. I graduated from the University of Oxford in Chemistry in 1984. I undertook postgraduate research with Professor Brian Howard in the Physical Chemistry Laboratory at the University of Oxford leading to the award of a D.Phil in 1988. From February 1988 until the present I have been employed by AstraZeneca, (formerly Zeneca and ICI) initially as a Senior Research Scientist and subsequently as a Team Leader/Manager, Principal Scientist and, since 2004, a Senior Principal Scientist.

2. I have worked in the formulation and drug delivery area throughout my career with AstraZeneca, where my research and development work has covered a range of formulation types including sustained released injections, including fulvestrant.

3. During the course of my study of the subject application (hereinafter "the Evans Application") and the underlying data, I have become aware of several transcription or other errors between certain disclosures of the subject application and the underlying laboratory notebook data. One purpose of this Declaration is to point out the existence

and nature of these errors and to report further testing that has been carried out under my guidance to obtain additional data (paragraphs 4-10 below and Attachments A-D). A further purpose of this Declaration is to set out and document the manner in which an experienced formulator would likely have approached the task of developing a sustained release injectable formulation suitable for human use for a steroidal compound such as fulvestrant in about early 2000, which I understand is when the priority applications supporting the Evans Application were filed (paragraphs 11 - 25 below and Attachment E). Citations to literature and patent references in this Declaration will be in the format Lead Author (Date), and the full citations are given in the Table of References at the end of this Declaration. A copy of each cited reference (or cited portions of the longer references) is included in Attachment F under the Tab number noted in the Table of References.

4. In Table 2 of the Evans Application, the solubility of fulvestrant in castor oil appears to have been transcribed incorrectly from the original source, the laboratory notebook. The value in the latter is 24.5 mg/ml and not 20 mg/ml. In other experiments to determine the solubility of fulvestrant in castor oil and also in benzyl benzoate, some variability was observed.

5. In Table 3 of the Evans Application, the given solubility values were generated at 4°C and not at 25°C as is stated in the title of Table 3. For fulvestrant formulations, it is preferable that the fulvestrant remains completely in solution at both 4°C and 25°C. The 4°C temperature corresponds to the storage temperature (2°C to 8°C in the FDA approved label for Faslodex), and the 25°C temperature corresponds to the administration temperature (ambient temperature). In addition, the specified solubility values on this Table 3 are mean values calculated from analysis of replicate samples from one or more trials. The individual values are shown in handwriting in the amended version of Table 3 in Attachment A. In addition, it appears that the mean values for the last three compositions have been incorrectly calculated. The corrected mean values, together with the correction of the temperature from "25°C" to read "4°C", are also shown in handwriting in the amended version of Table 3 in Attachment A.

6. I have evaluated the transcription and other errors against the original application disclosures and conclude that these do not change the ultimate conclusions made from the data as originally reported. The addition of 15% w/v benzyl benzoate to compositions having total alcohol concentrations in castor oil of 10%, 15%, 20% and 30% w/v unexpectedly provides a positive effect on fulvestrant solubility, significantly increasing the solubility of fulvestrant in the compositions despite fulvestrant having a lower solubility in benzyl benzoate than in either alcohol or castor oil.

7. An additional set of experiments has been conducted at 25°C under my guidance to obtain consistent data with reduced variability from a single set of rigorously controlled solubility experiments and to demonstrate that the unexpected increase of solubility of fulvestrant by adding benzyl benzoate into compositions containing ethanol, benzyl alcohol and castor oil, is present across the broader range of composition encompassed by the claims being presented with this Declaration. The solubility of fulvestrant in benzyl benzoate and in castor oil was also measured in the same set of experiments using the same batch of benzyl benzoate and the same batch of castor oil as were used to make up the compositions. The Experimental Test Procedure is described in Attachment B.

8. The results from these solubility experiments are shown in the table in Attachment C. These results show that the solubility of fulvestrant in castor oil alone (21.4 mg/ml) is significantly greater than the solubility of fulvestrant in benzyl benzoate alone (3.8 mg/ml) and demonstrate the unexpected increase in fulvestrant solubility on the addition of 10, 15 and 25% w/v benzyl benzoate, in place of an equivalent amount of castor oil, to compositions having total alcohol concentrations in castor oil of 10%, 15%, 20%, 25% and 30% w/v.

9. Thus, the results that were obtained from experiments conducted under rigorously controlled conditions and with an expanded range of compositions, as shown in Attachment C, confirm the ultimate conclusions drawn from the results shown in Table 3 of the original application disclosure, namely that the addition of 10% to 25% w/v benzyl

benzoate to compositions having total alcohol concentrations in castor oil of between 10% to 30% w/v unexpectedly provides a positive effect on fulvestrant solubility, significantly increasing the solubility of fulvestrant in the compositions despite fulvestrant having a lower solubility in benzyl benzoate than in either alcohol or castor oil.

10. During the course of my study of the Evans Application and the underlying source materials it was drawn to my attention that some of the composition data given for Delestrogen and Delalutin somehow had been shifted one column to the right. Thus, for Delestrogen, the 78% and 58% figures shown under the BzBz column should have been under the OIL column; the 20% and 40% figures shown under the BzOH column should have been under the BzBz column; and the 2% figures shown under EtOH should have been under the BzOH column. Similarly for Delalutin, the "up to 2%" shown under the EtOH column should have been under the BzOH column. This table reports that the source of this data was J.Pharm.Sci (1964) 53(8) 891, which is Riffkin (1964) elsewhere referred to in this Declaration, and I have also verified the corrected data from the entries for Delalutin and Delestrogen in PDR (1973). A copy of Table 1 from the Evans Application is reproduced as Attachment D, on which these corrections have been made in handwriting, and I have additionally more correctly noted that Delalutin is 17-hydroxy progesterone *caproate*, and that the "COMP" designation for Delalutin should be "BMS" (Bristol-Myers Squibb). Attachment D also includes a one page explanation of the corrections to this Table 1.

11. In about early 2000, a person responsible for developing a sustained release injectable formulation suitable for administration to humans for a new steroidal compound such as fulvestrant, would have had specialized training and experience in developing pharmaceutical formulations and methods for their administration. In developing such a formulation for fulvestrant, the objective would have been to formulate an intramuscular (IM) injection that would provide for the satisfactory sustained release of fulvestrant over a period of at least two weeks and preferably over a period of at least four weeks to reduce the frequency of administration, and would have a target fulvestrant content of at

least 45 mg/mL so as to provide a fulvestrant dose of at least 250 mg in a single 5-6 mL injection. From my personal experience and knowledge of the literature at about that time, I believe that such an experienced formulator would likely have approached the task of developing a formulation for fulvestrant in about the following manner.

12. Given the foregoing objective, the experienced formulator would have appreciated that the traditional administration options to explore were intramuscular (IM) injection of a sustained release aqueous or oil suspension or an oil-based solution (depot) containing at least 250 mg of fulvestrant in a volume of vehicle that is tolerable for injection, *i.e.*, no more than 5 or 6 mL.

13. Because of the extremely low solubility of fulvestrant in water, a reasonable starting point would have been to investigate intramuscular injection of an aqueous or oil suspension of fulvestrant. However, the formulator would have found that injection of an aqueous suspension of fulvestrant resulted in extensive local tissue irritation at the injection site as well as a poor release profile, such as reported in paragraph [0042] of the Evans Application. Since suspensions thus were not an acceptable option for fulvestrant, the experienced formulator would have moved on to further explore whether 250 mg of fulvestrant could be solubilised in no more than 5-6 mL of an oil-based vehicle, *i.e.*, to achieve the target fulvestrant concentration of at least 45 mg/mL.

14. In the preformulation phase, the experienced formulator would have conducted a literature review or otherwise would have become familiar with commercially marketed injectable formulations, particularly injectable sustained release formulations of steroids or other relatively insoluble compounds such as those listed in Table 1 of the Evans Application, with the objective of identifying potential oil vehicles, co-solvents and other excipients that already had been found to be tolerated and/or to have passed through regulatory review, and which might be candidates for further consideration and testing for the fulvestrant formulation. This review also would have provided guidance with respect to concentration levels of such co-solvents and other excipients that generally had been found acceptable in sustained release oil-based intramuscular injections administered to

humans. This objective is confirmed, for example, in Nema (1997) at page 166:

> Generally, a knowledge of which excipients have been deemed safe by the FDA or are already present in a marketed product provides increased assurance to the formulator that these excipients will probably be safe for their new drug product. ... Regulatory bodies may view an excipient previously approved in an injectable dosage form favorably, and will frequently require less safety data.

The purpose of this Nema paper was thus "to present the various excipients that have been included in the formulation of injectable products marketed in the USA."[1] Similar objectives were intended to be served by the compilations of commercial formulations in Strickley I (1999), Strickley II (2000) and Strickley III (2000):

> This compilation will also be useful for those interested in knowing what additives are currently used in injectable products and at what concentrations they are administered in practice. This compilation only focuses on marketed formulations and does not delve into the subject of preclinical or drug discovery formulations associated with early-stages pharmacokinetics or proof-of-concept pharmacodynamics, where the formulation scientist is not bound by regulatory constraints.

(Strickley I (1999) at 324).

Powell (1998) similarly states at page 238 with respect to its compilation of commercially used excipients:

> Thus, the formulation scientist is often faced with a dilemma -- which excipients are truly available for use (based on what has been used previously), and which are not? ... And at what concentrations, and by what route? ...
>
> Herein are listed the excipients found in most of the approved and marketed parenteral formulations, given systematically by excipient name. In this format it is easy to determine what concentrations were used, the route of administration, the main rationale for addition of that excipient, the drug that was formulated, the manufacturer, brand name, etc.

15. From the literature review, the formulator would have noted reference to a number of intramuscular injectable sustained release oil-based steroidal formulations that had been

[1] Nema (1997) does caution, however, that there is no guarantee that the new drug product will be safe as excipients are combined with other additives and/or with a new drug, creating unforeseen potentiation or synergistic toxic effects.

commercially marketed:

- Strickley I (1999), Table VII:

  Haloperidol Decanoate/Haldol decanoate (50-100 mg/mL in sesame oil, benzyl alcohol 1.2%);

  Testosterone Enanthate/Delatestryl (200 mg/mL in sesame oil, chlorobutanol 5 mg/mL);

- PDR (1973) at pages 1277-1278

  Proluton/progesterone (50 mg/mL in sesame oil, 150 mg/ml benzyl benzoate, 5 mg/ml benzyl alcohol, 1 mg/ml propylparaben);

- PDR (1973) at pages 1349-1354

  Deladumone/Testosterone Enanthate & Estradiol Valerate (90 & 4 mg/mL in sesame oil, 0.5% chlorobutanol);

  Deladumone OB/Testosterone Enanthate & Estradiol Valerate (180 & 8 mg/mL in sesame oil, 2% benzyl alcohol);

  Delalutin/hydroxyprogesterone caproate (250 mg/mL in 52% castor oil, 46% benzyl benzoate, 2% benzyl alcohol);

  Delestrogen/estradiol valerate (20 mg/mL in 78% castor oil, 20% benzyl benzoate, 2% benzyl alcohol and 40 mg/mL in 58% castor oil, 40% benzyl benzoate, 2% benzyl alcohol);

  Delatestryl/Testosterone Enanthate (200 mg/mL in sesame oil, 0.5% chlorobutanol);

  Delaluteval 2X/hydroxyprogesterone caproate & estradiol valerate (250 mg/mL & 5 mg/mL in castor oil, 45% benzyl benzoate, 1.6% benzyl alcohol);

- PDR (1973) at pages 1391-1392

  Prolixin Enanthate/FluphenazineEnanthate (25 mg/mL in sesame oil, 1.5% benzyl alcohol);

- Wang (1980):

  Depo-Testosterone/testosterone cypionate (100 mg/mL in 87.4% cottonseed oil, 0.1 mL benzyl benzoate, 9.45 mg benzyl alcohol as a preservative);

- Mackey (1995):

  Testoviron Depot/testosterone enanthate (250 mg/mL in castor oil and benzyl

benzoate);

as well as a number of other commercialized oil based long-acting IM injectable formulations reported on Table 1 of the Evans Application.

16. As a further part of the preformulation phase, the experienced formulator would have conducted a preformulation solubility screen, separately measuring the solubility of fulvestrant in a range of pure solvents, including the potential oil and co-solvent candidates that had been identified in the above literature review as being suitable for inclusion in intramuscular injection formulations. See, for example, Gupta (1999), Chapter 17 at page 402, under the heading "Formulation Development":

> The activities necessary to develop a parenteral product can be placed into the following three broad areas: preformulation, formulation, and scale-up. While there are alternative development perspectives, all development ultimately needs to accomplish the same activities. Preformulation includes the characteristics of the bulk drug plus initial screening for excipient compatibility with the drug.

"Preformulation studies" are said to "provide fundamental data and experience necessary to develop formulations for a specific compound" including, as item 8.1 in the outline of areas of specific interest, a determination of "solubility" in "selected solvents" (at 403). "Significant formulation activities begin with initial preformulation data and knowledge of the specific route of administration" (at 405), which "formulation activities include the identification and selection of a suitable vehicle (aqueous, nonaqueous or co-solvent system) ..." (at 404). It is further noted that "injection volume is one of the most important considerations in the formulation development of a commercial product" (at 405). When carrying out such a preformulation solubility screen with fulvestrant, the formulator would have found that fulvestrant had extremely low solubility in water, low solubility in most oils (but highest in castor oil), low solubility in benzyl benzoate, and the highest solubility in ethanol and benzyl alcohol, such as reported in Table 2 of the Evans Application.

17. With the information on prior commercialized formulations and the fulvestrant solubility data from the preformulation screen (such as reported in Table 2 of the Evans

Application), the experienced formulator would have selected castor oil as the oil vehicle because of the higher solubility of fulvestrant in castor oil relative to the other oils tested. Nevertheless, he would have appreciated that the target fulvestrant concentration of at least 45 mg/mL could not be achieved with castor oil alone, and that a co-solvent would be required.

18. A number of the commercialized formulations that would have been identified in the literature review (including the castor oil-based formulations) have a substantial benzyl benzoate component, which may be present as a co-solvent. See, for example, Delalutin noted in paragraph 15 above, which is reported in PDR (1973) and noted in Table I of the Evans Application, and is one of the formulations discussed in Riffkin (1964), "Castor Oil as a Vehicle for Parenteral Administration of Steroid Hormones" (see Riffkin n. 6). Delalutin is 250 mg/mL 17-hydroxyprogesterone caproate dissolved in 52% castor oil, 46% benzyl benzoate and 2% benzyl alcohol. However, Riffkin Table II reports that the solubility of 17-hydoxyprogesterone caproate in castor oil alone is only 55.6 mg/mL, but the solutility of 17-hydroxyprogesterone caproate in benzyl benzoate is substantially higher, being at least 250 mg/mL (see example 4 of Huber (US '520) and Attachment E discussed below). Even if not needed as a cosolvent, Riffkin (1964) notes that "the addition of benzyl alcohol or benzyl benzoate to castor oil resulted in a lower and more favorable viscosity, making it easier to inject" (paragraph bridging pages 893-894).

19. However, the skilled formulator would have appreciated from the fulvestrant solubility data generated in the preformulation screen that fulvestrant had very different solubility characteristics relative to the steroids of previous commercial formulations. Attachment E is a compilation showing the chemical structures and relative solubilities in castor oil and sesame oil of the compounds named in Riffkin (1964) Table II compared to the structure and the solubility of fulvestrant in these oils. It can be seen that the solubility of fulvestrant in castor oil and in sesame oil (20 mg/mL and 0.58 mg/mL, respectively, from Table 2 of the Evans Application) is appreciably lower than the solubility of the other steroids in these oils (taken from Table II of Riffkin (1964)). The second page of Attachment E tabulates the concentration in benzyl benzoate of five named steroids, taken

from Examples 1-5 of Huber (US '520), ranging from 200 to 400 mg/ml.[2] By comparison, the solubility of fulvestrant in benzyl benzoate is reported in Table 2 of the Evans Application as being only 6.15 mg/mL, and only 3.8 mg/mL as determined in the recently conducted tests reported in Attachment C.

20. The experienced formulator thus would have expected that benzyl benzoate would *not* act as a co-solvent for fulvestrant in castor oil because the solubility of fulvestrant in benzyl benzoate was significantly lower than its solubility in castor oil. The addition of benzyl benzoate to castor oil, for whatever reason, would have been expected to *decrease, rather than increase*, the solubility of fulvestrant in the resulting castor oil/benzyl benzoate mixture. This is confirmed in Table 4 of the Evans Application, which reports a fulvestrant solubility of only 12.6 mg/mL in the castor oil vehicle containing only 15% benzyl benzoate, compared to the 20 mg/mL solubility of fulvestrant in castor oil alone as reported in Table 2.[3]

21. Based on the solubility data determined in the preformulation screen (such as reported in Table 2 of the Evans Application), ethanol and/or benzyl alcohol would have been seen as the best co-solvent candidates for raising the fulvestrant solubility to the 45 mg/mL target in the castor oil vehicle, and would also function to lower the viscosity of the resulting formulation and make it easier to inject. Consistent with this solubility data, Dukes (US '814) added 40% w/v benzyl *alcohol* in order to dissolve 50 mg/mL fulvestrant in the castor oil-based formulation used in the experimental rat studies of his Example 3. It thus would have been apparent that 40% w/v benzyl alcohol could function as a co-solvent in castor oil to achieve the target fulvestrant concentration. Nevertheless, the skilled formulator would have been concerned with using such a high alcohol content in intramuscular injectable formulations for administration to a human.

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[2] Data taken from the Examples of Huber (US '520); these are concentrations used in the examples and not necessarily the actual maximum solubility of each steroid in benzyl benzoate, which may be higher. Huber was a co-author on Riffkin (1964).

[3] It should be noted that in the further tests that were recently conducted under my guidance (paragraphs 7-9 above and Attachments B and C hereto), the solubility of fulvestrant in castor oil alone was again tested and found to be 21.4 mg/mL, and the solubility of fulvestrant in benzyl benzoate alone was again tested and found to be only 3.8 mg/mL, which further confirms that benzyl benzoate would not be expected to act as a cosolvent for fulvestrant in castor oil.

22. First of all, the experienced formulator would want to minimize the amount of co-solvents and excipients in any injectable formulation. For example, as stated in Gupta (1999), Chapter 17, "Formulation and Administration Techniques to Minimize Injection Pain and Tissue Damage Associated with Parental Products" at page 414:

> Cosolvents are commonly used to enhance drug solubility and stability. Cosolvents may include ethanol, propylene glycol, polyethylene glycols, and glycerine. These components have intrinsic effects on biologic tissue and can alter the properties of other excipients, thus influencing the tissue damage or pain caused by a product. There is a dearth of literature on the pain caused by cosolvents, but there is also a growing body of knowledge on the tissue damage that they can cause. It is not certain that tissue damage is always directly correlated with the injection pain, but minimization of both pain on injection and potential for tissue damage should be included in the product development plan.

See also Gupta (1999), Chapter 11, titled Cosolvent Use in Injectable Formulations, page 217:

> Ideally, it is best to select and use solvents that would maximize the solubility of the compound. Maximizing the solubility of a compound in a particular cosolvent system would result in lower total levels of the non-aqueous solvent(s) being administered to the patient, thereby lowering the chance for potential side effects.

This objective would have applied to aqueous and oil-based systems alike, in that the precedent of commercialized formulations identified in the literature review would have confirmed that fixed oils, such as castor oil, have long been commercially used and accepted as the major component of oil-based sustained release intramuscular injectable steroidal formulations. On the other hand, co-solvents such as ethanol or benzyl alcohol have generally been used only in far lesser concentrations, as discussed in the following paragraph.

23. Thus, use of such a high content of either benzyl alcohol or ethanol would have been contrary to precedent as shown from the review of commercialized oil-based intramuscular injectable sustained release formulations. The literature review as of early 2000 would have shown that any benzyl alcohol in such formulations was almost always

present as a preservative in a concentration of about 2% or less, occasionally at a concentration of up to 5%, but only rarely at higher concentrations. With respect to benzyl alcohol see, for example:

- Gupta (1999), Chapter 11 at page 229 stating that benzyl alcohol "is typically used in concentrations of up to 2 percent as a preservative and up to 5 present as a solvent," and then discussing reported toxicities.
- Nema (1997), Table V at page 168, reporting that benzyl alcohol was present as an antimicrobial preservative in 74 injectable formulations (not limited to oil-based IM formulations) at concentrations of from 0.75-5% (note that benzyl alcohol is not included at all in Nema Table I, "Solvents and Co-solvents";
- Powell (1998), the benzyl alcohol listing at pages 244-246, particularly those indicated as being used in IM formulations;
- Strickley I (1999) at page 329 notes the inclusion of 2% benzyl alcohol in an IM lorazapam formulation in a propylene glycol vehicle, but does not include benzyl alcohol at all in Table VI listing "Cosolvents Used in Parenteral Formulations;"
- Lopatin (1972) noting in Table 3 at page 727 opposite Benzyl alcohol, "Toxic. Used in concentration of not over 3%. Has irritant action in concentration of 5%;"
- Cornelius (US '863), col. 1, lines 30-35 stating, "It is known that the solubility of steroids in vegetable or animal oils can be increased by the addition of excipients such as benzyl alcohol and benzyl benzoate. An objection to the use of such excipients, and specifically benzyl alcohol in somewhat higher concentrations, is that these agents may irritate the tissues."

The literature review as of early 2000 also would have shown that, with few exceptions, ethanol was not included in such formulations in excess of about 10%. See, for example:

- Gupta (1999), Chapter 11 at page 225 noting that ethanol has been used at levels up to 50 percent, but these levels typically are associated with pain on injection;
- Strickley I (1999), Table VI, "List of Cosolvents Used in Parenteral Formulations" more specifically lists the ethanol content in IM formulations for specifically identified drugs, which concentrations range only from 2.5 to 10%; an IM/IV lorazapam formulation in a propylene glycol vehicle is noted at page 329 as having 18% alcohol, but is not included with the IM formulations in Table VI;

- Nema (1997), Table I, "Solvents and Co-solvents" at page 167, lists ethanol as being in 24 formulations with a concentration range of 0.6-80% (for Prograf); note that this is misleading, however, since Prograf is a *concentrate* for intravenous infusion only, and is to be diluted 250 to 1000 times before administration;
- Powell (1998), lists "alcohol" at page 242 and "ethyl alcohol" at page 255, wherein the ethanol concentration for IM formulations ranges from 0.61-10%.

24. Thus, even though Dukes (US '814) had demonstrated that the target 45 mg/mL fulvestrant concentration could be achieved by adding 40% benzyl alcohol to the castor oil vehicle, the precedent of commercialized IM oil-based systems would have motivated the experienced formulator to substantially reduce the benzyl alcohol content of the formulation intended for human use, and this commercial precedent would have made him very reluctant to replace benzyl alcohol with the substantial amount of ethanol that would be needed to maintain the target fulvestrant concentration. Benzyl benzoate clearly would not be considered to solve this dilemma, but rather would be expected to have a negative effect on fulvestrant solubility since fulvestrant was even less soluble in benzyl benzoate than in castor oil, that is, one would have expected that adding benzyl benzoate would require still *more* alcohol to maintain the target fulvestrant concentration.[4]

25. Under these circumstances, the discovery by Evans *et al.*, that the addition of benzyl benzoate to the castor oil/alcohol mixture actually increases the solubility of fulvestrant such that more fulvestrant could be dissolved in a given volume of formulation, was unexpected and truly surprising. This positive benzyl benzoate effect on fulvestrant solubility in the resulting formulation is shown in Table 3 of the specification (and is not changed by the above-noted corrections), and is confirmed and demonstrated over a broader range of formulation composition by the additional set of experiments conducted under my guidance and discussed in paragraphs 7-9 above, the results of which are reported in Attachments C.

[4] It should be noted that even apart from this solubility issue, there would have been no motivation to add benzyl benzoate for viscosity reduction since the significant quantity of alcohol would serve the dual function of acting as a co-solvent as well as reducing the injection viscosity and making it easier to inject, whereas the benzyl benzoate would be expected to have a negative effect on the fulvestrant solubility.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punished by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Signature: P. R. Mulick.

Date: 8th August 2008.

TABLE OF REFERENCES

Tab	Author/Inventor	Reference Citation/Patent
1	Cornelius (US '863)	US Patent 4,212,863
2	Dukes (EP '014)	EP 0 346 014 A1 (corresponds to US Patent 5,183,814)
3	Dukes (US '814)	US Patent 5,183,814 (corresponds to EP 0 346 013 A1)
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5	Huber (US '520)	US Patent 3,164,520
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8	Nema (1997)	S. Nema, R.J. Washkuhn, and R.J. Brendel. Excipients and their use in injectable products. *PDA J. Pharm. Sci. Technol.* **51**:166-71 (1997)
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14	Strickley III (2000)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part III. *PDA J. Pharm. Sci. Technol.* **54**:152–169 (2000)
15	Wang (1980)	Y.C. J. Wang and R. R. Kowal. Review of excipients and pH's for parenteral products used in the United States. *J. Parenteral Drug Assoc.* **34**:452–462 (1980).

ATTACHMENT A

TABLE 3

EFFECT OF BENZYL BENZOATE ON FULVESTRANT SOLUBILITY IN CASTOR OIL AT 25°C 4°C

		% w/v							
	Ethanol (96%)	5	5	10	10	10	10	15	15
	Benzyl Alcohol	5	5	5	5	10	10	15	15
	Benzyl Benzoate		15		15		15		15
	Castor Oil	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100
Mean	Fulvestrant Solubility [mgml^{-1}]	27	36	46	54	45	68 64	78 74	102 103
	Individual values [mgml^{-1}]	27.8 25.8	35.5 36.1	54.0 38.0	64.1 49.3 58.0 50.3	49.4 41.3	68.9 66.8 63.2 92.9	65.8 86.9 90.0 73.4	80.6 101.9 121.6 103.4

ATTACHMENT B:

Experimental Test Procedure for measuring the solubility of fulvestrant in different solvent vehicles at 25°C

1. Solvent vehicles for the solubility experiments were prepared by weighing the required amount of benzyl benzoate, benzyl alcohol and ethanol into a 20 ml volumetric flask and then diluting to volume with castor oil.
2. For each solvent vehicle in which the solubility of fulvestrant was to be determined, 1.0-1.5g of fulvestrant was weighed into each of 3 separate vials (2 dram size) and 5mls of the solvent vehicle was added to each vial, except for the pure castor oil vehicle, where 80mg of fulvestrant were weighed into each of the 3 separate vials and 2mls of the castor oil added to each vial. The reduced amount of fulvestrant and lower volume of solvent vehicle was needed to maintain stirring and achieve adequate mixing with the pure castor oil vehicle due to the combination of its higher viscosity and lower fulvestrant solubility/higher undissolved fulvestrant levels compared to the other solvent vehicles.
3. A magnetic stirrer bar was placed into each vial and the vials were capped and then placed on a magnetic stirrer block maintained at 25 ± 0.5°C.
4. After 5 days of stirring at 25 ± 0.5°C, an aliquot of each fulvestrant/solvent vehicle mixture was removed from each vial and placed into an Eppendorf tube which was then centrifuged at 12000 rpm for 5 minutes at ambient temperature.
5. For all but the fulvestrant/castor oil mixture, 1 ml of the supernatant was then removed from the Eppendorf tube and pipetted into a 10ml or 20ml volumetric flask and then diluted to volume with methanol and mixed to give a sample for analysis. The choice of whether to use a 10ml or 20ml volumetric flask for a particular sample was dependent on the likely concentration of fulvestrant in the sample and the quantifiable concentration range of the HPLC assay method used. For the fulvestrant/castor oil mixture, 100µl of the supernatant was removed from the Eppendorf tube and pipetted into a 1ml volumetric flask and then diluted to volume with methanol and mixed to give a sample for analysis.
6. Step 5 was repeated to give a duplicate sample for analysis. Thus, this gave 2 samples for each of the 3 vials, giving a total of 6 samples for analysis for each solvent vehicle tested.
7. The resultant samples were analysed for fulvestrant content by reverse phase High

Performance Liquid Chromatography (HPLC). The HPLC method that was used is described below at point 9. The fulvestrant content obtained for each sample was used to calculate a value for the concentration of fulvestrant dissolved in the corresponding solvent vehicle after stirring for 5 days at 25°C.

8. The mean solubility of fulvestrant for each different solvent vehicle tested was calculated as the arithmetic mean of the 6 individual values for the concentration of fulvestrant dissolved in the corresponding solvent vehicle.
9. HPLC Method details:

Gradient HPLC Method

Eluent A : 27% Methanol / 32% Acetonitrile / 41% Water

Eluent B : 41% Methanol / 49% Acetonitrile / 10% Water

Column : 15cm 3.5um Symmetry C8 4.6mm i.d.

Detection wavelength : 225 nm

Flow rate : 2 mL min-1

Temperature : 40°C

Injection volume : 10 µL

Gradient programme :

Time (min)	Eluent A (%)	Eluent B (%)
0	100	0
25	100	0
55	0	100
65	0	100
66	100	0
70	100	0

Retention time of fulvestrant: 21minutes approximately

ATTACHMENT C: EFFECT OF BENZYL BENZOATE ON FULVESTRANT SOLUBILITY IN CASTOR OIL AT 25°C

	% w/v																					
Ethanol (96%)	0	0	5	5	5	5	10	10	10	10	10	10	10	10	12.5	12.5	12.5	12.5	15	15	15	15
Benzyl Alcohol	0	0	5	5	5	5	5	5	5	5	10	10	10	10	12.5	12.5	12.5	12.5	15	15	15	15
Benzyl Benzoate	0	100		10	15	25		10	15	25		10	15	25		10	15	25		10	15	25
Castor oil	100	0	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100	to 100
Mean Fulvestrant solubility [mgml^{-1}]	**21.4**	**3.8**	**27.6**	**29.2**	**43.3**	**47.5**	**64.6**	**71.6**	**84.2**	**94.0**	**68.1**	**87.2**	**93.4**	**118.9**	**96.6**	**107.7**	**116.1**	**139.6**	**121.3**	**144.6**	**143.8**	**166.2**
Individual values [mgml^{-1}]	23.2	3.9	29.5	31.2	43.9	48.3	64.2	76.2	83.8	95.2	68.6	90.0	92.5	122.1	104.1	106.1	115.5	138.9	110.0	129.8	148.2	163.3
	17.8	4.0	28.3	26.3	45.1	50.7	66.8	72.1	81.9	97.8	68.9	84.9	92.1	120.3	74.0	86.6	117.9	141.0	120.0	133.5	147.1	164.8
	21.5	3.9	24.5	31.5	44.3	45.4	61.2	66.2	93.2	95.6	71.6	87.6	93.9	120.4	102.0	112.6	118.8	139.4	124.4	150.1	144.4	168.5
	21.8	3.8	26.6	29.3	45.4	45.2	66.0	65.7	84.6	96.1	67.6	88.1	93.0	118.3	98.6	117.9	116.1	142.1	125.6	151.7	144.4	169.7
	22.2	4.0	27.0	29.1	36.9	47.6	65.8	75.4	82.4	88.2	67.0	90.7	93.8	116.8	102.1	107.9	117.0	138.7	123.3	151.2	139.5	165.5
	22.0	3.2	29.6	27.8	44.3	47.6	63.6	73.9	79.1	91.0	64.8	82.1	95.3	115.7	98.4	115.1	111.5	137.9	124.6	151.1	139.1	165.5

ATTACHMENT D

TABLE 1

OIL BASED LONG-ACTING INTRAMUSCULAR INJECTIONS

PRODUCT NAME	STEROID	DOSE	TYPE	COMP.	SOURCE	OIL	BzBz	BzOH	EtOH	DOSE	DOSING
SUSTANON 250	Testosterone propionate	30 mg	Androgen	Organon	ABPI Data Sheet Comp. 1998	Arachis		0.1 ml		1 ml	3 weeks
	Testosterone phenyl-propionate	60 mg									
	Testosterone isocaproate	60 mg									
	Testosterone decanoate	100 mg									
PROLUTON DEPOT	Hydroxy progesterone hexanoate	250 mg ml^{-1}	Progestogen	Schering HC	ABPI Data Sheet Comp. 1998	Castor	up to 46%			1 or 2 ml	1 week
TOCOGESTAN	Hydroxy progesterone enantate	200 mg	Progestogen	Theramex	Dict. Vidal 1999	Ethyl oleate	*40%			2 ml	<1 week
	Progesterone	50 mg									
	Tocopherol	250 mg									
TROPHOBOLENE	Estrapronicate	1.5 mg	Mixed	Theramex	Dict. Vidal 1997	Olive	40%			1 ml	15 to 30 days
	Nandrolone undecanoate	50 mg									
	Hydroxy-progesterone heptanoate	80 mg									
NORISTERAT	Norethisterone enanthate	200 mg	Contra-ceptive	Schering HC	ABPI Data Sheet Comp. 1998	Castor	YES			1 ml	8 weeks
BENZO-GYNOESTRYL	Estradiol hexahydro-benzoate	5 mg	Estradiol	Roussel	Dict. Vidal 1998	Arachis				1 ml	1 week
PROGESTERONE-RETARD	Hydroxy progesterone caproate	250 mg ml^{-1}	Progestogen	Pharlon	Dict. Vidal 1999	Castor	YES			1 or 2 ml	1 week
GRAVIBINAN	Estradiol 17-β-valerate	5 mg ml^{-1}	Mixed	Schering HC	Dict. Vidal 1998	Castor	YES			1 or 2 ml	1-2 weeks
	Hydroxy-progesterone caproate	250 mg ml^{-1}									
PARABOLAN	Trenbolone	76 mg	Androgen	Negma	Dict. Vidal 1997	Arachis		76 mg	45 mg	1.5 ml	2 weeks
DELESTROGEN	Estradiol valerate	20 mg ml^{-1} 40 mg ml^{-1}	Estradiol	BMS	J Pharm. Sci (1964) 53(8) 891	Castor 99% 55%	~~20%~~ ~~35%~~ 20% 40%	~~20%~~ ~~40%~~ 2% 2%	~~2%~~ ~~2%~~		
DELALUTIN	17-Hydroxy progesterone caproate	250 mg ml^{-1}	Progestogen	~~DMS~~ BMS	J Pharm. Sci (1964) 53(8) 891	Castor	YES	YES up to 2%	~~up to 2%~~		

BzBz = benzylbenzoate
BzOH = benzylalcohol
EtOH = ethanol Dict.
Vidal = Dictionnaire Vidal % are w/v and
*approximate as measured directly from a single sample

Corrections to Table 1

In Table 1, the given values for the benzyl benzoate, benzyl alcohol and ethanol levels for the Delestrogen and Delalutin products have been incorrectly entered into the wrong columns. The entries are shown in their correct form in the attached corrected version of Table 1. The error is apparent from a review of the reference J.Pharm Sci (1964) 53 (8) 891 (Riffkin) which is stated in Table 1 as being the Source of the information for the Delestrogen and Delalutin products:

- In the Summary on page 895 of Riffkin, Delestrogen and Delalutin are identified as castor oil based commercially available products containing estradiol valerate at 20 & 40 mg/ml and 17-hydroxy-progesterone caproate at 250 mg/ml respectively.
- Furthermore, details of particular vehicle compositions for estradiol valerate and 17-hydroxy-progesterone caproate are given in Tables V and VI

    - In Table VI, the only 20 mg/ml formulation of estradiol valerate, also referred to as commercially available, has the composition castor oil 78%, benzyl benzoate 20% and benzyl alcohol 2%.
    - In Table VI, the only 40 mg/ml castor oil based formulation of estradiol valerate, has the composition castor oil 58%, benzyl benzoate 40% and benzyl alcohol 2%.
    - In Table V, there are three 250/mg/ml castor oil based formulations of 17-hydroxy-progesterone caproate that all contain benzyl benzoate. Two of these formulations also contain 2% benzyl alcohol and the other formulation does not contain benzyl alcohol ie they all contain up to 2% benzyl alcohol.

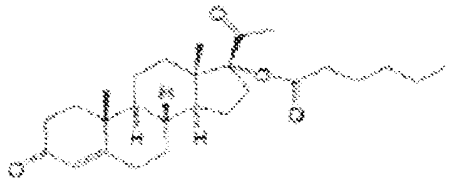
- None of the vehicle compositions disclosed in Tables V and VI in Riffkin contain ethanol. Therefore the entries in the Ethanol column of Table 1 for the Delestrogen and Delalutin products must have been incorrectly entered in the wrong column and should have been entered into the Benzyl Alcohol column.
- It is also apparent from Table VI that the 78% and 58% entries in the Benzyl Benzoate column of Table 1 for the Delestrogen products should have been entered into the Oil column and the 20% and 40% entries in the Benzyl Alcohol column should have been entered into the Benzyl Benzoate column
- The exact compositions for the Delestrogen and Delalutin products are confirmed in the Physicians Desk Reference (Edition 27, 1973) on page 1352.

In addition, the name of the steroid given in Table 1 for the Delalutin product should have been 17-hydroxy-progesterone caproate and not just 17-hydroxy-progesterone. Also the entry under the Company column for the same product should read BMS rather than DMS.

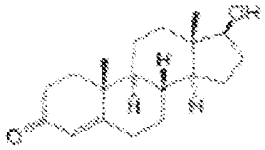
ATTACHMENT E

Structure of compounds disclosed in Riffkin et al.

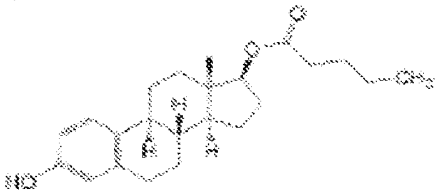
17-Hydroxyprogesterone caproate:



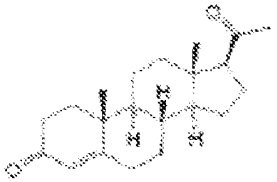
Testosterone:



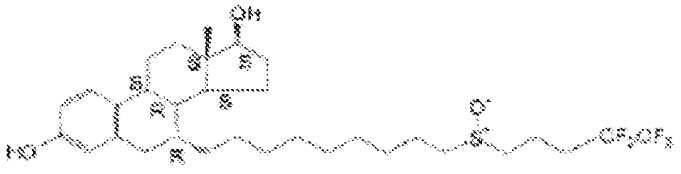
Estradiol valerate:



Progesterone:



On the other hand, fulvestrant has the following structure:



From Riffkin et al. Table II:

Steroid	Solubility [mg/ml] at 25°C	
	Castor oil	Sesame oil
Fulvestrant	20	0.58
17-Hydroxyprogesterone caproate	55.5	23.4
Testosterone	38.6	5.4
Estradiol valerate	60.6	16.1
Progesterone	52.0	22.9

Tabulation of data from Examples of Huber, 3,164,520:

Example	Steroid	Steroid concentration in benzyl benzoate (mg/ml)
1	16,17-dihydroxyprogesterone	200
2	testosterone palmitate	200
3	progesterone	250
4	Progesterone + 17-hydroxyprogesterone caproate	250 + 250
5	Testosterone enanthate	400

TABLE OF REFERENCES

Tab	Author/Inventor	Reference Citation/Patent
1	Cornelius (US '863)	US Patent 4,212,863
2	Dukes (EP '014)	EP 0 346 014 A1 (corresponds to US Patent 5,183,814)
3	Dukes (US '814)	US Patent 5,183,814 (corresponds to EP 0 346 013 A1)
4	Gupta (1999)	P.K. Gupta and G.A. Brazeau (eds). *Injectable Drug Development:Techniques to Reduce Pain and Irritation.* Chapters 11 & 17 Interpharm Press, Denver, Colorado (1999)
5	Huber (US '520)	US Patent 3,164,520
6	Lopatin (1972)	P.V. Lopatin, V. P. Safonov, T. P. Litvinova and L. M. Yakimenko. Use of nonaqueous solvents to prepare injection solutions. *Pharm. Chem. J.* ***6***:724-733 (1972)
7	Mackey (1995)	M.A. Mackey, A.J. Conway and D.J. Handelsman. Tolerability of intramuscular injections of testosterone ester in oil vehicle. *Hum. Reprod.***10**: 862-865 (1995)
8	Nema (1997)	S. Nema, R.J. Washkuhn, and R.J. Brendel. Excipients and their use in injectable products. *PDA J. Pharm. Sci. Technol.* **51**:166-71 (1997)
9	PDR (1973)	*Physicians' Desk Reference (27th edition).* 1277-1278, 1350-1354, 1391-1392 Medical Economics Company, Oradell, NJ (1973)
10	Powell (1998)	M. F. Powell, T. Nguyen, and L. Baloian. Compendium of excipients for parenteral formulations. *PDA J. Pharm. Sci. Technol.* **52**:238–311 [pages 238-255 provided] (1998)
11	Riffkin (1964)	C. Riffkin, R. Huber and C.H. Keysser. Castor oil as a vehicle for parenteral adminstation of steroid hormones. *J.Pharm.Sci.* **53**: 891-5 (1964)
12	Strickley I (1999)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) -Part I. *PDA J. Pharm. Sci. Technol.* **53**:324–349 (1999)
13	Strickley II (2000)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part II *PDA J. Pharm. Sci. Technol.* **54**:69–96 (2000)
14	Strickley III (2000)	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part III. *PDA J. Pharm. Sci. Technol.* **54**:152–169 (2000)
15	Wang (1980)	Y.C. J. Wang and R. R. Kowal. Review of excipients and pH's for parenteral products used in the United States. *J. Parenteral Drug Assoc.* **34**:452–462 (1980).

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/285,887	Filing Date 10/15/2008	☐ To be Mailed

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☐		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **01/17/2012**

	(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 20	Minus	** 30	= 0	X $ =		OR	X $60=	0
Independent (37 CFR 1.16(h))	* 2	Minus	***8	= 0	X $ =		OR	X $250=	0
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1)		(Column 2)	(Column 3)					
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))									
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/DESHONNE T. MARTINO/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

AMENDMENTS TO THE CLAIMS

Please amend claims 24, 32, 34, 36, 44, and 46. Please add new claims 54-57. Please cancel claims 25, 28, 31, 33, 37, 40, 43, 45, and 48-53 without prejudice or disclaimer. This listing of claims will replace all prior versions and listings of claims in the application.

Claims 1-23 (Cancelled)

24. (Currently amended) A method for treating a hormonal dependent benign or malignant disease of the breast or reproductive tract comprising administering intramuscularly to a human in need of such treatment a formulation comprising:

~~at least 45 mgml^{-1} of fulvestrant;~~

~~a mixture of from 17 – 23% w/v of ethanol and benzyl alcohol;~~

~~12 – 18% w/v of benzyl benzoate; and~~

about 50 mgml^{-1} of fulvestrant;

about 10% w/v of ethanol;

about 10% w/v of benzyl alcohol;

about 15% w/v of benzyl benzoate; and

a sufficient amount of castor oil vehicle;

wherein the method achieves a therapeutically significant blood plasma fulvestrant concentration of at least 2.5 ngml^{-1} for at least ~~two~~ four weeks.

25. (Cancelled)

26. (Previously presented) The method of claim 24, wherein the therapeutically significant blood plasma fulvestrant concentration is at least 8.5 $ngml^{-1}$.

27. (Previously presented) The method of claim 24, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

28. (Cancelled)

29. (Previously presented) The method of claim 24, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

30. (Previously presented) The method of claim 24, wherein the method further comprises once monthly administration of the formulation.

31. (Cancelled)

32. (Currently amended) The method of ~~claim 31~~ claim 26, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

33. (Cancelled)

34. (Currently amended) The method of ~~claim 33~~ claim 32, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

35. (Previously presented) The method of claim 34, wherein the method further comprises once monthly administration of the formulation.

36. (Currently amended) A method for treating a hormonal dependent benign or malignant disease of the breast or reproductive tract comprising administering intramuscularly to a human in need of such treatment a formulation consisting essentially of:

~~at least 45 mgml^{-1} of fulvestrant;~~

~~a mixture of from 17 – 23% w/v of ethanol and benzyl alcohol;~~

~~12 – 18% w/v of benzyl benzoate; and~~

~~a sufficient amount of castor oil vehicle;~~

about 50 mgml^{-1} of fulvestrant;

about 10% w/v of ethanol;

about 10% w/v of benzyl alcohol;

about 15% w/v of benzyl benzoate; and

wherein the method achieves a therapeutically significant blood plasma fulvestrant concentration of at least 2.5 ngml^{-1} for at least ~~two~~ four weeks.

37. (Cancelled)

38. (Previously presented) The method of claim 36, wherein the therapeutically significant blood plasma fulvestrant concentration is at least 8.5 ngml^{-1}.

39. (Previously presented) The method of claim 36, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

40. (Cancelled)

41. (Previously presented) The method of claim 36, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

42. (Previously presented) The method of claim 36, wherein the method further comprises once monthly administration of the formulation.

43. (Cancelled)

44. (Currently amended) The method of ~~claim 43~~ claim 38, wherein the hormonal dependent benign or malignant disease of the breast or reproductive tract is breast cancer.

45. (Cancelled)

46. (Currently amended) The method of ~~claim 45~~ claim 44, wherein the method comprises administering intramuscularly to a human in need of such treatment 5 mL of the formulation.

47. (Previously presented) The method of claim 46, wherein the method further comprises once monthly administration of the formulation.

Claims 48-53 (Cancelled)

54. (New) The method according to claim 24, wherein the formulation is administered in a divided dose.

55. (New) The method according to claim 35, wherein the formulation is administered in a divided dose.

56. (New) The method according to claim 36, wherein the formulation is administered in a divided dose.

57. (New) The method according to claim 47, wherein the formulation is administered in a divided dose.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	10/15/2008	John R. Evans	11285.0056-00000	1199

22852 7590 03/20/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

EXAMINER	
HUI, SAN MING R	
ART UNIT	PAPER NUMBER
1628	

MAIL DATE	DELIVERY MODE
03/20/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/285,887	EVANS ET AL.	
	Examiner	Art Unit	
	SAN-MING HUI	1628	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 January 2012*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *24,26,27,29,30,32,34-36,38,39,41,42,44,46,47 and 54-57* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *24, 26-27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46-47, and 54-57* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/2011, 1/17/12*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 1/17/2012 has been entered.

Claims 24, 26-27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46-47, and 54-57 are pending.

The outstanding rejection under 35 USC 103(a) is withdrawn in view of the arguments along with the declaration of Dr. Sawchuk filed 1/17/2012.

However, in view of the decision dated 2/15/2012 with regard to the terminal disclaimer, the obviousness double patenting rejections are maintained with the new added claims are also rejected.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims

are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 24, 26-27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46-47, and 54-57 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-9 of U.S. Patent No. 6,774,122 (‘122). Although the conflicting claims are not identical, they are not patentably distinct from each other because '122 teaches the method of treating hormonal dependent benign or malignant disease of reproductive tract by employing the herein claimed composition. The ratio of

the solvents and the excipients are within the range taught in '122. The optimization of result effect parameters (e.g., dosing regimen [single dosing or multiple divided dosing], weight ratio of the actives and the excipients) is obvious as being within the skill of the artisan. The optimization of known effective amounts of known active agents to be administered, is considered well in the competence level of an ordinary skilled artisan in pharmaceutical science, involving merely routine skill in the art. It has been held that it is within the skill in the art to select optimal parameters, such as amounts of ingredients, in a composition in order to achieve a beneficial effect. See *In re Boesch*, 205 USPQ 215 (CCPA 1980). It is also noted that "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Claims 24, 26-27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46-47, and 54-57 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 7,456,160 ('160). Although the conflicting claims are not identical, they are not patentably distinct from each other because '160 teaches the method of treating hormonal dependent benign or malignant disease of reproductive tract by employing the herein claimed composition. The ratio of the solvents and the excipients are within the range taught in '160. The optimization of result effect parameters (e.g., dosing regimen [single dosing or multiple divided dosing], weight ratio of the actives and the excipients) is obvious as being within the skill of the

artisan. The optimization of known effective amounts of known active agents to be administered, is considered well in the competence level of an ordinary skilled artisan in pharmaceutical science, involving merely routine skill in the art. It has been held that it is within the skill in the art to select optimal parameters, such as amounts of ingredients, in a composition in order to achieve a beneficial effect. See *In re Boesch*, 205 USPQ 215 (CCPA 1980). It is also noted that "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAN-MING HUI whose telephone number is (571)272-0626. The examiner can normally be reached on Mon - Fri from 9:00 to 5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brandon Fetterolf can be reached on (571) 272-2919. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

San-ming Hui
Primary Examiner
Art Unit 1628

/San-ming Hui/
Primary Examiner, Art Unit 1628

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	12/285,887
				Filing Date	October 15,2008
				First Named Inventor	John R. EVANS
				Art Unit	1628
				Examiner Name	HUI, San Ming R.
Sheet	1	of	1	Attorney Docket Number	11285.0056-00000

U.S. PATENTS AND PUBLISHED U.S. PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

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 Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[6]
	1	WO 03/006064	23-JAN-2003	Astrazeneca AB		

NONPATENT 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.	Translation[6]
	2	The Merck Index, 12th Ed., Merck & Co., Inc., pgs. xiv, 189-190, 641-642 and 1715 (1996).	
	3	Guerrini, et al., "Pharmacokinetics of probenecid in sheep", J Vet Pharmacol Ther., 128-135 (1985).	
	4	Lavy, et al., "Pharmacokinetics of clindamycin HCl administered intravenously, intramuscularly and subcutaneously to dogs", J Vet Pharmacol Ther., 22(4):261-265 (1999).	
	5	Ismail, "Disposition kinetics of difloxacin after intravenous, intramuscular and subcutaneous administration in calves", Vet Res Commun., 31(4):467-476 (2007).	
	6	Documents from the prosecution of European Application No. 01900186.6 (EP 1 250 138) from August 27, 2009 to December 15, 2011.	
	7	Documents from the prosecution of European Application No. 10180667.7 (EP 2 266 573) from November 23, 2010 to December 19, 2011.	
	8	Documents from the prosecution of European Application No. 10180661.0 (EP 2 286 818) from January 19, 2011 to December 19, 2011.	
	9	Declaration Under 35 U.S.C §1.132 of Dr. Paul Gellert filed in August 2008 in U.S. Application No. 10/872,784.	
Examiner Signature	/San Ming Hui/	Date Considered	03/09/2012

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.H./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	12/285,887
				Filing Date	October 15, 2008
				First Named Inventor	John R. EVANS
				Art Unit	1628
				Examiner Name	San Ming R. Hui
Sheet	1	of	1	Attorney Docket Number	11285.0056-00000

U.S. PATENTS AND PUBLISHED U.S. PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
		US-			
		US-			
		US-			
		US-			

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

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 Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[6]

NONPATENT 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.	Translation[6]
	1	McLeskey et al., "Tamoxifen-resistant fibroblast growth factor-transfected MCF-7 cells are cross-resistant *in vivo* to the antiestrogen ICI 182,780 and two aromatase inhibitors," Clin. Cancer Res., 4:697-711 (1998).	
	2	JRF Robertson, et al., "Fulvestrant: pharmacokinetics and pharmacology," British Journal of Cancer, 90(1):S7-S10 (2004).	
	3	John F. R. Robertson, "Fulvestrant (Faslodex®)--how to make a good drug better," The Oncologist, 12:774-784 (2007).	
	4	Search Report for European Patent Application No. 10180667.7 dated November 23, 2010.	
	5	Search Report for European Patent Application No. 10180661.0 dated January 19, 2011.	
	6	Documents from the Opposition against European Patent Application No. 01900186.6 from April 21, 2009 to September 7, 2009.	

Examiner Signature	/San Ming Hui/	Date Considered	03/09/2012

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.H./

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	86164	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L2	451	fulvestrant and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L3	2807	oil and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L4	3	"4659516".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L5	7	"346014".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L6	16129	(benzyl adj benzoate) or (phenylmethyl adj benzoate)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L7	1884456	solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L8	8391	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L9	4	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (estrogen or estradiol or estrone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L10	7	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (testosterone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L11	14	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L12	1971	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) and (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33

L13	2	"6774122".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L14	982	514/177.ccls.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L15	1418	514/178.ccls.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L16	2168462	castor oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L17	86164	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L18	451	fulvestrant and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L19	2807	oil and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L20	16129	(benzyl adj benzoate) or (phenylmethyl adj benzoate)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L21	1884456	solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L22	8391	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L23	7	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (testosterone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L24	14	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L25	1971	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) and (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L26	86164	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L27	5153	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT;	OR	ON	2012/03/09 16:33

			IBM_TDB			
L28	2905	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L29	1495	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L30	3	((((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent) same steroid	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L31	3641	fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L32	3641	fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L33	86164	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L34	451	fulvestrant and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L35	2807	oil and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L36	3	"4659516".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L37	7	"346014".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L38	16129	(benzyl adj benzoate) or (phenylmethyl adj benzoate)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L39	1884456	solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L40	8391	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L41	4	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (estrogen or estradiol or estrone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L42	7	(((benzyl adj benzoate) or	US-PGPUB;	OR	ON	2012/03/09

		(phenylmethyl adj benzoate)) same solvent) same (testosterone)	USPAT; EPO; JPO; DERWENT; IBM_TDB			16:33
L43	14	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L44	1971	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) and (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L45	86164	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L46	5153	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L47	2905	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L48	1495	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L49	3	((((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent) same steroid	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L50	3641	fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L51	96080	breast adj cancer	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L52	2487	breast adj cancer and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L53	366	breast adj cancer same fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L54	1549	cancer same fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L55	2	"7456160".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33
L56	2	"6,774,122".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/03/09 16:33

EAST Search History (Interference)

<This search history is empty>

3/9/2012 4:41:31 PM
C:\Users\shui\Documents\EAST\Workspaces\12-285887.wsp

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12285887	EVANS ET AL.
	Examiner	Art Unit
	San-ming Hui	1628

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	12/19/2010	09/06/2011	03/09/2012						
	1	✓								
	2	✓								
	3	✓								
	4	✓								
	5	✓								
	6	✓								
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	32		✓	✓						
	33		✓	-						
	34		✓	✓						
	35		✓	✓						
	36		✓	✓						

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12285887	EVANS ET AL.
	Examiner	**Art Unit**
	San-ming Hui	1628

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	12/19/2010	09/06/2011	03/09/2012						
	37		✓	-						
	38		✓	✓						
	39		✓	✓						
	40		✓	-						
	41		✓	✓						
	42		✓	✓						
	43		✓	-						
	44		✓	✓						
	45		✓	-						
	46		✓	✓						
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	52		✓	-						
	53		✓	-						
	54			✓						
	55			✓						
	56			✓						
	57			✓						

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12285887	EVANS ET AL.
	Examiner	**Art Unit**
	San-ming Hui	1628

SEARCHED			
Class	**Subclass**	**Date**	**Examiner**
514	177, 178	12/19/10	SH
514	177, 178	9/6/11	SH
514	177, 178	3/9/12	SH

SEARCH NOTES		
Search Notes	**Date**	**Examiner**
EAST and inventor search in PALM	12/19/10	SH
update search in EAST and inventor search in PALM	9/6/11	SH
EAST in EAST and inventor search in PALM	3/9/12	SH

INTERFERENCE SEARCH			
Class	**Subclass**	**Date**	**Examiner**

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	12/285,887	EVANS ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 01/17/12	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

Lawana Hixon

U.S. Patent and Trademark Office

PATENT
Attorney Docket No. 11285.0056-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)
	)
John R. Evans et al.	) Group Art Unit: 1628
	)
Application No.: 12/285,887	) Examiner: HUI, San Ming R.
	)
Filed: October 15, 2008	) Confirmation No.: 1199
	)
For: FORMULATION	) **VIA EFS-WEB**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RESPONSE UNDER 37 C.F.R. § 1.111

In reply to the non-final Office Action mailed March 20, 2012 ("Office Action"), and pursuant to 37 C.F.R. § 1.111, Applicants hereby respectfully request reconsideration of this application in view of the following remarks.

REMARKS

I. Status of the claims and amendments

Claims 24, 26, 27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46, 47, and 54-57 are pending in this application. No claims are being amended in this response.

II. Statement of Substance of Interview under 37 C.F.R. § 1.133(b)

Applicants would like to thank Examiner San Ming Hui for granting a personal interview to Applicants on March 1, 2012. Applicants present this Statement of Substance of Interview in connection with that interview conducted between Examiner San Ming Hui, Dr. Ronald J. Sawchuk, Dr. Paul R. Gellert (AstraZeneca Pharmaceuticals), Mr. Allen F. Giles (AstraZeneca Pharmaceuticals), and the undersigned.

During the interview, the participants discussed the outstanding rejections, the arguments Applicants presented in the Response filed on January 17, 2012, as well as the contents of the declaration under 37 C.F.R. §1.132 by Dr. Sawchuk filed on January 17, 2012.

No agreement was reached at the interview and the Examiner indicated he would consider the information submitted with the January 17th Response and discussed at the interview in the preparation of the next Office Action.

III. Double Patenting Rejection

The Office rejected claims 24, 26, 27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46, 47, and 54-57 under the nonstatutory obviousness-type double patenting doctrine as

being unpatentable over: (a) claims 1-9 of U.S. Patent No. 6,774,122 ("the '122 patent") and (b) claims 1-12 of U.S. Patent No. 7,456,160 ("the '160 patent").

Applicants filed a Terminal Disclaimer on January 17, 2012, to obviate a double patenting rejection. In the Office Action, the Examiner refers to a decision dated February 15, 2012, which disapproved Applicants' Terminal Disclaimer for failure to comply with 37 C.F.R. 3.73(b).

On March 23, 2012, the undersigned contacted Ms. Lawana Hixon, who was the paralegal who issued the decision dated February 15, 2012. During the call with Ms. Hixon, the undersigned pointed out that Applicants complied with 37 C.F.R. 3.73(b) because the Terminal Disclaimer: (1) recites the chain of title from the inventors to the current assignee, AstraZeneca AB, including citation to the relevant assignment records, and (2) states that the person signing the Terminal Disclaimer is authorized to act on behalf of AstraZeneca AB. Ms. Hixon agreed that the requirements of Section 3.73 had been met and a review of the image file wrapper of this application available through PAIR confirms Ms. Nixon approved the Terminal Disclaimer.

Thus, the instant double patenting rejections are moot and Applicants respectfully request that they be withdrawn.

IV. Conclusion

In view of the foregoing remarks, Applicants respectfully request reconsideration of this application and the timely allowance of the pending claims.

The Office is encouraged to contact the undersigned at the phone number below should the Office consider a telephonic conversation will facilitate prosecution of the application.

Please grant any extensions of time required to enter this response and charge any required fees not included with this Response to Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: April 9, 2012

By: _____

Carlos M. Téllez
Reg. No. 48,638
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PATENT
Attorney Docket No. 11285.0056-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
John R. Evans et al.	)	Group Art Unit: 1628
	)	
Application No.: 12/285,887	)	Examiner: HUI, San Ming R.
	)	
Filed: October 15, 2008	)	Confirmation No.: 1199
	)	
For: FORMULATION	)	**VIA EFS-WEB**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97(c)

Pursuant to 37 C.F.R. §§ 1.56 and 1.97(c), Applicant brings to the attention of the Examiner the documents on the attached listing. This Information Disclosure Statement is being filed after the events recited in Section 1.97(b) but, to the undersigned's knowledge, before the mailing date of either a Final action, Quayle action, or a Notice of Allowance. Under the provisions of 37 C.F.R. § 1.97(c), this Information Disclosure Statement is accompanied by a fee of $180.00 as specified by Section 1.17(p)].

Copies of the listed foreign and non-patent literature documents are attached. It is the undersigned's understanding that the cited U.S. copending application is available to the Examiner through the PTO's Image File Wrapper system. Accordingly, a copy of that application is not enclosed. See M.P.E.P. § 609.04.

Applicants respectfully request that the Examiner consider the listed documents and indicate they were considered by making appropriate notations on the attached form.

This submission does not represent that a search has been made or that no better art exists and does not constitute an admission that each or all of the listed documents are material or constitute "prior art." If the Examiner applies any of the documents as prior art against any claims in the application and Applicants determine that the cited documents do not constitute "prior art" under United States law, applicants reserve the right to present to the office the relevant facts and law regarding the appropriate status of such documents.

Applicants further reserve the right to take appropriate action to establish the patentability of the disclosed invention over the listed documents, should one or more of the documents be applied against the claims of the present application.

If there is any fee due in connection with the filing of this Statement, please charge the fee to Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: April 9, 2012

By: _____

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	12/285,887
				Filing Date	October 15,2008
				First Named Inventor	John R. EVANS
				Art Unit	1628
				Examiner Name	HUI, San Ming R.
Sheet	1	of	1	Attorney Docket Number	11285.0056-00000

U.S. PATENTS AND PUBLISHED U.S. PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	U.S. Application No. 13/387,584	Filing date: 27-Jan-2012	DIMERY et al.	

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

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 Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[6]
	2	WO 2011/012885	03-Feb-2011	AstraZeneca UK Ltd.		

NONPATENT 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.	Translation[6]
	3	Buzdar, A. U., "Fulvestrant - A novel estrogen receptor antagonist for the treatment of advanced breast cancer," *Drugs of Today*, 44(9):679-692 (2008).	
	4	"Comparison of fulvestrant (faslodex) 250 mg and 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer progressing or relapsing after previous endocrine therapy," Clinicaltrials.gov (20-May-2009) retrieved 24-Jan- 2012.	
	5	Di Leo A., et al., "Confirm: a phase III, randomized, parallel-group trial comparing fulvestrant 250 mg vs fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer," Cancer Res., 69(24) Supp. 3, (2009).	
	6	International Search Report for PCT Application No. PCT/GB10/51228 (WO 2011/012885) mailed December 20, 2012.	
	7	International Preliminary Report on Patentability for PCT Application No. PCT/GB10/51228 (WO 2011/012885) mailed December 20, 2012.	
	8	Documents from the prosecution of European Application No. 01900186.6 (EP 1 250 138) dated December 15, 2011.	
	9	Documents from the prosecution of European Application No. 01900186.6 (EP 1 250 138) dated February 27, 2012.	

Examiner Signature		Date Considered	

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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(54) Title: FULVESTRANT IN A DOSAGE OF 500MG FOR THE TREATMENT OF ADVANCED BREAST CANCER

(57) Abstract: The present invention relates to fulvestrant at a dosage of 500mg for use in the treatment of a postmenopausal woman with advanced breast cancer who has progressed or recurred on endocrine therapy.

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FULVESTRANT IN A DOSAGE OF 500MG FOR THE TREATMENT OF ADVANCED BREAST CANCER

The present invention relates to fulvestrant at a dosage of 500mg for use in the treatment of a postmenopausal woman with advanced breast cancer who has progressed or recurred on endocrine therapy.

Breast cancer is one of the most common malignancies in women, comprising 18% of female cancers worldwide (Mcpherson et al 2000), and the most common cause of cancer deaths. The incidence varies among populations with about half of all cases occurring in North America and Western Europe. It has long been acknowledged that many breast cancers are hormone dependent and that hormonal manipulation can affect the progress of the disease (Beatson 1896). The most important factor determining response to hormonal manipulation is the presence of the oestrogen receptor (ER) in the target tissue (Fisher et al 2001).

The antioestrogen (AO) tamoxifen has been the most widely used endocrine therapy for breast cancer in both premenopausal and postmenopausal women. However, despite its demonstrated efficacy, *de novo* or acquired resistance may occur during treatment. In some patients, the disease progresses during therapy because tumour growth may be stimulated by tamoxifen, due to its partial agonist activity on the ER (Wiebe et al 1993).

The search for a pure AO, devoid of the agonist activity of tamoxifen, resulted in the discovery and clinical development of ICI 182,780 (also known as fulvestrant or FASLODEX™). Fulvestrant is an ER antagonist without known agonistic properties that down-regulates cellular levels of the ER in a dose-dependent manner (Howell et al 2000, Robertson et al 2001, Wakeling et al 1991). Fulvestrant is well tolerated and has demonstrated efficacy in women whose breast cancer had progressed following endocrine therapy (Howell et al 2002, Osborne et al 2002, Chia et al 2008).

Women diagnosed with early breast cancer are generally treated with tamoxifen or an aromatase inhibitor if endocrine therapy is appropriate. However if the cancer recurs or progresses there is a need for alternative therapies. Fulvestrant (FASLODEX™) is presently approved at a dose of 250mg as an alternative endocrine therapy. The present

invention is based on the discovery that increasing the dose of fulvestrant to 500mg is more advantageous for patients than the 250mg dose.

One feature of the invention provides fulvestrant at a dosage of 500mg for use in the treatment of a postmenopausal woman with advanced breast cancer who has progressed or recurred on endocrine therapy. Preferably the fulvestrant is administered monthly. Preferably an additional dose of 500mg is administered during the first month of treatment. Preferably the additional dose is administered at about day 14. Preferably the woman is oestrogen receptor positive or progesterone receptor positive; more preferably oestrogen receptor positive. Preferably the progression or recurrence on endocrine therapy comprised therapy with tamoxifen or an aromatase inhibitor. Preferably the aromatase inhibitor is selected from anastrozole, letrozole or exemestane; more preferably anastrozole or letrozole. Preferably the use use of fulvestrant at 500mg dosage provides an increase the time to progression compared with fulvestrant at a dosage of 250mg; in particular the doses are preferably administered monthly with an additional dose at 500mg in the first month. Tamoxifen, anastrozole, letrozole and exemestane are all commercially available drugs with regulatory approval for administration to women with breast cancer.

Another feature of the invention provides the use fulvestrant at a dosage of 500mg for preparation of a medicament for treatment of a postmenopausal woman with advanced breast cancer who has progressed or recurred on endocrine therapy. This feature may be combined with any of the preferred features described herein.

Another feature of the invention provides the treatment of a postmenopausal woman with advanced breast cancer who has progressed or recurred on endocrine therapy with fulvestrant at a dosage of 500mg. This feature may be combined with any of the preferred features described herein.

The invention is exemplified by the following non-limiting Example, in which Figure 1 shows a Kaplan-Meier plot of time to progression comparing fulvestrant at 250mg with 500mg. The x-axis shows the time in months and y-axis shows proportion of patients progression free. Tick marks indicate censored observations.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or special term	Explanation
AE	Adverse event
AI	Aromatase inhibitor
ALT	Alanine aminotransferase
AO	Antioestrogen
AST	Aspartate aminotransferase
BOR	Best objective/overall response
CBR	Clinical benefit rate
CI	Confidence interval
CR	Complete response
CRA	Clinical research associate
CRF	Case report form
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CT	Computed tomography
CTCAE	Common terminology criteria for adverse events
DAE	Premature discontinuation of treatment with investigational product due to an adverse event (adverse events).
DCO	Data cut-off
DoCB	Duration of clinical benefit
DoR	Duration of response
ECG	Electrocardiogram
EDoCB	Expected duration of clinical benefit
EDoR	Expected duration of response
Endpoint	A status of the patient that constitutes the 'endpoint' of a patient's participation in a clinical study and that is used as the final outcome.
ER	Oestrogen receptor
EU	European Union
FACT-B	Functional Assessment of Cancer Therapy - breast cancer
FSH	Follicle stimulating hormone
GCP	Good clinical practice
HER	Human epidermal growth factor receptor

Abbreviation or special term	Explanation
HRQoL	Health-related quality of life
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
im	Intramuscular
INR	International normalised ratio
IRB	Institutional Review Board
International Co-ordinating investigator	An Investigator assigned the responsibility for the co-ordination of investigators across all Study Sites participating in a multinational, multicentre study.
LD	Longest diameter
LHRH	Luteinising hormone releasing hormone
MedDRA	Medical dictionary for regulatory activities
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
OAE	Other significant adverse event (ie, significant AEs, other than SAEs and DAEs, which are of particular clinical importance in this development program).
OR	Objective response
ORR	Objective response rate
OS	Overall survival
Outcome variable	A variable (usually a derived variable) specifically defined to be used in the analysis of a study objective.
Patient identifier	Only one variable is used to identify each patient within the reporting database. This identifier is a concatenation of the Study Number, and the enrolment Code (eg, D1234C00001/E0010001). Within an individual study report, the enrolment code alone (eg, E0010001) may be used to reference individual patients in-text within the CSR, including tables and listings. With respect to individual Patient Narratives, and the higher level documents, the full unique patient identifier should be used.
PD	Progressive disease
PgR	Progesterone receptor
PPS	Per Protocol Set
PR	Partial response
Principal investigator	A person responsible for the conduct of a clinical study at an investigational study site. Every investigational study site has a principal investigator.
PRO	Patient reported outcomes
PT	Preferred term

Abbreviation or special term	Explanation
RECIST	Response evaluation criteria in solid tumours
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Stable disease
sd	Standard deviation
SE	Standard error
SOC	System organ class
TOI	Trial outcome index
TTP	Time to progression. The definition of TTP used in this clinical study is also commonly termed progression free survival (PFS).
TTR	Time to response
ULRR	Upper limit reference range
US	United States of America
Variable	A characteristic or a property of a patient that may vary eg from time to time or between patients.
WHO	World Health Organisation

Example 1

A Randomised, Double-Blind, Parallel-group, Multicentre, Phase III Study Comparing the Efficacy and Tolerability of Fulvestrant (FASLODEX™) 500 mg with Fulvestrant (FASLODEX™) 250 mg in Postmenopausal Women with Oestrogen Receptor Positive Advanced Breast Cancer Progressing or Relapsing after Previous Endocrine Therapy

This study assessed the relationship between fulvestrant dose and efficacy. It compared the current approved dose and dosing schedule of fulvestrant (250 mg every 28 days) with a higher dose regimen (500 mg every 28 days plus an additional 500 mg on Day 14 of the first month only). The study is also referred to as CONFIRM.

Study centres

One-hundred and twenty-eight centres in 17 countries (Belgium, Brazil, Chile, Colombia, Czech Republic, Hungary, India, Italy, Malta, Mexico, Poland, Russia, Slovakia, Spain, USA, Ukraine and Venezuela). The US, Mexico, Italy, Brazil, Spain, Chile, Colombia and

Venezuela also participated in health-related quality of life (HRQoL) assessments during the study.

Objectives

The primary objective of the study was to compare the efficacy of fulvestrant 500 mg treatment with fulvestrant 250 mg treatment in terms of time to progression (TTP).

The secondary objectives of the study were:

- To compare the objective response rate (ORR) of patients treated with fulvestrant 500 mg with the objective response rate of patients treated with fulvestrant 250 mg.
- To compare clinical benefit rate (CBR) of patients treated with fulvestrant 500 mg with the clinical benefit rate of patients treated with fulvestrant 250 mg.
- To compare duration of response (DoR) of patients treated with fulvestrant 500 mg with the duration of response of patients treated with fulvestrant 250 mg.
- To compare the duration of clinical benefit (DoCB) of patients treated with fulvestrant 500 mg with the duration of clinical benefit of patients treated with fulvestrant 250 mg.
- To compare the overall survival (OS) of patients treated with fulvestrant 500 mg with the overall survival of patients treated with fulvestrant 250 mg.
- To assess the tolerability of fulvestrant 500 mg treatment compared with fulvestrant 250 mg treatment.
- To assess the health-related quality of life (HRQoL) of patients treated with fulvestrant 500mg as compared to fulvestrant 250 mg in a subgroup of patients.

Study design

This was a randomised, double-blind, parallel-group, multicentre, phase III study to compare 2 dose levels of fulvestrant in postmenopausal women with oestrogen receptor positive (ER+ve) advanced breast cancer who had either relapsed whilst on adjuvant endocrine therapy, or progressed whilst on first endocrine therapy for advanced disease.

Target patient population and sample size

A total of 720 postmenopausal women with histological/cytological confirmation of ER+ve breast cancer who had relapsed or progressed on previous endocrine therapy were planned to be recruited; a total of 736 were actually randomised.

The sample size calculation was based on the primary variable, TTP, and assumed exponential progression times. The sample size was driven by the number of required events. In order to detect a hazard ratio of ≤0.8 (or ≥1.25) for fulvestrant 500 mg compared to fulvestrant 250 mg, at a 2-sided significance level of 5%, with 80% power, approximately 632 events were required to have occurred in the study (ie, approximately 632 patients to have progressed or died).

Investigational product and comparator: dosage, mode of administration and batch numbers

Fulvestrant 500 mg was given as two 5 ml intramuscular (im) injections, one in each buttock, on days 0, 14, 28 and every 28 (±3) days thereafter.

Fulvestrant 250 mg was given as two 5 ml im injections (1 fulvestrant injection plus 1 placebo injection), one in each buttock, on days 0, 14 (2 placebo injections only), 28 and every 28 (±3) days thereafter.

Duration of treatment

Treatment was to continue until disease progression occurred, unless any of the criteria for treatment discontinuation were met first.

Criteria for evaluation - efficacy and pharmacokinetics (main variables)

Efficacy

The primary outcome variable TTP; secondary variables were ORR, CBR, DoR, DoCB and OS.

Patient reported outcomes

The primary patient reported outcome for HRQoL was the Trial Outcome Index (TOI) derived from the Functional Assessment of Cancer Therapy - Breast cancer (FACT-B) questionnaire.

Criteria for evaluation - safety (main variables)

Outcome variables for safety were frequency and severity of adverse events (AEs), including pre-specified AEs of interest.

Statistical methods

For the primary endpoint TTP, the primary analysis was an unadjusted log-rank test and the secondary analysis was a Cox proportional hazard model, adjusted for treatment and other predefined covariates.

For OS, the unadjusted log-rank test was performed. For ORR and CBR, a logistic regression model with treatment factor only was fitted. DoR and DoCB were analysed in those patients who had an OR and CB, respectively. For HRQoL endpoints, a longitudinal model with treatment and other covariates was used.

The hypotheses for TTP, ORR, CBR, DoR, DoCB, OS, FACT-B score and TOI score were:

H_0: fulvestrant 500 mg is not different from fulvestrant 250 mg, vs.

H_1: fulvestrant 500 mg is different from fulvestrant 250 mg

For efficacy and HRQoL endpoints, summaries and analyses were carried out according to the randomised treatment ie, using the Full Analysis Set. For safety endpoints, summaries and analyses were carried out according to the treatment actually received, ie, using the safety analysis set. The primary endpoint was also analysed in the per protocol set (PPS).

Patient population

A total of 720 patients were planned to be recruited; 736 were actually randomised. Diagram S1 shows the number of patients randomised to each of the 2 treatment groups and the number in each of the populations analysed. In addition, HRQoL was analysed in 145 of the patients in the Full Analysis Set (72 patients in the fulvestrant 500 mg group and 73 patients in the fulvestrant 250 mg group). The patient population was consistent with the one intended to be recruited. In the fulvestrant 500 mg group, 41 patients were ongoing study treatment at data cut off (DCO) compared with 31 patients in the fulvestrant 250 mg group.

1.1 Selection of study population

Before entering the study, patients were assessed to ensure that they met the eligibility criteria. Investigators had to keep a record of patients who were considered for enrolment but were never randomised (patient screening log). This information is necessary to establish that the patient population was selected without bias. The patient screening log had to be filed in the Investigator study file at each centre.

1.1.1 Inclusion criteria

For inclusion in the study patients had to fulfil all of the following criteria:

1. Provision of written informed consent
2. Histological/cytological confirmation of breast cancer

3. Documented ER+ve status of primary or metastatic tumour tissue, according to the local laboratory parameters

4. Requiring endocrine therapy:
   - Relapsing during, or within 12 months of completion of, adjuvant endocrine therapy (tamoxifen, toremifene or AIs such as anastrozole, letrozole and exemestane), or
   - Progressing on an endocrine therapy (tamoxifen, toremifene or AIs such as anastrozole, letrozole and exemestane) provided that this endocrine treatment was started at least 12 months after the completion of adjuvant endocrine treatment, or
   - Progressing on an endocrine therapy (tamoxifen, toremifene or AIs such as anastrozole, letrozole and exemestane) given as first treatment for patients with *de novo* advanced[1] breast cancer

5. Fulfilling one of the following criteria:
   - Patients with measurable disease as per RECIST criteria. This is defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan.
   - Patients with bone lesions, lytic or mixed (lytic and sclerotic), in the absence of measurable disease as defined by RECIST.

6. Postmenopausal woman, defined as a woman fulfilling any 1 of the following criteria:
   - Age ≥60 years.
   - Age ≥45 years with amenorrhoea ≥ 12 months with an intact uterus.
   - Having undergone a bilateral oophorectomy
   - Follicle stimulating hormone (FSH) and oestradiol levels in postmenopausal range (utilising ranges from the local laboratory facility).
   - In patients who had previously been treated with a luteinising hormone releasing hormone (LHRH) analogue, the last depot must have been

[1] Advanced breast cancer: Metastatic disease or locally advanced disease which is not amenable to treatment with curative intent.

administered more than 4 months prior to randomisation, menses must not have restarted, and FSH and oestradiol levels must also have been in the postmenopausal range (utilising ranges from the local laboratory facility).

7. WHO performance status 0, 1 or 2.

Rationale for inclusion criteria

1. This criterion was set as part of the ethical conduct of the study, which complies with GCP.
2. This criterion was set to objectively confirm breast cancer.
3. This criterion was set to select a patient population expected to respond to fulvestrant based on its mechanism of action.
4. This criterion was set to clarify the history of hormonal therapy for breast cancer in this study.
5. This criterion was set to enable the conduct of efficacy assessments according to modified RECIST.
6. This criterion was set because the effect of fulvestrant on pre-menopausal breast cancer patients had not been fully assessed.
7. This criterion was set to conduct efficacy assessments properly and to ensure the safety of patients.

1.1.2 Exclusion criteria

Any of the following was regarded as a criterion for exclusion from the study:

1. Presence of life-threatening metastatic visceral disease, defined as extensive hepatic involvement, or any degree of brain or leptomeningeal involvement (past or present), or symptomatic pulmonary lymphangitic spread. Patients with discrete pulmonary parenchymal metastases were eligible, provided their respiratory function was not compromised as a result of disease.
2. More than one regimen of chemotherapy for advanced disease.[2]
3. More than one regimen of endocrine therapy for advanced disease.[3]

[2] Patients previously treated with one regimen of chemotherapy for advanced disease were allowed as long as their last treatment was an AO or an AI.

[3] Oophorectomy, ovarian ablation, or LHRH analogue therapy did not count as endocrine treatments in this context and also did not render the patient ineligible for this study.

4. Extensive radiation therapy within the last 4 weeks (greater than or equal to 30% marrow or whole pelvis or spine) or cytotoxic treatment within the past 4 weeks prior to screening laboratory assessment, or strontium-90 (or other radiopharmaceuticals) within the past 3 months.
5. Treatment with a non-approved or experimental drug within 4 weeks before randomisation.
6. Current or prior malignancy within previous 3 years (other than breast cancer or adequately treated basal cell or squamous cell carcinoma of the skin or in-situ carcinoma of the cervix).
7. Any of the following laboratory values:
   - Platelets $<100 \times 10^9$/L
   - Total bilirubin >1.5×upper limit reference range (ULRR)
   - ALT or AST >2.5×ULRR if no demonstrable liver metastases or >5×ULRR in presence of liver metastases.
8. History of:
   - Bleeding diathesis (ie, disseminated intravascular coagulation, clotting factor deficiency), or
   - Long-term anticoagulant therapy (other than antiplatelet therapy and low dose warfarin (see Section 3.7 of the CSP [Appendix 12.1.1 of this report]).
9. History of hypersensitivity to active or inactive excipients of fulvestrant and/or castor oil.
10. Any severe concomitant condition which made it undesirable for the patient to participate in the trial or which would jeopardize compliance with the CSP, eg, uncontrolled cardiac disease or uncontrolled diabetes mellitus.

Rationale for exclusion criteria

The exclusion criteria for concurrent diseases, concomitant drugs and patients' conditions were set because they were considered to affect the safety of patients or the efficacy assessment of fulvestrant in hormone receptor positive, postmenopausal advanced or recurrent breast cancer.

1.1.3 Restrictions

The following restrictions were applied to patients in this trial:

1. Patients who were blood donors were not to donate blood during the study and for 12 weeks following their last dose of randomised treatment.
2. Patients who had confirmed disease progression must have been discontinued from their randomised treatment.
3. Concomitant treatments listed in Section 3.7 of the CSP.

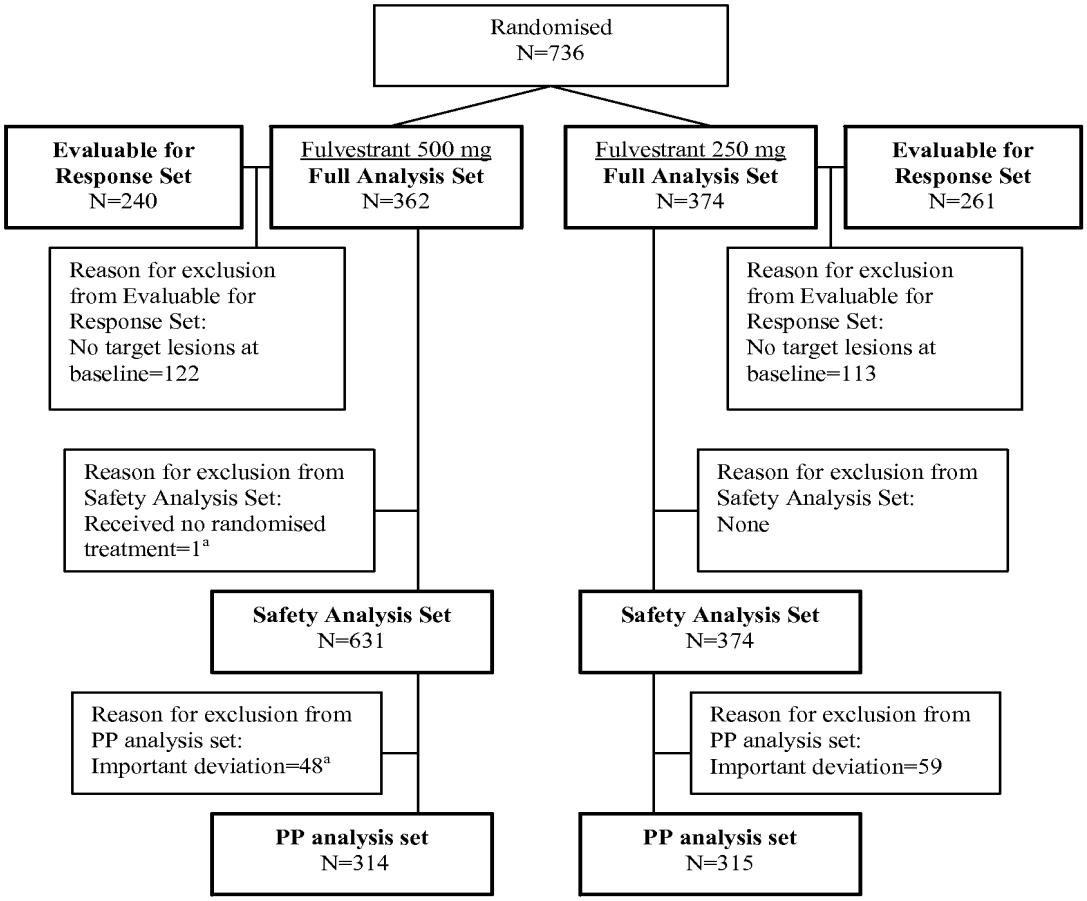
Rationale for restrictions

1. This restriction was included to ensure that anaemia was not induced by blood donation following the additional blood sampling requirement of the study.
2. This restriction was included to protect patients who were not receiving or who ceased to receive clinical benefit from their study treatment and is in line with current clinical practice.
3. This restriction was included because the concomitant treatments listed in Section 3.7 of the CSP were considered to effect the safety of patients or the efficacy assessment of the study drugs.

1.1.4 Discontinuation of patients from treatment or assessment

Patients could be discontinued from study treatment and assessments at any time at the discretion of the investigators. Patients were also free to discontinue their participation in the study at any time, without prejudice to further treatment. Specific reasons for discontinuing a patient from this study, and the procedures to be followed when a patient discontinued or was incorrectly enrolled, are listed in Section 3.3.5 of the CSP. For patients who discontinued, it was noted whether they were assessed after study medication was stopped, and whether they were asked about the reason(s) for their discontinuation and about the presence of any adverse events (AEs). If possible, they were seen and assessed by an investigator. AEs were followed up for 56 days after the last injection.

Diagram S1 Analysis sets



[a] The patient who was excluded from the safety analysis set was also classified as a deviator, therefore these n values are not mutually exclusive.

Summary of demographics and baseline characteristics

A total of 96.1% of patients randomised into the study were Caucasian. The mean age of patients was 60.9 years and the mean weight of patients was approximately 70 kg. Tumour characteristics were well balanced across the 2 treatment groups. Most patients (507 [68.9%]) were ER+ve and PgR+ve at primary diagnosis and almost all patients (721 [98%]) had metastatic disease at baseline. In this study, 42.5% of patients had relapsed or progressed on AI therapy and 57.5% had relapsed or progressed on AOs. Most patients had relapsed or progressed either during previous adjuvant endocrine cancer therapy (344 patients [46.7%]) or during endocrine therapy given as a first treatment for *de*

novo advanced disease (255 patients [34.6%]). Approximately two thirds of patients had shown a response[4] to their last endocrine therapy.

Summary of efficacy results

A summary of efficacy data is presented in Table S1.

Table S1 Summary of efficacy results for the main outcome variables

Variable	Result
Primary outcome variable	
TTP[a]	Hazard ratio=0.80 (95% CI 0.68–0.94); p=0.006 Median TTP: fulvestrant 500 mg =6.5 months; fulvestrant 250 mg =5.5 months % patients progression free at 12 months: fulvestrant 500 mg=34%; fulvestrant 250 mg = 25%
Secondary outcome variables	
ORR	Odds ratio=0.94 (95% CI 0.57–1.55); p=0.795 ORR: fulvestrant 500 mg=13.8%; fulvestrant 250 mg=14.6%
CBR	Odds ratio=1.28 (95% CI 0.95–1.71); p=0.100 CBR: fulvestrant 500 mg=45.6%; fulvestrant 250 mg=39.6%
DoR[b]	Ratio of EDoR=0.894 (95% CI 0.479–1.667); p=0.724 Median DoR[c]: fulvestrant 500 mg=19.4 months; fulvestrant 250 mg=16.4 months
DoCB	Ratio of EDoCB=1.357 (95% CI 1.067–1.726); p=0.013 Median DoCB: fulvestrant 500 mg=16.6 months; fulvestrant 250 mg=13.9 months
OS	Hazard ratio=0.84 (95% CI 0.69–1.03); p=0.091 Median OS: fulvestrant 500 mg=25.1 months; fulvestrant 250 mg=22.8 months % patients alive at 24 months: fulvestrant 500 mg=53%; fulvestrant 250 mg=49%

[a] TTP ≡ progression-free survival. At data cut-off, 84% of patients had progressed or died in the absence of progression.

[b] measured from randomisation to progression

[c] from randomisation.

[4] Defined as patients who experienced recurrence after ≥2 years on adjuvant endocrine therapy and/or patients who received clinical benefit (CR, PR or SD ≥24 weeks) from first-line therapy for advanced disease.

TTP:time to progression; ORR:objective response rate; CBR:clinical benefit rate; DoR:duration of response; DoCB:duration of clinical benefit; OS:overall survival; EDoR:expected duration of response; EDoCB:expected duration of clinical benefit.

Fulvestrant 500 mg was associated with a significantly longer TTP compared with fulvestrant 250 mg (hazard ratio=0.80 [95% CI 0.68–0.94]; p=0.006) corresponding to a reduction in risk of progression of 20%. Subgroup analyses showed a consistent treatment effect across all 6 predefined baseline covariates, including patients treated previously with either an aromatase inhibitor (AI) or antioestrogen (AO).

The ORR for fulvestrant 500 mg and fulvestrant 250 mg were similar (13.8% and 14.6% respectively, odds ratio=0.94 [95% CI 0.57 to 1.55]; p=0.795) but there was a trend for an increased CBR in patients receiving fulvestrant 500 mg compared to those receiving fulvestrant 250 mg (45.6% vs. 39.6%, odds ratio=1.28 [95% CI 0.95 to 1.71]; p=0.100).

There was no statistically significant difference between the 2 treatment groups in expected DoR (EDoR); however, there was a statistically significant improvement in expected DoCB (EDoCB) in patients randomised to receive fulvestrant 500 mg compared with patients randomised to receive fulvestrant 250 mg (9.83 months vs. 7.24 months, ratio of EDoCB=1.357 [95% CI 1.067 to 1.726]; p=0.013).

There was a trend for improved survival for patients treated with fulvestrant 500 mg compared with fulvestrant 250 mg (hazard ratio=0.84 [95% CI 0.69 to 1.03]; p=0.091); this corresponds to a 16% reduction in risk of death.

In the subgroup of patients where it was measured, on-treatment HRQoL for both fulvestrant 500 mg and fulvestrant 250 mg was good (mean TOI score of approximately 60 out of 92). Patients treated with fulvestrant 500 mg had a similar on-treatment HRQoL to patients treated with fulvestrant 250 mg and there were no statistically significant differences between the 2 treatment groups in terms of change in on treatment HRQoL as measured by both the TOI and FACT-B score, although there was a numerical advantage in TOI in favour of fulvestrant 500 mg.

Efficacy results

Primary variable: Time to progression

The primary objective of this study was to compare TTP between patients treated with fulvestrant 500 mg and those treated with fulvestrant 250 mg. The primary analysis set was the Full Analysis Set. An analysis of TTP in the PPS was also performed as a

secondary analysis. Table S2 shows the TTP data for patients in the fulvestrant 500 mg and fulvestrant 250 mg groups in the Full Analysis Set; Figure 1 shows a Kaplan-Meier plot of these data.

At DCO 618/736 (84.0%) patients had progressed or died in the absence of progression (297 [82.0%] in the fulvestrant 500 mg group and 321 [85.8%] in the fulvestrant 250 mg group). The unadjusted log rank test indicates that the TTP for patients in the fulvestrant 500 mg group was significantly longer than for those in the fulvestrant 250 mg group (hazard ratio=0.80 [95% CI 0.68 to 0.94]; p=0.006). Median TTP was 6.5 months in the fulvestrant 500 mg group and 5.5 months in the fulvestrant 250 mg group. The Kaplan-Meier plot for TTP in the Full Analysis Set shows a separation between the 2 treatment groups from approximately 3 months, favouring the fulvestrant 500 mg group.

Month	0	4	8	12	16	20	24	28	32	36	40	44	48
Fulvestrant 500mg at risk	362	216	163	113	90	54	37	19	12	7	3	2	0
Fulvestrant 250mg at risk	374	199	144	85	60	35	25	12	4	3	1	1	0

Table S2 Summary of time to progression: Full Analysis Set

	Fulvestrant 500 mg N=362	**Fulvestrant 250 mg N=374**
Number progressed (%)	297 (82.0)	321 (85.8)
Median (months)	6.5	5.5
Time to progression (months): 25% quartile	2.8	2.7
Time to progression (months): 75% quartile	16.6	11.9
Percentage of patients progression free at:		
6 months	51%	45%
12 months	34%	25%
18 months	23%	14%
24 months	16%	11%
Hazard ratio (95% CI)	0.80 (0.68–0.94)	
p-value	0.006	

Time to progression is the time between randomisation and the earliest of progression or death from any cause.

A hazard ratio <1 indicates fulvestrant 500 mg is associated with a longer time to disease progression than fulvestrant 250 mg

A hazard ratio >1 indicates fulvestrant 500 mg is associated with a shorter time to disease progression than fulvestrant 250 mg

Data source: Tables 11.2.1.1, 11.2.1.2 and 11.2.1.5.

The primary analysis of TTP is supported by the Cox proportional hazards regression analysis, adjusted for treatment and 6 specified covariates (hazard ratio=0.78 [95% CI 0.67 to 0.92]; p=0.003).

Summary of safety results

Fulvestrant 500 mg was well tolerated and its safety profile was consistent with the known safety profile of fulvestrant 250 mg. The most commonly reported pre-specified AEs of interest were gastrointestinal disturbances and joint disorders (approximately 20% and 19% of patients, respectively, in each of the treatment groups). There were no differences between treatment groups in the incidence or type of AEs, serious AEs and AEs leading to discontinuation. There was no evidence for dose dependence for any AE. There were no clinically important changes in haematology, clinical chemistry, vital signs or physical findings.

Conclusions

This study demonstrates that fulvestrant 500 mg provides a clinically meaningful benefit over fulvestrant 250 mg, in terms of TTP, in the treatment of postmenopausal women with ER+ve advanced breast cancer who have progressed or recurred on endocrine therapy. Further analyses demonstrated that the TTP data obtained in the study are robust. The results show that fulvestrant 500 mg reduces the risk of disease progression by 20% compared with fulvestrant 250 mg. The risk in progression appears to be reduced in the fulvestrant 500 mg group compared to the 250 mg group by 3 observed factors:

- a reduction in the proportion of patients with a best objective response of progressive disease (38.7% in the fulvestrant 500 mg group vs 44.7% in the fulvestrant 250 mg group)
- an increase in the proportion of patients who achieved clinical benefit (45.6% vs 39.6%, respectively)

- an increase in the duration of clinical benefit in patients receiving clinical benefit (median of 16.6 months vs 13.9 months, respectively).

There was also a trend towards improved survival in the fulvestrant 500 mg group (median of 25.1 months compared with 22.8 months in the 250 mg group), indicating that the observed treatment comparison for overall survival supports the advantage observed for TTP and suggesting that the benefit provided by treatment, in terms of progression, is maintained past progression.

In the subgroup of patients where it was measured, on-treatment HRQoL remained stable while patients were receiving study treatment; there was no detrimental effect of the fulvestrant 500 mg dose compared with 250 mg.

In the registration trials for fulvestrant, Studies 20/21, fulvestrant 250 mg was shown to be non-inferior to anastrozole (Robertson et al 2003). Demographic characteristics of patients in the CONFIRM study were broadly similar to those of patients in the combined analysis of Studies 20/21 and the efficacy results for fulvestrant 250 mg were consistent across the studies (median TTP of 5.5 months in CONFIRM and the combined analysis of Studies 20/21). Data from these studies give further reassurance of the significant benefit that fulvestrant 500 mg offers over an already effective 250 mg dose.

The treatment effect for TTP, favouring fulvestrant 500 mg, was consistent across all subgroups analysed. The consistency of the TTP treatment effect in the aromatase inhibitor (AI) and antioestrogen (AO) subgroups is of particular interest, given that in many markets the current regulatory approval for fulvestrant 250 mg is limited to patients who have progressed on AO therapy. Since the first regulatory approval for the use of non-steroidal AIs in breast cancer, changes in clinical practice have meant that there has been a considerable increase in the proportion of patients being treated upfront with these drugs in both the adjuvant and the advanced setting (see National Comprehensive Cancer Network [NCCN], Inc. 2009 and references therein for more details). There are few endocrine treatment options available to patients who progress on AI therapy and it is therefore important to identify agents that effectively prolong the time to progression after failing on such therapy. Although guidelines like NCCN support the use of a same class agent with a steroidal structure (steroidal AIs) in patients who have progressed on a non-steroidal AI, there are currently no agents of this type with regulatory approval for this treatment sequence. Fulvestrant 500 mg has a different mechanism of action to AIs and is

the first agent to show consistent benefit in a phase III setting in patients who have progressed during either AO or AI therapy.

The safety profile of fulvestrant 500 mg is consistent with the known safety profile of fulvestrant 250 mg with no evidence for dose dependence for any AE. The 2 SAEs that were considered by the investigator to be possibly causally related to study treatment were confounded by other factors in the patients' medical histories and concomitant medications. The incidence of pre-specified AEs was well balanced between the 2 treatment groups. Although the incidence of injection site reactions was similar between treatment groups, a full assessment of the injection procedure was not possible to evaluate due to the double blind design. However, it is reassuring to observe that there is no increase in the AE incidence with doubling the dose of fulvestrant.

Overall, fulvestrant 500 mg provides improved efficacy without any detrimental effect on safety, tolerability or HRQoL compared with fulvestrant 250 mg.

Overall conclusions

The CONFIRM study demonstrated a clear improvement in the efficacy of fulvestrant 500 mg when compared with the currently approved dose of fulvestrant 250 mg. There was a statistically significant prolongation of the TTP with a 20% reduction in the risk of progressing for patients receiving fulvestrant 500mg. Given the superior efficacy, similar safety, tolerability and HRQoL that fulvestrant 500mg offers over fulvestrant 250mg we conclude that there is a superior benefit-risk profile for fulvestrant 500mg in patients recurring or progressing on endocrine therapy.

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Claims:

1. Fulvestrant at a dosage of 500mg for use in the treatment of a postmenopausal woman with advanced breast cancer who has progressed or recurred on endocrine therapy.

2. A use according to claim 1 wherein the fulvestrant is administered monthly.

3. A use according to claim 2 wherein an additional dose of 500mg is administered during the first month of treatment.

4. A use according to claim 3 wherein the additional dose is administered at about day 14.

5. A use according to any preceding claim wherein the woman is oestrogen receptor positive or progesterone receptor positive.

6. A use according claim 5 wherein the woman is oestrogen receptor positive.

7. A use according to any preceding claim wherein the progression or recurrence on endocrine therapy comprised therapy with tamoxifen or an aromatase inhibitor.

8. A use according to claim 7 wherein the aromatase inhibitor is selected from anastrozole, letrozole or exemestane.

9. A use according to any preceding claim whereby to increase the time to progression compared with fulvestrant at a dosage of 250mg.

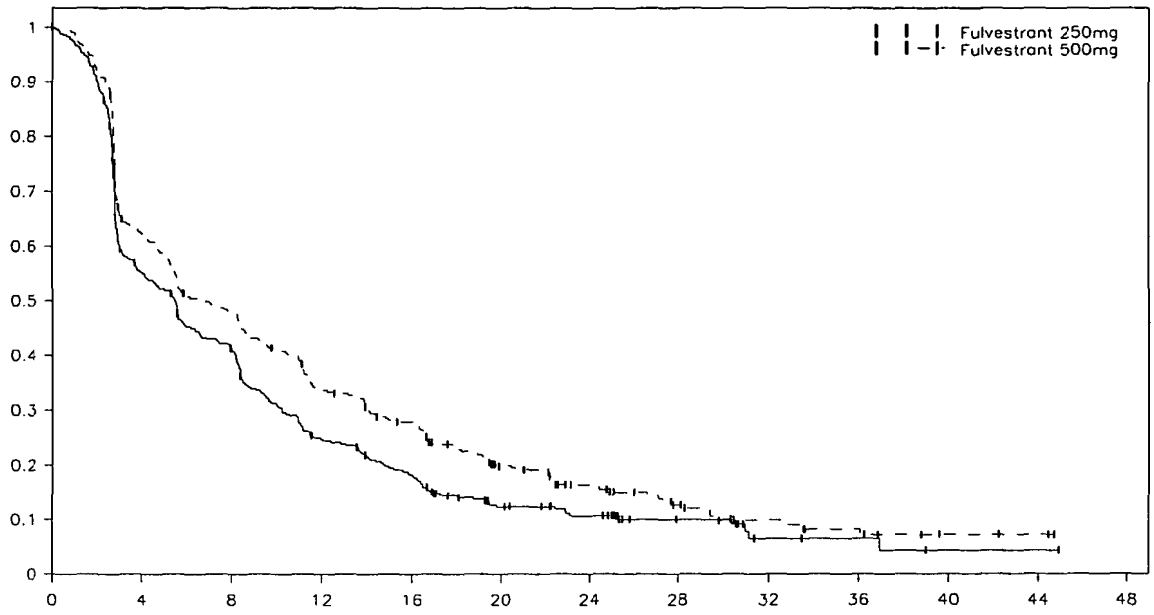

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/051228

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/565 A61P35/00

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)
A61K A61P

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 practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUZDAR AMAN U: "FULVESTRANT - A NOVEL ESTROGEN RECEPTOR ANTAGONIST FOR THE TREATMENT OF ADVANCED BREAST CANCER" DRUGS OF TODAY, vol. 44, no. 9, September 2008 (2008-09), pages 679-692, XP002612295 ISSN: 1699-3993 page 684 page 686, column 2, paragraph 1 page 688, column 2, paragraph 2-3 figure 8 ----- -/--	1-9

[X] 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
"E" earlier document but 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
2 December 2010	20/12/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Rodríguez-Palmero, M

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROBERTSON J F R: "Fulvestrant (Faslodex(R)) - How to make a good drug better" ONCOLOGIST 200707 US LNKD-DOI:10.1634/THEONCOLOGIST.12-7-774, vol. 12, no. 7, July 2007 (2007-07), pages 774-784, XP002612296 ISSN: 1083-7159 figure 1 page 778, column 1, paragraph 2 page 780, column 2, last paragraph page 782, column 1, last paragraph - column 2, paragraph 1	1-9
X	"Comparison of Fulvestrant (Faslodex) 250 mg and 500 mg in postmenopausal women with oestrogen receptor-positive advanced breast cancer progressing or relapsing after previous endocrine therapy."[Online] 20 May 2009 (2009-05-20), XP002612297 Clinicaltrials.gov Retrieved from the Internet: URL:http://clinicaltrials.gov/archive/NCT00099437/2009_05_20> [retrieved on 2010-11-26] the whole document	1-9
X,P	Di Leo A et al.: "CONFIRM: A Phase III, Randomized, Parallel-Group Trial Comparing Fulvestrant 250 mg vs Fulvestrant 500 mg in Postmenopausal Women with Estrogen Receptor-Positive Advanced Breast Cancer." Cancer Res, [Online] vol. 69, no. 24 Suppl 3, 25, 15 December 2009 (2009-12-15), pages 1-2, XP002612298 DOI: 10.1158/0008-5472.SABCS-09-25 Retrieved from the Internet: URL:http://cancerres.aacrjournals.org/cgi/content/abstract/69/24_MeetingAbstracts/25 > [retrieved on 2010-12-01] abstract	1-9

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 103803-1P WO	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/GB2010/051228	International filing date *(day/month/year)* 26 July 2010 (26.07.2010)	Priority date *(day/month/year)* 27 July 2009 (27.07.2009)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant ASTRAZENECA AB		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 31 January 2012 (31.01.2012)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pt04.pct@wipo.int

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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2010/051228	26.07.2010	27.07.2009

International Patent Classification (IPC) or both national classification and IPC
INV. A61K31/565 A61P35/00

Applicant
ASTRAZENECA AB

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Rodríguez-Palmero, M Telephone No. +49 89 2399-7871

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1, 5-9
	No:	Claims	2-4
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 **Documents**

Reference is made to the following documents; the numbering will be adhered to in the rest of the procedure:

D1 BUZDAR AMAN U: "FULVESTRANT - A NOVEL ESTROGEN RECEPTOR ANTAGONIST FOR THE TREATMENT OF ADVANCED BREAST CANCER",
DRUGS OF TODAY,
vol. 44, no. 9, September 2008 (2008-09), pages 679-692,
ISSN: 1699-3993

D2 ROBERTSON J F R: "Fulvestrant (Faslodex(R)) - How to make a good drug better",
ONCOLOGIST 200707 US LNKD- DOI:10.1634/THEONCOLOGIST.12-7-774,
vol. 12, no. 7, July 2007 (2007-07), pages 774-784,
ISSN: 1083-7159

D3 "Comparison of Fulvestrant (Faslodex) 250 mg and 500 mg in postmenopausal women with oestrogen receptor-positive advanced breast cancer progressing or relapsing after previous endocrine therapy.",
Clinicaltrials.gov
, 20 May 2009 (2009-05-20),
Retrieved from the Internet:
URL:http://clinicaltrials.gov/archive/NCT00099437/2009_05_20
[retrieved on 2010-11-26]

D4 Di Leo A et al.: "CONFIRM: A Phase III, Randomized, Parallel-Group Trial Comparing Fulvestrant 250 mg vs Fulvestrant 500 mg in Postmenopausal Women with Estrogen Receptor-Positive Advanced Breast Cancer.",
Cancer Res,
vol. 69, no. 24 Suppl 3, 25, 15 December 2009 (2009-12-15), pages 1-1,
DOI: 10.1158/0008-5472.SABCS-09-25
Retrieved from the Internet:

URL:http://cancerres.aacrjournals.org/cgi/content/abstract/69/24_MeetingAbstracts/25
[retrieved on 2010-12-01]

1.1 Unless indicated, reference is made to the passages indicated in the international search report.

2 Novelty (Art. 33(2) PCT)

2.1 Both D1 and D2 report on the results of the EFECT study. This study evaluated fulvestrant administered in a loading-dose regimen (500 mg at day 0, 250 mg at days 14 and 28, and then 250 mg every 28 days thereafter) for the treatment of postmenopausal women with oestrogen receptor positive advanced breast cancer progressing or relapsing after previous endocrine therapy. The results indicate that the treatment is effective and safe. Since a dose of 500 mg fulvestrant is used in this study, D1 and D2 take away the novelty of present claim 1. The subject-matter of dependent claims 5-8 is also disclosed in D1 or D2 and is thus also not novel (see in particular page 684 in D1 and page 778, column 1, paragraph 2 in D2).

Claim 9 concerns the result of a comparison which has not been done in the EFECT study. However, this result does not characterize the claimed use (fulvestrant at 500 mg for the treatment of postmenopausal women with oestrogen receptor positive breast cancer progressing or relapsing after previous endocrine therapy). Therefore, this claim does not comprise any feature that further characterizes the use defined in claim 1 and anticipated in D1 and D2. Consequently, claim 9 is also not novel in the light of D1 or D2.

3 Inventive Step (Art. 33(3) PCT)

D1-D3 all mention the CONFIRM study, which evaluates the use of fulvestrant at 500 mg/month) for the treatment of postmenopausal women with oestrogen receptor positive advanced breast cancer progressing or relapsing after previous endocrine therapy with anti-oestrogen hormonal treatment such as tamoxifen or an aromatase inhibitor. Therefore, these documents are the closest prior art for the claimed subject-matter. The difference with present claims 1-9 is that the results of this study are not mentioned in D1-D3. The **problem** can therefore be defined as to provide a regimen for the treatment of

breast cancer progressing or relapsing after previous endocrine therapy in postmenopausal women. The **solution** given in present claims 1-9 is to use fulvestrant at 500mg. Since this solution is already mentioned in D1-D3, the person skilled in the art only needed to perform the instructions given in D1-D3 to obtain the results included in the present application. This does not involve an inventive step and Art. 33(3) PCT is thus not fulfilled.

4 **Industrial applicability (Art. 33(4) PCT)**

Present claims 1-9 are susceptible of industrial application and thus do not contravene Art. 33(4) PCT.

Re Item VI

Certain documents cited

The current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not the case, D4 could become relevant for the questions of novelty and/or inventive step of the present patent application.

Re Item VIII

Certain observations on the international application

5 The term "advanced" in "advanced breast cancer" in claim 1 is not considered to be clear. This term is vague and there is no general accepted definition in the field, so that the scope of the claim is uncertain. The description does not provide any further clarification of the term. For the purpose of the present opinion, this term has been ignored.

6 It appears that the word "whereby" in present claim 9 had to be deleted.

Electronic Patent Application Fee Transmittal

Application Number:	12285887
Filing Date:	15-Oct-2008
Title of Invention:	Formulation
First Named Inventor/Applicant Name:	John R. Evans
Filer:	Carlos M. Tellez
Attorney Docket Number:	11285.0056-00000

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

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:	12500599
Application Number:	12285887
International Application Number:	
Confirmation Number:	1199
Title of Invention:	Formulation
First Named Inventor/Applicant Name:	John R. Evans
Customer Number:	22852
Filer:	Carlos M. Tellez
Filer Authorized By:	
Attorney Docket Number:	11285.0056-00000
Receipt Date:	09-APR-2012
Filing Date:	15-OCT-2008
Time Stamp:	17:18:20
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	3893
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1		11285-0056-00000--09-APR-2012--Response_20-Mar-2012_OA.pdf	110796 ef73257122a429e023f5524934f990a0713936f1	yes	4

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Applicant Arguments/Remarks Made in an Amendment	2	4

Warnings:

Information:

2		11285-0056-00000--09-APR-2012--IDS-SB-08.pdf	130853 d001d0d3f802db7b45efba454f210ffd85929b3c	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:

3		11285-0056-00000-09-APR-2012--WO-2011-012885--spec_for-0059_with_ISR.pdf	1122686 dbb1b7f1640ac4a25c243083dd978bb8794303bc	yes	27

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Foreign Reference	1	25
	Non Patent Literature	26	27

Warnings:

Information:

4	Non Patent Literature	11285-0056-00000-09-APR-2012--Fulvestrant-Buzdar.pdf	877405 6e842fa192b8e62c613a5f0b7382c9ed29c2787e	no	14

Warnings:

Information:

5	Non Patent Literature	11285-0056-00000--09-APR-2012-ComparisonofFulvestrant.pdf	103280 1dc5c1fd7b3c3cee08cb7060c69ef6da5c811a69	no	3

Warnings:

Information:					
6	Non Patent Literature	11285-0056-00000--09-APR-2012--DiLeo.pdf	75882 ae06797d8cbf7b1e30df9dcc0c314a3ffb978f73	no	2
Warnings:					
Information:					
7	Non Patent Literature	11285-0056-00000-09-APR-2012--IPRP_related_app-0059.pdf	281691 488c6a22ae56ca12023dcf82377994633d06d146	no	7
Warnings:					
Information:					
8	Non Patent Literature	11285-0056-00000--09-APR-2012--EP12501383rdpartyobservationsreMcLeskeypaper.pdf	1813766 de1d96bb02a6281ed8113271edd52b9c3a8c1c75	no	30
Warnings:					
Information:					
9	Non Patent Literature	11285-0056-00000--09-APR-2012--EP205138Appealstatement27Feb2012.pdf	2988672 3a22cf51f7e2e38617325437d48f9f47643c379e	no	82
Warnings:					
Information:					
10	Fee Worksheet (SB06)	fee-info.pdf	29656 e0e62a09a961af8a5964380985546f778384f3b6	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	7534687		

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.

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/285,887	Filing Date 10/15/2008	☐ To be Mailed

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☐		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	04/09/2012	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 30	= 0	X $ =		OR	X $60=	0
	Independent (37 CFR 1.16(h))	* 2	Minus	***8	= 0	X $ =		OR	X $250=	0
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/MARGARET BYARS/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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UNITED STATES DEPARTMENT OF COMMERCE
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NOTICE OF ALLOWANCE AND FEE(S) DUE

22852 7590 06/04/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

EXAMINER	
HUI, SAN MING R	
ART UNIT	PAPER NUMBER
1628	

DATE MAILED: 06/04/2012

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	10/15/2008	John R. Evans	11285.0056-00000	1199

TITLE OF INVENTION: FORMULATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1740	$300	$0	$2040	09/04/2012

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

22852 7590 06/04/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	10/15/2008	John R. Evans	11285.0056-00000	1199

TITLE OF INVENTION: FORMULATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1740	$300	$0	$2040	09/04/2012

EXAMINER	ART UNIT	CLASS-SUBCLASS
HUI, SAN MING R	1628	514-177000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
- ❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	10/15/2008	John R. Evans	11285.0056-00000	1199

22852 7590 06/04/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

EXAMINER	
HUI, SAN MING R	

ART UNIT	PAPER NUMBER
1628	

DATE MAILED: 06/04/2012

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 248 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 248 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No.	Applicant(s)	
	12/285,887	EVANS ET AL.	
	Examiner	Art Unit	
	SAN-MING HUI	1628	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *4/9/2012*.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *24,26,27,29,30,32,34-36,38,39,41,42,44,46,47 and 54-57*.
4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☒ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☒ Certified copies of the priority documents have been received in Application No. *10/872784* .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 4/9/12, 6/4/12
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

/San-ming Hui/
Primary Examiner, Art Unit 1628

DETAILED ACTION

Applicant's response filed 4/9/2012 has been entered. Claims 24, 26, 27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46, 47, and 54-57 are pending.

The outstanding double patenting rejection is withdrawn in view of the terminal disclaimer filed 1/17/2012.

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance: The outstanding rejection is withdrawn and therefore, the pending claims 24, 26, 27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46, 47, and 54-57 are in condition of allowance.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Allowable Subject Matter

Claims 24, 26, 27, 29, 30, 32, 34-36, 38, 39, 41, 42, 44, 46, 47, and 54-57 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAN-MING HUI whose telephone number is (571)272-0626. The examiner can normally be reached on Mon - Fri from 9:00 to 5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brandon Fetterolf can be reached on (571) 272-2919. The fax phone

number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

San-ming Hui
Primary Examiner
Art Unit 1628

/San-ming Hui/
Primary Examiner, Art Unit 1628

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12285887	EVANS ET AL.
	Examiner	Art Unit
	San-ming Hui	1628

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	12/19/2010	09/06/2011	03/09/2012	04/17/2012					
	1	✓								
	2	✓								
	3	✓								
	4	✓								
	5	✓								
	6	✓								
	7	✓								
	8	✓								
	9	✓								
	10	✓								
	11	✓								
	12	✓								
	13	✓								
	14	✓								
	15	✓								
	16	✓								
	17	✓								
	18	✓								
	19	✓								
	20	✓								
	21	✓								
	22	✓								
	23	✓								
	24		✓	✓	=					
	25		✓	-						
	26		✓	✓	=					
	27		✓	✓	=					
	28		✓	-						
	29		✓	✓	=					
	30		✓	✓	=					
	31		✓	-						
	32		✓	✓	=					
	33		✓	-						
	34		✓	✓	=					
	35		✓	✓	=					
	36		✓	✓	=					

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12285887	EVANS ET AL.
	Examiner	**Art Unit**
	San-ming Hui	1628

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	12/19/2010	09/06/2011	03/09/2012	04/17/2012					
	37		✓	-						
	38		✓	✓	=					
	39		✓	✓	=					
	40		✓	-						
	41		✓	✓	=					
	42		✓	✓	=					
	43		✓	-						
	44		✓	✓	=					
	45		✓	-						
	46		✓	✓	=					
	47		✓	✓	=					
	48		✓	-						
	49		✓	-						
	50		✓	-						
	51		✓	-						
	52		✓	-						
	53		✓	-						
	54			✓	=					
	55			✓	=					
	56			✓	=					
	57			✓	=					

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12285887	EVANS ET AL.
	Examiner	**Art Unit**
	San-ming Hui	1628

SEARCHED

Class	Subclass	Date	Examiner
514	177, 178	12/19/10	SH
514	177, 178	9/6/11	SH
514	177, 178	3/9/12	SH
514	177, 178	4/17/12	SH

SEARCH NOTES

Search Notes	Date	Examiner
EAST and inventor search in PALM	12/19/10	SH
update search in EAST and inventor search in PALM	9/6/11	SH
EAST in EAST and inventor search in PALM	3/9/12	SH
update search in EAST and inventor search in PALM	4/17/12	SH

INTERFERENCE SEARCH

Class	Subclass	Date	Examiner
514	177, 178	4/17/12	SH

INFORMATION DISCLOSURE CITATION (Use several sheets if necessary)	Attorney Docket No. **056291-5004-02**	Application No. **12/285,887**
	Applicants: **John R. EVANS *et al.***	
PTO Form 1449 June 4, 2009	Filing Date: **October 15, 2008**	Group Art Unit: **1617**

U.S. PATENT DOCUMENTS

Initial		Document No.	Date	Name	Class	Sub-Class	Filing Date
	1.	US 3,164,520	January 5, 1965	Huber			
	2.	US 4,212,863	July 15, 1980	Cornelius			
	3.	US 4,388,307	June 14, 1983	Cavanak			

FOREIGN PATENT DOCUMENTS

		Document No.	Date	Country	Class	Sub-Class	Translation
	4.	EP 0310542A1	April 5, 1989	EPO			Yes

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

	5.	P.K. Gupta and G.A. Brazeau (eds). *Injectable Drug Development:Techniques to Reduce Pain and Irritation.* Chapters 11 & 17 Interpharm Press, Denver, Colorado (1999)
	6.	P.V. Lopatin, V. P. Safonov, T. P. Litvinova and L. M. Yakimenko. Use of nonaqueous solvents to prepare injection solutions. *Pharm. Chem. J.* ***6***:724-733 (1972)
	7.	S. Nema, R.J. Washkuhn, and R.J. Brendel. Excipients and their use in injectable products. *PDA J. Pharm. Sci. Technol.* **51**:166-71 (1997)
	8.	*Physicians' Desk Reference (27th edition).* 1277-1278, 1350-1354, 1391-1392 Medical Economics Company, Oradell, NJ (1973)
	9.	M. F. Powell, T. Nguyen, and L. Baloian. Compendium of excipients for parenteral formulations. *PDA J. Pharm. Sci. Technol.* **52**:238–311 [pages 238-255 provided] (1998)
	10.	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) -Part I. *PDA J. Pharm. Sci. Technol.* **53**:324–349 (1999)
	11.	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part II *PDA J. Pharm. Sci. Technol.* **54**:69–96 (2000)
	12.	R. G. Strickley. Parenteral formulations of small molecule therapeutics marketed in the United States (1999) - Part III. *PDA J. Pharm. Sci. Technol.* **54**:152–169 (2000)
	13.	Y.C. J. Wang and R. R. Kowal. Review of excipients and pH's for parenteral products used in the United States. *J. Parenteral Drug Assoc.* **34**:452–462 (1980).

Examiner /San Ming Hui/	Date Considered /S.H./

Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

DB1/63065447.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.H./

Issue Classification

Application/Control No.	Applicant(s)/Patent Under Reexamination
12285887	EVANS ET AL.
Examiner	**Art Unit**
SAN-MING HUI	1628

ORIGINAL		INTERNATIONAL CLASSIFICATION									
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED				
514	177	A	6	1	K	31 / 56 (2006.01.01)					

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
514	178				

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	24		40	19	56										
	25	12	41	20	57										
2	26	13	42												
3	27		43												
	28	14	44												
4	29		45												
5	30	15	46												
	31	16	47												
6	32		48												
	33		49												
7	34		50												
8	35		51												
9	36		52												
	37		53												
10	38	17	54												
11	39	18	55												

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	20	
/SAN-MING HUI/ Primary Examiner.Art Unit 1628	04/17/2012	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	12/285,887
				Filing Date	October 15,2008
				First Named Inventor	John R. EVANS
				Art Unit	1628
				Examiner Name	HUI, San Ming R.
Sheet	1	of	1	Attorney Docket Number	11285.0056-00000

U.S. PATENTS AND PUBLISHED U.S. PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	U.S. Application No. 13/387,584	Filing date: 27-Jan-2012	DIMERY et al.	

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

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 Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[6]
	2	WO 2011/012885	03-Feb-2011	AstraZeneca UK Ltd.		

NONPATENT 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.	Translation[6]
	3	Buzdar, A. U., "Fulvestrant - A novel estrogen receptor antagonist for the treatment of advanced breast cancer," *Drugs of Today*, 44(9):679-692 (2008).	
	4	"Comparison of fulvestrant (faslodex) 250 mg and 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer progressing or relapsing after previous endocrine therapy," Clinicaltrials.gov (20-May-2009) retrieved 24-Jan- 2012.	
	5	Di Leo A., et al., "Confirm: a phase III, randomized, parallel-group trial comparing fulvestrant 250 mg vs fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer," Cancer Res., 69(24) Supp. 3, (2009).	
	6	International Search Report for PCT Application No. PCT/GB10/51228 (WO 2011/012885) mailed December 20, 2012.	
	7	International Preliminary Report on Patentability for PCT Application No. PCT/GB10/51228 (WO 2011/012885) mailed December 20, 2012.	
	8	Documents from the prosecution of European Application No. 01900186.6 (EP 1 250 138) dated December 15, 2011.	
	9	Documents from the prosecution of European Application No. 01900186.6 (EP 1 250 138) dated February 27, 2012.	

Examiner Signature	/San Ming Hui/	Date Considered	04/17/2012

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /S.H./

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S264	86836	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S265	459	fulvestrant and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S266	2864	oil and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S267	3	"4659516".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S268	7	"346014".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S269	16301	(benzyl adj benzoate) or (phenylmethyl adj benzoate)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S270	1895366	solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S271	8503	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S272	4	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (estrogen or estradiol or estrone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S273	7	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (testosterone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S274	14	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S275	2008	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) and (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47

S276	2	"6774122".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S277	982	514/177.ccls.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S278	1426	514/178.ccls.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S279	2183348	castor oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S280	86836	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S281	459	fulvestrant and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S282	2864	oil and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S283	16301	(benzyl adj benzoate) or (phenylmethyl adj benzoate)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S284	1895366	solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S285	8503	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S286	7	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (testosterone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S287	14	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S288	2008	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) and (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S289	86836	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S290	5214	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT;	OR	ON	2012/04/16 14:47

			IBM_TDB			
S291	2934	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S292	1513	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S293	3	((((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent) same steroid	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S294	3712	fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S295	3712	fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S296	86836	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S297	459	fulvestrant and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S298	2864	oil and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S299	3	"4659516".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S300	7	"346014".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S301	16301	(benzyl adj benzoate) or (phenylmethyl adj benzoate)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S302	1895366	solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S303	8503	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S304	4	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (estrogen or estradiol or estrone)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S305	7	(((benzyl adj benzoate) or	US-PGPUB;	OR	ON	2012/04/16

		(phenylmethyl adj benzoate)) same solvent) same (testosterone)	USPAT; EPO; JPO; DERWENT; IBM_TDB			14:47
S306	14	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) same (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S307	2008	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same solvent) and (steroid)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S308	86836	castor adj oil	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S309	5214	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) and (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S310	2934	((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S311	1513	(((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S312	3	((((benzyl adj benzoate) or (phenylmethyl adj benzoate)) same (castor adj oil)) same solvent) same steroid	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S313	3712	fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S314	97131	breast adj cancer	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S315	2541	breast adj cancer and fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S316	368	breast adj cancer same fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47
S317	1579	cancer same fulvestrant	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2012/04/16 14:47

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	973	514/177.ccls.	US-PGPUB; USPAT	OR	ON	2012/04/17 09:28
L2	1395	514/178.ccls.	US-PGPUB; USPAT	OR	ON	2012/04/17 09:28

4/17/2012 9:28:47 AM
C:\Users\shui\Documents\EAST\Workspaces\12-285887.wsp

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	10/15/2008	John R. Evans	11285.0056-00000	1199

22852 7590 07/13/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

EXAMINER	
HUI, SAN MING R	

ART UNIT	PAPER NUMBER
1628	

MAIL DATE	DELIVERY MODE
07/13/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

UNITED STATES PATENT AND TRADEMARK OFFICE

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Application No. : 12285887
Applicant : Evans
Filing Date : 10/15/2008
Date Mailed : 07/13/2012

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given 1 month(s) from the mail date of this Notice, or the time remaining from the Notice of Allowance and Fee(s) Due, whichever is longer, within which to respond.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED. **This period for reply is NOT extendable under 37 CFR 1.136(a).**

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee,Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Patricia Pacenski/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 12285887

IDENTIFICATION OF APPLICATION DEFICIENCIES

☐ Applicant must provide legible text for the following item(s).

- ☐ Specification filed , page(s) .
- ☐ Claims filed , claim(s) .
- ☐ Oath/declaration filed .
- ☐ Other: .

☒ Applicant must provide missing information on the following page(s) of the specification by amending the specification to add the missing text. No new matter may be added. line 1 of page 6 appears to be incomplete

☐ The specification refers to one or more applications by attorney docket number and does not show the U.S. application number(s). Applicant must supply the U.S. application number in place of each attorney docket number.

☐ Applicant must provide an Abstract of the Disclosure.

☐ Applicant has submitted a DECLARATION (37 CFR 1.63) FOR A UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76) (e.g., form PTO/SB/01A). The Application Data Sheet, however, is not present with the filed application. Applicant must submit an Application Data Sheet or file a new oath or declaration (e.g., PTO/SB/01) executed by the inventors and containing the information required in 37 CFR 1.63.

☐ Applicant must provide an executed declaration.

☐ Applicant must provide the missing page(s) of the oath/declaration or Application Data Sheet filed

☐ Applicant must provide a declaration signed by inventor(s) .

☐ The oath/declaration filed shows non-initialed and/or non-dated alterations. Applicant must file a new oath/declaration in compliance with 37 CFR 1.67(a).

☐ Applicant(s) in the latest-filed oath/declaration or Application Data Sheet (ADS) did not show the inventor's residence at all, or did not show both a city and state in the U.S. inventor's residence, or did not show both a city and country in the non-U.S. inventor's residence. Applicant must supply an oath/declaration or Application Data Sheet (ADS) that shows each U.S. inventor's city and state of residence and each non-U.S. inventor's city and country of residence.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
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or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

22852 7590 06/04/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	10/15/2008	John R. Evans	11285.0056-00000	1199

TITLE OF INVENTION: FORMULATION

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1740	$300	$0	$2040	09/04/2012

EXAMINER	ART UNIT	CLASS-SUBCLASS
HUI, SAN MING R	1628	514-177000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Finnegan, Henderson,
2 Farabow, Garrett &
3 Dunner LLP

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: AstraZeneca AB

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Södertälje, Sweden

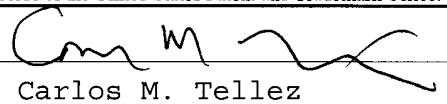
Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☒ Issue Fee
☒ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☐ A check is enclosed.
☒ Payment by credit card. ~~Form PTO-2038 attached~~ EFS Web
☒ The Director is hereby authorized to charge ~~the required fee,~~ any deficiency, or credit any overpayment, to Deposit Account Number 06-0916 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature 
Date September 4, 2012

Typed or printed name Carlos M. Tellez
Registration No. 48,638

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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PATENT
Attorney Docket No. 11285.0056-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	) Group Art Unit: 1628
John R. Evans et al.	) Examiner: HUI, San Ming R.
Application No.: 12/285,887	) Confirmation No.: 1199
Filed: October 15, 2008	) **Mail Stop Issue Fee**
For: FORMULATION	) VIA EFS-WEB

Commissioner for Patents
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Sir:

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

This paper is a reply to the Notice to File Corrected Application Papers mailed July 13, 2012 ("Notice"). The Notice requested that Applicants "provide missing information . . . by amending the specification to add the missing text" on line 1, page 6 of the specification.

The missing text, however, was missing at the time of filing and is also missing from the parent and grandparent applications from which this application claims priority (Application Nos. 10/872, 784 and 09/756,291, respectively). Thus, the missing text cannot be added at this point and **Applicants are not amending the specification in this Response**. Applicants point out, however, that the missing text does not affect the disclosure of the invention claimed in the application.

Please grant any extensions of time required to enter this response and charge any required fees not included with this Response to Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: September 4, 2012

By: _____

Carlos M. Téllez
Reg. No. 48,638
(202) 408-4123

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Application No. : 12285887
Applicant : Evans
Filing Date : 10/15/2008
Date Mailed : 07/13/2012

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given 1 month(s) from the mail date of this Notice, or the time remaining from the Notice of Allowance and Fee(s) Due, whichever is longer, within which to respond.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED. **This period for reply is NOT extendable under 37 CFR 1.136(a).**

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee,Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Patricia Pacenski/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 12285887

IDENTIFICATION OF APPLICATION DEFICIENCIES

☐ Applicant must provide legible text for the following item(s).

- ☐ Specification filed , page(s) .
- ☐ Claims filed , claim(s) .
- ☐ Oath/declaration filed .
- ☐ Other: .

☒ Applicant must provide missing information on the following page(s) of the specification by amending the specification to add the missing text. No new matter may be added. line 1 of page 6 appears to be incomplete

☐ The specification refers to one or more applications by attorney docket number and does not show the U.S. application number(s). Applicant must supply the U.S. application number in place of each attorney docket number.

☐ Applicant must provide an Abstract of the Disclosure.

☐ Applicant has submitted a DECLARATION (37 CFR 1.63) FOR A UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76) (e.g., form PTO/SB/01A). The Application Data Sheet, however, is not present with the filed application. Applicant must submit an Application Data Sheet or file a new oath or declaration (e.g., PTO/SB/01) executed by the inventors and containing the information required in 37 CFR 1.63.

☐ Applicant must provide an executed declaration.

☐ Applicant must provide the missing page(s) of the oath/declaration or Application Data Sheet filed

☐ Applicant must provide a declaration signed by inventor(s) .

☐ The oath/declaration filed shows non-initialed and/or non-dated alterations. Applicant must file a new oath/declaration in compliance with 37 CFR 1.67(a).

☐ Applicant(s) in the latest-filed oath/declaration or Application Data Sheet (ADS) did not show the inventor's residence at all, or did not show both a city and state in the U.S. inventor's residence, or did not show both a city and country in the non-U.S. inventor's residence. Applicant must supply an oath/declaration or Application Data Sheet (ADS) that shows each U.S. inventor's city and state of residence and each non-U.S. inventor's city and country of residence.

Substituted page 6

described which comprises 50mg of fulvestrant, 400mg of benzyl alcohol and sufficient castor oil to bring the solution to a volume of 1 ml. Manufacture at a commercial scale of a formulation as described in US 5,183,814 will be complicated by the high alcohol concentration. Therefore, there is a need to lower the alcohol concentration in fulvestrant formulations whilst preventing precipitation of fulvestrant from the formulation.

SUMMARY OF THE INVENTION

The invention relates to a novel sustained release pharmaceutical formulation adapted for administration by injection containing the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol, more particularly to a formulation adapted for administration by injection containing the compound 7α-[9-(4,4,5,5,5-pentafluoropentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol in solution in a ricinoleate vehicle which additionally comprises at least one alcohol and a non-aqueous ester solvent which is miscible in the ricinoleate vehicle.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the release profile *in vivo* of the four formulations from the second part of Table 4 below, and shows the effect of the fixed oil component on fulvestrant plasma profile over five days following intramuscular administration in rabbits.

DETAILED DESCRIPTION OF THE INVENTION

Table 2 shows the solubility of fulvestrant in a number of different solvents.

Table 2 - SOLUBILITY OF FULVESTRANT

SOLVENT	SOLUBILITY ($mgml^{-1}$ at 25°C)
Water	0.001
Arachis oil	0.45
Sesame oil	0.58
Castor oil	20
Miglyol 810	3.06

Electronic Patent Application Fee Transmittal

Application Number:	12285887
Filing Date:	15-Oct-2008
Title of Invention:	FORMULATION
First Named Inventor/Applicant Name:	John R. Evans
Filer:	Carlos M. Tellez
Attorney Docket Number:	11285.0056-00000

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

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:				
Utility Appl issue fee	1501	1	1740	1740
Publ. Fee- early, voluntary, or normal	1504	1	300	300

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**2040**

Electronic Acknowledgement Receipt

EFS ID:	13649794
Application Number:	12285887
International Application Number:	
Confirmation Number:	1199
Title of Invention:	FORMULATION
First Named Inventor/Applicant Name:	John R. Evans
Customer Number:	22852
Filer:	Carlos M. Tellez
Filer Authorized By:	
Attorney Docket Number:	11285.0056-00000
Receipt Date:	04-SEP-2012
Filing Date:	15-OCT-2008
Time Stamp:	15:03:13
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$2040
RAM confirmation Number	1646
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Issue Fee Payment (PTO-85B)	11285-0056-00000ISSUEFEEPAYMENT.pdf	36618 4abbd63ba121833238721bbc8c421b8fc4733ea8	no	1
Warnings:					
Information:					
2	Post Allowance Communication - Incoming	11285-0056-00000RESPONSETONOTICEANDCOPYOFNOTICETOFILECORRECTEDAPPLICATION.pdf	78402 d1f784346701d68691d6581e727f6e46e8027f7b	no	6
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	31526 eee9ce3badbbbdf35eb96456cca5ffd70bfdab3d	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	146546		

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.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	10/15/2008	John R. Evans	11285.0056-00000	1199

22852 7590 09/06/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

EXAMINER	
HUI, SAN MING R	

ART UNIT	PAPER NUMBER
1628	

MAIL DATE	DELIVERY MODE
09/06/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Response to Rule 312 Communication	Application No.	Applicant(s)
	12/285,887	JOHN R.
	Examiner	Art Unit

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

1. ☒ The amendment filed on *04 September 2012* under 37 CFR 1.312 has been considered, and has been:

a) ☒ entered.

b) ☐ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

B.Crittenden

Publishing Division

U.S. Patent and Trademark Office
PTOL-271 (Rev. 04-01) Reponse to Rule 312 Communication Part of Paper No.

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UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/285,887	12/11/2012	8329680	11285.0056-00000	1199

22852 7590 11/21/2012

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER
LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 338 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

John R. Evans, Macclesfield, UNITED KINGDOM;
Rosalind U. Grundy, Macclesfield, UNITED KINGDOM;

PATENT
Customer No. 22,852
Attorney Docket No. 11285.0056-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)
	) Application No.: 12/285,887
U.S. Patent No. 8,329,680	)
	) Filed: October 15, 2008
Issue Date: December 11, 2012	)
	) Confirmation No.: 1199
Inventors: John R. EVANS et al.	)
	)
For: FORMULATION	) **EFS-Web Filing**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

FEE ADDRESS FOR MAINTENANCE FEE PURPOSES
IN ACCORDANCE WITH 37 C.F.R. § 1.363

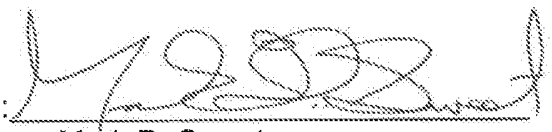
In accordance with the provisions of 37 C.F.R. § 1.363, the fee address set forth below is being supplied for purposes of receiving notices, receipts, and other correspondence relating to the payment of maintenance fees:

Thomson Scientific IP Management Services
300 Franklin Center, 29100 Northwestern Highway
Southfield, Michigan 48034
Payor Number: 00124

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, LLP

Dated: January 17, 2013

By: 
Mark D. Sweet
Reg. No. 41,469
(202) 408-4162

Electronic Acknowledgement Receipt

EFS ID:	14725009
Application Number:	12285887
International Application Number:	
Confirmation Number:	1199
Title of Invention:	FORMULATION
First Named Inventor/Applicant Name:	John R. Evans
Customer Number:	22852
Filer:	Abhay Ashok Watwe/andrea pinkney
Filer Authorized By:	Abhay Ashok Watwe
Attorney Docket Number:	11285.0056-00000
Receipt Date:	17-JAN-2013
Filing Date:	15-OCT-2008
Time Stamp:	13:39:18
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Change of Address	feeaddress.pdf	49101 936c65a9d42a66083d27210c0fbc9b453577275a	no	1

Warnings:	
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Total Files Size (in bytes):	49101

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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.

PATENT
Customer No. 22,852
Attorney Docket No. 11285.0056-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
U.S. Patent No. 8,329,680	Application No.: 12/285,887
Issue Date: December 11, 2012	Filed: October 15, 2008
Inventors: John R. EVANS et al.	Confirmation No.: 1199
For: FORMULATION	**EFS-Web Filing**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

FEE ADDRESS FOR MAINTENANCE FEE PURPOSES
IN ACCORDANCE WITH 37 C.F.R. § 1.363

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Castle Street
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Channel Islands
Customer Number: 000197

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, LLP

Dated: February 15, 2013

By: 

Mark D. Sweet
Reg. No. 41,469
(202) 408-4162

Electronic Acknowledgement Receipt

EFS ID:	14976524
Application Number:	12285887
International Application Number:	
Confirmation Number:	1199
Title of Invention:	FORMULATION
First Named Inventor/Applicant Name:	John R. Evans
Customer Number:	22852
Filer:	Abhay Ashok Watwe/Margie Harris
Filer Authorized By:	Abhay Ashok Watwe
Attorney Docket Number:	11285.0056-00000
Receipt Date:	15-FEB-2013
Filing Date:	15-OCT-2008
Time Stamp:	17:49:31
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Change of Address	FeeAddress.pdf	49405 6cb1e2c68f5730b06c81a99b6de1beb246375150	no	1

Warnings:	
Information:	

Total Files Size (in bytes):	49405

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New International Application Filed with the USPTO as a Receiving Office
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AO 120 (Rev. 08/10)

TO:	**Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313–1450**	**REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK**

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the **U.S. District Court for the District of New Jersey** on the following:
____ Trademarks or **X** Patents. (____ the patent action involves 35 U.S.C. § 292.)

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
3:14–cv–03547–FLW–LHG	6/3/2014	TRENTON, NJ

PLAINTIFF	DEFENDANT
ASTRAZENECA PHARMACEUTICALS LP	SANDOZ INC.

PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 US 6,774,122 B2	August 10, 2004	AstraZeneca AB
2 US 7,456,160 B2	November 25, 2008	AstraZeneca AB
3 US 8,329,680 B2	December 11, 2012	AstraZeneca AB
4 US 8,466,139 B2	June 18, 2013	AstraZeneca AB
5		

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ___ Amendment ___ Answer ___ Cross Bill ___ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1		
2		
3		
4		
5		

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
William T. Walsh	s/ Marlene Kalbach	6/3/2014

Copy 1—Upon initiation of action, mail this copy to Director Copy 3—Upon termination of action, mail this copy to Director
Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy