liraglutide increased absolute and relative weight of pancreas at all doses in both sexes in 28-day and 52-week studies (HEM ≥ 1X). In 5 mg/kg high dose monkeys in the 52-week study, increased pancreas weight was due to an increase in the mass of pancreas exocrine cells and ducts, but liraglutide had no effect on beta cell mass.

Postmarketing cases of acute pancreatitis have been reported in exenatide treated patients. There was a numerical imbalance in the number of reported pancreatitis cases in clinical trials of liraglutide with a larger number of cases and a higher rate in liraglutide treated groups.

Other Effects

In the 104-week carcinogenicity study in mice, liraglutide caused tubular cystic hyperplasia in thymus in males at all doses (0.03, 0.2, 1, 3 mg/kg/day, HEM ≥ 0.2X) and in females at ≥ 0.2 mg/kg/day (HEM ≥ 2X). In the 52-week toxicity study in monkeys, group mean relative thymus weight (normalized to body weight) was 21.5 – 26.8% lower than controls in males at all liraglutide doses (0.05, 0.5, 5 mg/kg/day) (HEM ≥ 1X) with correlative microscopic findings of atrophy.

In the 104 week mouse carcinogenicity study, centrilobular hypertrophy of hepatocytes and diffuse centrilobular hepatocyte vacuolation occurred in males at all liraglutide doses (0.03, 0.2, 1, 3 mg/kg/day, HEM ≥ 0.2X)). In the same study, femoro-tibial degenerative joint disease occurred in male and female mice at all doses (HEM ≥ 0.2X).

Genetic Toxicity

Liraglutide was not mutagenic or clastogenic, with or without metabolic activation, in an Ames bacterial mutagenicity assay or chromosomal aberrations assay in human peripheral blood lymphocytes. Liraglutide did not induce micronuclei in bone marrow polychromatic erythrocytes from rats treated with up to 30 mg/kg liraglutide for 4 days or up to 1 mg/kg (HEM 11X) for 28 days.

Carcinogenicity

In 2-year life-time exposure carcinogenicity studies in mice and rats, liraglutide was a non-genotoxic, multisex, multispecies rodent carcinogen causing thyroid C-cell tumors in male and female rats and mice and fibrosarcomas on the dorsal skin and subcutis in male mice.

In the mouse carcinogenicity study, the NOAEL for neoplastic findings was 0.2 mg/kg/day liraglutide (safety margin 2) based on thyroid C-cell adenomas in males and females and combined C-cell adenomas and carcinomas in females at ≥ 1 mg/kg/day liraglutide (HEM ≥ 10X). Liraglutide caused focal C-cell hyperplasia, a preneoplastic lesion, at ≥ 0.2 mg/kg/day in males and females (HEM ≥ 2X). C-cells secrete calcitonin, and in mice, plasma calcitonin increased at ≥ 0.2 mg/kg/day and it was a biomarker for increased C-cell focal hyperplasia and tumors. In males, fibrosarcomas on the dorsal skin and subcutis occurred at 3 mg/kg/day liraglutide. Equivocal finding of dose-related dorsal skin and subcutis rhabdomyosarcoma and injection site fibrosarcomas in males, and incidences in the 3 mg/kg/day group were above the historical control range for both tumors, but the increased incidence for either finding never reached statistical significance in any liraglutide group. Dorsal skin and subcutis tumors were likely related to the high local concentration of liraglutide at or near injection sites, so comparison of systemic exposure is not relevant for risk assessment. The liraglutide concentrations in high dose drug formulation was 0.6 mg/mL, 10-times lower than the 6 mg/mL concentration in the clinical formulation.

In the rat carcinogenicity study, liraglutide dose-dependently caused thyroid C-cell adenomas at ≥ 0.25 mg/kg/day in males (HEM ≥ 2X) and at ≥ 0.075 mg/kg/day in females (HEM ≥ 0.5X), C-cell carcinomas at 0.75 mg/kg/day in males (HEM 8X), and combined C-cell adenomas or carcinomas at ≥ 0.25 mg/kg/day in males (HEM ≥ 2X) and at ≥ 0.075 mg/kg in females (HEM ≥ 0.5X). The incidence of C-cell carcinomas, a rare tumor in rats, was above concurrent and historical controls at ≥ 0.075 mg/kg/day liraglutide in males (HEM ≥ 0.5X) and at ≥ 0.25 mg/kg/day in females (HEM ≥ 2X).

To determine human relevance of liraglutide-induced rodent thyroid C-cell tumors, Novo Nordisk proposed a novel mode-of-action based on drug-induced, GLP-1R-mediated calcitonin secretion and synthesis driving C-cell hyperplasia with progression of hyperplasia to tumors, and they performed an

extensive series of mechanistic studies to evaluate it. However, mechanistic studies did not adequately support the proposed mode of action. CDER's Executive Carcinogenicity Assessment Committee (December 2008 meeting) and a large majority of members from a April 2009 Advisory Committee convened to evaluate the safety of liraglutide both concluded there was insufficient evidence to determine that liraglutide-induced C-cell tumors in mice and rats are not relevant to humans.

Until recently, liraglutide was the only investigational or marketed drug known to cause C-cell tumors in both mice and rats, but data from other marketed and investigational GLP-1R agonists suggest persistent GLP-1R activation cause C-cell tumors in both species. The human relevance of liraglutide-induced thyroid C-cell tumors in rodents is unknown.

Reproductive and Developmental Toxicity

Reproductive Toxicity

In a definitive combined fertility and embryofetal developmental toxicity study in rats, the NOAEL for reproductive toxicity was 1 mg/kg liraglutide in males (HEM 11X) and 0.25 mg/kg in females (HEM 2X). .Although liraglutide decreased the absolute weight of seminal vesicles, prostate, and epididymis at $\geq$ 0.25 mg/kg (estimated HEM $\geq$ 2X), it had no effect on reproductive performance. Furthermore, in a 13-week toxicity study in rats, up to 1 mg/kg/day liraglutide (HEM 14X) did not increase abnormalities of eosin-stained sperm from cauda epididymis. Four weeks of treatment with 1 mg/kg/day liraglutide decreased CYP2A1 (hepatic steroid hydroxylase, testosterone 7 α-hydroxylase activity) ~2-fold in liver of male rats, but a relationship between decreased liver CYP2A1 activity and decreased weight of male reproductive organs in rats was not established. Decreased testosterone hydroxylase activity should increase circulating levels of testosterone. In the definitive combined fertility and embryofetal development study, liraglutide increased early embryonic deaths in females at 1 mg/kg (HEM 11X).

In the 104-week mouse carcinogenicity study, lymphocyte infiltration occurred in seminal vesicles at $\geq$ 0.03 mg/kg/day liraglutide (HEM $\geq$ 0.2X).

Developmental Toxicity

Distribution and excretion studies show rat and rabbit fetuses were exposed to liraglutide *in utero* and liraglutide was excreted intact in milk of lactating rats. Although intact liraglutide was secreted in milk, since it's a lipidated peptide, oral bioavailability was likely to be very low in nursing pups. In a definitive combined fertility and embryofetal development toxicity study in rats, the maternal NOAEL was 0.25 mg/kg liraglutide (safety margin 2) due to clinical signs of toxicity (hunched posture, rolling gate) at 1 mg/kg (HEM 11X). The NOAEL for fetal toxicity was < 0.1 mg/kg liraglutide based on fetal abnormalities of displaced kidneys, displaced azygous vein, and small additional ossified area within the cranial structure or fontanel at $\geq$ 0.1 mg/kg. A more complete state of ossification in fetuses from liraglutide treated groups compared to controls was noted. Major fetal abnormalities were misshaped oropharynx and/or narrowed opening to the larynx at 0.1 mg/kg and umbilical hernia at 0.1 and 0.25 mg/kg, but they occurred without relation to liraglutide dose.

In a definitive embryofetal development toxicity study in New Zealand White rabbits, all tested doses yielded estimated liraglutide plasma $AUC_{0\text{-}24h}$ below clinical exposure. The maternal NOAEL was 0.05 mg/kg, the highest dose tested. Reduced food consumption, body weight gain, and body weight were considered pharmacologic effects in pregnant females, but reduced maternal weight may have impacted fetal development andreducing fetal weight. The NOAEL for fetal toxicity was < 0.01 mg/kg liraglutide based on decreased fetal weight compared to controls, dose-related increased fetal and litter incidence in total fetal malformations (2.1%, 3.7%, 5.7%, and 7.6% of fetuses and 18%, 30%, 35%, and 32% of litters affected by major abnormalities at 0, 0.01, 0.025, and 0.05 mg/kg/day liraglutide, respectively), fetal malformations (microopthalmia with or without retinal fold, forelimb flexure, right kidney represented by small area of tissue with attached cyst, curved scapula), and minor abnormalities (bilobed or bifurcated gall bladder, intermediate lung lobe absent, jugals fused to maxilla, superior angle or lamina of axis

incompletely ossified, slight downward pelvic shift, slight asymmetric alignment of pelvic bones) at 0.01 mg/kg or ≥ 0.01 mg/kg. Fetal malformations occurred at 0.025 mg/kg (2 fetuses from 2 different litters with hydrocephaly, hepatic duct diverticulum, displaced or herniated umbilica, branchyury, dilated pulmonary trunk with incomplete aortic arch and malrotated heart, displaced umbilicus with part of the intestines fused to the umbilical vein, and split sternum). A minor abnormality of an additional liver lobe within the median cleft also occurred at 0.025 mg/kg. At 0.05 mg/kg, Malformations were connected parietal bones in 5 fetuses from 1 litter and dilated ascending aorta with narrow pulmonary trunk. Minor abnormalities at 0.05 mg/kg were corneal opacity, esophageal cyst, and kinked tail.

Fetal abnormalities occurred in rats and rabbits at low multiples of human exposure (based on estimated AUC in pregnant rats or rabbits). Herniated umbilica, a malformation, occurred in both rats and rabbits, but the incidence was not related to liraglutide dose in either species. Increased fetal bone ossification was likely due to liraglutide treatment.

Prenatal and Postnatal Development Toxicity

Prenatal and postnatal toxicity of liraglutide was assessed in a multigenerational study in rats. The F_0 maternal NOAEL was < 0.1 mg/kg liraglutide (HEM < 1X) based on clinical signs of toxicity at all doses. The NOAEL for F_0 reproductive toxicity was < 0.1 mg/kg liraglutide (HEM < 1X) based on a dose-related increased incidence of continuing gestation to day 22 (33%, 58%, 67%, and 96% of litters delivered on day 22 at 0, 0.1, 0.25, and 1 mg/kg liraglutide, respectively) with increased gestation duration from 21.3 to 22.0 days at 1 mg/kg (HEM 11X). Liraglutide had no effect on maternal behavior or F_1 pup survival, post-natal development prior to weaning (physical development, functional development, or sexual maturation), or post-weaning sexual maturation rate. The NOAEL for postnatal toxicity in the F_1 generation was < 0.1 mg/kg based on dose-dependent decreased body weight from lactation day 7 to day 21. In the postweaning period, body weight of F_1 rats was lower than controls at all liraglutide doses (≥ 0.1 mg/kg) in males from postpartum day 7 to week 16 and in females at 0.1 and 1 mg/kg from postpartum day 7 to week 10. In weaned F_1 rats, clinical observations of greasy coat occurred at ≥ 0.1 mg/kg in males and females. Bleeding scab and agitated behavior occurred in weaned 1 mg/kg F_1 males and an increased incidence of scabs in males and females treated with 1 mg/kg liraglutide was noted at necropsy. The NOAEL for fertility, mating performance, or reproductive performance of F_1 rats was 1 mg/kg liraglutide (administered to F_0 rats only). The NOAEL for development toxicity of F_2 rats was 1 mg/kg (administered to F_0 rats only).

Qualification of Impurities and Excipients

Liraglutide, the active pharmaceutical ingredient (API) in Victoza, is produced by acylating recombinant human Arg^{34}GLP-1(7-37), produced in *Saccharomyces cerevisiae*, with hexadecanoic acid (palmitic acid) at lysine 26 using a glutamate linker to yield liraglutide ($N^{\varepsilon 26}$-(N-hexadecanoyl-L-γ-glutamyl)-Arg^{34}GLP-1(7-37)). Impurities in liraglutide were [redacted] -related impurities, [redacted] -related impurities, (impurities [redacted] impurities. b(4)

Liraglutide-related impurities were categorized as liraglutide-related impurities A, B, or C, or other [redacted] related impurities based on HPLC elution characteristics relative to liraglutide. [redacted] In [redacted] degradation studies, elevated temperatures and humidity increase [redacted] -related impurities ([redacted] fold) and [redacted] related impurities [redacted] fold), category B impurities ([redacted] fold), and total impurities ([redacted] fold). Exposing the drug substance (packaged in glass vials) to light [redacted] molecular weight protein [redacted] fold), other [redacted] impurities ([redacted] fold), other [redacted] impurities [redacted] fold), and total impurities ([redacted] fold), but there were no unique photo-degradation products. Changes in the drug substance manufacturing process during development resulted in some changes in the impurity profile. Repeat-dose toxicity of drug substance impurities in late-stage development batch that had undergone forced [redacted] were assessed in a 4-week study in rats. b(4)

Process-related impurities were not detected in the drug substance. The step manufacturing process includes fermentation (steps), recovery (steps), purification of the liraglutide precursor (steps), acylation (step), and purification and (steps). The acylating agent is b(4)

pilot and production scale batches tested (see Chemistry Review 1, page 37). Levels of these process-related impurities are below the threshold of toxicological concern for genotoxic impurities (1.5 mcg/daily dose).

The final drug product, liraglutide injection, is an aqueous formulation of 6 mg/mL liraglutide, 1.4 mg/mL disodium phosphate dihydrate, 14 mg/mL propylene glycol, and 5.5 mg/mL phenol at pH 8.15 in an -filled glass cartridge containing 3 mL liraglutide solution. The glass cartridge is inserted into a multiple dose pen injector for subcutaneous injection. The drug product is stable for . Liraglutide is light sensitive and should be stored in the capped pen injector protected from light. There were no unique impurities in the drug product, so impurities qualification are the same as for the drug substance. Excipients are qualified by existing safety data. b(4)

Toxicity of impurities in the final to-be-marketed formulation, formulation 4,containing 6.0 mg/mL liraglutide in solution at pH 8.15 was adequately assessed in subcutaneous repeat-dose toxicity studies, but not in genetic toxicity studies. In chronic repeat-dose toxicity studies, liraglutide caused irreversible injection site reactions in monkeys using drug formulations that were at least 3-fold more dilute than the clinical formulation. Fibrosarcomas occurred in the dorsal skin and subcutis of male mice. This carcinogenic effect of liraglutide at or near the injection site in mice may not be GLP-1R-mediated and it occurred using a liraglutide dosing formulation that was 10-times less concentrated than the clinical formulation. Genetic toxicity of liraglutide impurities at levels consistent with drug substance and drug product acceptance criteria should be evaluated.

Unresolved Toxicology Issues

1. Human relevance of liraglutide-induced thyroid C-cell tumors in rats and mice is unknown.

2. Local toxicity after repeat dosing with liraglutide was not adequately assessed in nonclinical studies because liraglutide concentrations in nonclinical formulations used in repeat-dose toxicity and carcinogenicity studies were substantially lower than the liraglutide concentration in the clinical formulation.

3. Genetic toxicity of some liraglutide impurities were not adequately assessed i*n vitro*.

Recommendations: Not approvable. Please see 'Recommendations of Approvability' on page 1.

Suggested Labeling: Please see 'Recommendations on labeling' starting on page 1.

APPENDICIES

Appendix A: Mouse Carcinogenicity Study Review

Study title: NC 90-1170: 104-week carcinogenicity study in mice with subcutaneous administration

NNC 90-1170 Tumor Findings in Male Mice

Result	Organ/Tissue	Neoplasm	Historical Incidence	Parameter	Sex: Males; NNC 90-1170 Dose (mg/kg/day): 0 (Trend analysis)	0.03	0.2	1	3
Positive	Thyroid	c-cell adenoma	< 1%	incidence (%)	0	0	0	13.4	19.0
				p-value	0.000	-	-	0.000	0.000
	Dorsal skin & subcutis	fibrosarcoma	> 1%	incidence (%)	0	3.0	1.5	3.0	8.8
				p-value	0.003	> 0.05	> 0.05	> 0.05	0.008
Equivocal (+ dose response, lacking statistically significant increase in at least the HD group)	Injection site	fibrosarcoma	< 1%	incidence (%)	0	1.5	1.5	0	5.1
				p-value	0.019	> 0.05	> 0.05	-	> 0.05
	Dorsal skin & subcutis	rhabdomyosarcoma	< 1%	incidence (%)	0	0	3.0	1.5	5.1
				p-value	0.018	-	> 0.05	> 0.05	> 0.05
Equivocal (- dose response, statistically significant increase in at least 1 dose group)	Vascular (all sites)	hemangioma or hemangiosarcoma	> 1%	incidence (%)	1.3	3.0	14.9	0	8.9
				p-value	0.194	> 0.05	0.001	> 0.05	**0.036**

Underlined values considered positive based on trend analysis p-value for rare ($p < 0.025$) or common ($p < 0.005$) tumors, p-value for pairwise comparison to the control group for rare ($p < 0.05$) or common ($p < 0.01$) tumors, and the incidence in the historical control group.

NNC 90-1170 Tumor Findings in Female Mice

Result	Organ/Tissue	Neoplasm	Historical Incidence	Parameter	Sex: Females; NNC 90-1170 Dose (mg/kg/day): 0 (Trend analysis)	0.03	0.2	1	3
Positive	Thyroid	c-cell adenoma	< 1%	incidence (%)	0	0	0	6.0	19.7
				p-value	0.000	-	-	0.051	0.000
		c-cell carcinoma	< 1%	incidence (%)	0	0	0	0	2.6
				p-value	0.063	-	-	-	> 0.05
		c-cell adenoma or carcinoma	< 1%	incidence (%)	0	0	0	6.0	22.4
				p-value	0.000	-	-	0.051	0.000

Underlined values considered positive based on trend analysis p-value for rare ($p < 0.025$) or common ($p < 0.005$) tumors, p-value for pairwise comparison to the control group for rare ($p < 0.05$) or common ($p < 0.01$) tumors, and the incidence in the historical control group.

Key study findings:

- Subcutaneously injected NNC 90-1170 (dorsal surface) was a non-genotoxic carcinogen in male and female mice with treatment related neoplasms occurring in **thyroid c-cells (males and females)** and **dorsal skin and subcutis (males)**.
- The NOAEL for neoplastic findings was 0.2 mg/kg/day NNC 90-1170 (uncorrected SM 1.8) based on increased incidence of thyroid c-cell adenomas in males and females and combined c-cell adenomas / carcinomas at $\geq$ 1 mg/kg/day NNC 90-1170. Focal thyroid c-cell hyperplasia, a preneoplastic finding, occurred at $\geq$ 0.2 mg/kg/day.

- NNC 90-1170 dose-dependently increased the incidence of **focal thyroid c-cell hyperplasia**, a preneoplastic lesion, at ≥ 0.2 mg/kg/day in males and females, dose-dependently increased the incidence of **thyroid c-cell adenomas** at ≥ 1 mg/kg/day in males and females (uncorrected human exposure multiple (uHEM) 10), and increased the incidence of combined **c-cell adenomas / carcinomas** at ≥ 1 mg/kg/day in females (uHEM 10).
    - Between weeks 25 and 104, plasma calcitonin levels increased > 2 fold at 3 mg/kg/day in males and females.
- A positive finding of **dorsal skin and subcutis fibrosarcomas** at 3 mg/kg/day NNC 90-1170 in males (uHEM 45). There was an equivocal finding of dose-related **dorsal skin and subcutis rhabdomyosarcoma** and **injection site fibrosarcomas** in males, and incidences in the 3 mg/kg/day group (uHEM 45) were above the historical control range for both tumors, but the increased incidence for either finding never reached statistical significance in any NNC 90-1170 group. The sponsor's analysis of tumor incidence data grouping **total sarcomas dorsal surface skin and subcutis** was statistically significant for trend ($p < 0.001$) and pair-wise analysis compared to controls at 3 mg/kg/day NNC 90-1170 in males ($p < 0.001$, uHEM 45). In control group females, there was a high incidence of total sarcomas in the skin and subcutis.
- Equivocal findings in males occurred in the **vasculature (hemangiomas / hemangiosarcomas at all sites** at 0.2 mg/kg/day), but the increased incidence was not dose related.
- The NOAEL for non-neoplastic findings was < 0.03 mg/kg/day. Non-neoplastic findings occurred in **thyroid** (inflammatory cell infiltrate at ≥ 0.03 mg/kg/day in males and at 0.03 and 3 mg/kg/day in females; focal c-cell hyperplasia, considered a preneoplastic lesion, at ≥ 0.2 mg/kg/day in males and females), **liver** (pigmented Kupffer cells (attributed to hemosiderin accumulation) at ≥ 0.03 mg/kg/day in males and at ≥ 0.2 mg/kg/day in females, centrilobular hypertrophy, diffuse centrilobular hepatocyte vacuolation at ≥ 0.03 mg/kg/day in males), **spleen** (hemosiderin accumulation at ≥ 0.03 mg/kg/day in females), **femoro-tibial joint** (degenerative disease at ≥ 0.03 mg/kg/day in males and at 0.03, 1, and 3 mg/kg/day in females), **seminal vesicles** (lymphocytic infiltration at ≥ 0.03 mg/kg/day and inflammation at 0.03 and 3 mg/kg/day in males), and **thymus** (tubular cystic hyperplasia at ≥ 0.03 mg/kg/day in males and at ≥ 0.2 mg/kg/day in females).
- Methodological / Protocol isues:
    - Due to low survival of control group females in the main study group, termination of the 78 week interim sacrifice group was cancelled and treatment was continued for 104 weeks. Tumor analysis was performed after combining results from both main study and week 78/104 groups.
    - Actual NNC 90-1170 concentrations were up to 3 fold lower than the nominal concentration for the 0.03 mg/kg/day dosing solution. However, human risk assessment is based on comparative exposure.
    - Mice used for assessment of anti-liraglutide antibodies in week 104 survived 104 weeks of treatment (week 78/104 week group with treatment of 78 week interim sacrifice group extended to week 104), but these mice were sacrificed 10 days after the last dose to washout residual liraglutide that could potentially interfere with the anti-liraglutide antibody assay. These mice were included in the carcinogenicity assessment.
    - Ophthalmoscopic examinations were not performed.
    - Validation of the commercial rat plasma immunoradiometric assay to measure mouse plasma calcitonin was not submitted in the NDA, although the report references 2 assay validation reports (reports 205089 and restandardization report 205189).
    - Although transient weight loss and food consumption occurred in the first weeks of the study, a pharmacodynamic effect of NNC 90-1170 was not sustained over the entire study period.

Adequacy of the carcinogenicity study and appropriateness of the test model:

Mice are pharmacologically responsive to subcutaneously administered NNC 90-1170 (transiently decreased body weight and food consumption in CD-1 mice, lowered blood glucose in diabetic ob/ob and diabetic db/db mice, and increased beta cell mass in db/db mice) and in the carcinogenicity study, mice did not mount a neutralizing antibody response. Protein binding of NNC 90-1170 is slightly higher in mice than in humans. There are no major metabolites of lipid-labeled ^{3}H-[Pal]-liraglutide in humans, but metabolism of ^{3}H-[Pal]-liraglutide is similar in vivo and in vitro in mice and humans. In vitro metabolism of peptide-labeled ^{3}H-[tyr]-liraglutide is similar in mice and humans, but in vivo metabolism was not characterized in either species.

Evaluation of tumor findings:

Treatment-related neoplastic lesions occurred in thyroid (c-cell adenomas at ≥ 1 mg/kg/day in males and females, c-cell adenomas / carcinomas at ≥ 1 mg/kg/day in females) and dorsal skin and subcutis (fibrosarcomas at 3 mg/kg/day in males).

CAC concurrence:

- The Committee concurred that the study was acceptable based on tumor findings in males and females.
- The Committee concurred that thyroid C-cell adenomas, C-cell adenomas or carcinomas (combined), and dorsal skin and subcutis fibrosarcomas were drug related. Liraglutide significantly increased the incidence of thyroid c-cell adenomas at ≥ 1 mg/kg in males and females, C-cell adenomas and carcinomas (combined) at ≥ 1 mg/kg in females, and dorsal skin and subcutis fibrosarcomas at 3 mg/kg in males.

Study no.: 204229 (sponsor), 457274

Submission, Module, and page #: N000 4.2.3.4.1.1, pages 1 - 3096 b(4)

Conducting laboratory and location:

Date of study initiation: 23 November 2004

Study ending date: 1 December 2007

GLP compliance: Yes (OECD compliance claimed)

QA report: yes (X) no ( )

Drug, lot #, and % purity: NNC 90-1170 lots shown in the table below. Purity of 97.1% by RP-HPLC reported for lot PQ50365 only (certificate of analysis in Appendix B).

Test Item	Batch No.	Units	Arrival Date	Expiry Date
NNC 90-1170 6.25 or 6.0 mg/ml liraglutide	PQ50102	934	07 October 2004	12 August 2005
	PQ50365	19	16-June-05	11-March-07
	PQ50367	546	21 July 2005	14 March 2006
	PQ50365	500	18 January 2006	11 September 2006
	PQ50365	250	06 July 2006	11 September 2007
	RQ50574	300	06 September 2006	28 March 2007

Typical Certificates of Analysis for a batch of test item and vehicle used are presented in Appendix B and Appendix C

[N000 4.2.3.4.1.1 P15]

Methods

Doses: 0 (vehicle), 0.03, 0.2, 1.0, 3.0 mg/kg/day NNC 90-1170

Basis of dose selection (MTD, MFD, AUC etc.): AUC ratio > 25 in males and females.

Species/strain: CD-1 mice (Crl:CD-1™(ICR)BR)

Number/sex/group (main study): 50 /sex/dose main study. Due to mortality in main study control females, treatment of week 78 interim sacrifice group was extended to 104 weeks (29/sex/ 0 or 3.0 mg/kg/day and 17/sex/ 0.03, 0.2, or 1.0 mg/kg/day)

Group	Treatment	(mg/kg/day)	Animal Numbers			
			Main Study		Week 78 (Treatment extended to 104 Weeks)	
			Males	Females	Males	Females
1	Control	0	1-50	251-300	501-529	610-638
2	Low	0.03	51-100	301-350	530-546	639-655
3	Intermediate I	0.2	101-150	351-400	547-563	656-672
4	Intermediate II	1.0	151-200	401-450	564-580	673-689
5	High	3.0	201-250	451-500	581-609	690-718

Extra animals were numbered sequentially from 719 onwards.

[N000 4.2.3.4.1.1 P18]

Route, formulation, volume: subcutaneous injection (dorsal surface, rump and between scapula), 6.0 – 6.25 mg/mL NNC 90-117 solution diluted in vehicle (1.4 mg/mL monosodium phosphate dehydrate, 14 mg/mL propylene glycol, 5.5 mg/mL phenol), 5.0 mL/kg
Frequency of dosing: once a day
Satellite groups used for toxicokinetics or special groups:
78 week interim sacrifice group was planned, but treatment continued for 104 weeks due to decreased survival in main study control females.

Satellite group for toxicokinetics and plasma calcitonin consisting of 17/sex/dose in each group terminated on weeks 26, 52, and 104.

Group	Treatment (mg/kg/day)		Animal Numbers					
			Week 26		Week 52		Week 104	
			Males	Females	Males	Females	Males	Females
1	Control	0	1001-1017	1086-1102	1171-1187	1256-1272	1341-1357	1426-1442
2	Low	0.03	1018-1034	1103-1119	1188-1204	1273-1289	1358-1374	1443-1459
3	Intermediate I	0.2	1035-1051	1120-1136	1205-1221	1290-1306	1375-1391	1460-1476
4	Intermediate II	1.0	1052-1068	1137-1153	1222-1238	1307-1323	1392-1408	1477-1493
5	High	3.0	1069-1085	1154-1170	1239-1255	1324-1340	1409-1425	1494-1510

A further 10 males (Numbers 1511-1520) and 10 females (1521-1530) were used for pretrial antibody level assessment. Extra animals were numbered from the last number used.

[N000 4.2.3.4.1.1 P18]

Age and weight: 5 weeks on arrival with males weighing 27.8-42.9g, and females weighing 20.7-39.6 g at the start of the study
Animal housing: One male and 2 or 3 females per cage by dose group were housed in suspended polypropylene cages (48 x 15 x 13 cm) with solid bottoms and stainless steel grid tops (including integral food hopper), sterilized white wood shavings (analysis revealed no significant contaminants), and a polycarbonate water bottle with a stainless steel nozzle (page 17).
Restriction paradigm for dietary restriction studies: None.
Drug stability/homogeneity: Two different SOPs were used to determine stability of NNC 90-1170 solution for subcutaneous injection. Drug concentrations in 0, 0.2, 1.0, and 3.0 mg/kg/day dosing solutions were analyzed using method 434-1018 and the 0.03 mg/kg/day dosing solution was analyzed using method 878-LP-08005. Dose solution samples were taken in week 1, week 39, and week 103 on days 1 and 7.
Dual controls employed: No, but controls included in satellite TK/calcitonin and 78 week interim sacrifice groups

Interim sacrifices: Due to reduced survival in main study control group females, dosing was continued to week 104 for 78 week interim sacrifice group.
Deviations from original study protocol:
Week 78 interim sacrifice group mice were rescheduled for sacrifice in week 104 with treatment continued to week 104 because of reduced survival in main study control group females. Therefore, a week 78 interim sacrifice group report was not issued.

Sixteen males in group 5 were under-dosed on 17 February 2005 and 6 females in group 4 were underdosed on 28 April 2005. Both dosing errors were corrected by giving an additional dose volume to give the correct dose.

The following TK group mice were dosed twice during toxicokinetic sampling in study week 52:

Group/Sex	Animal No.
1M	1172
1F	1259
2F	1276
3M	1208
4M	1223
4F	1310
5F	1325

[N000 4.2.3.4.1.1 P24]

Due to scheduling errors, no samples were taken for the following study doses/time points in week 26:

Group 2 predose
Group 4 2 hour

Additional thyroid processing in week 104 was going to be based on results from thyroid histology from mice from week 78 interim sacrifice group, but this group was sacrificed in week 104.

The following summary table lists mice replaced during the first 2 weeks of dosing.

Due to a number of deaths and clinical signs, the following animal replacements were made during the first two weeks of the study.

Date (Provantis Day)	Animal to be Replaced	Grp/ Sex	Replacement Animal	Reason for Replacement
08 Dec 04 (0)	369	3F	728	Large Swelling
08 Dec 04 (0)	115	3M	721	Lesion
13 Dec 04 (0)	209	5M	722	Found Dead
14 Dec 04 (0)	184	4M	724	Found Dead
20 Dec 04 (12)	125	3M	723	Found Dead
20 Dec 04 (12)	138	3M	720	Found Dead
20 Dec 04 (12)	548	3M	719	Tremors and Slow Respiration
20 Dec 04 (12)	397	3F	725	Lesion
20 Dec 04 (12)	711	5F	726	Found Dead

Day 0 = First day of dosing

[N000 4.2.3.4.1.1 P19]

The following summary table lists toxicokinetic and antibody satellite group mice replaced during the first week of dosing.

The following animal replacements were also made during Week 1 of the staggered satellite and antibody study.

Date (Provantis Day)	Animal to be Replaced	Grp/ Sex	Replacement Animal	Reason for Replacement
12 Jan 05 (0)	1133	3F	1538	Found Dead
12 Jan 05 (0)	1141	4F	1537	Suprascapular swelling prior to dosing

Day 0 = First day of dosing

These replacements did not affect the integrity or outcome of the study.

[N000 4.2.3.4.1.1 P19]

Observation times

Mortality: Twice a day.

Clinical signs: Main study mice checked twice a day with detailed examination performed once a week. Starting in week 83, clinical signs and detailed were recorded for week 78/104 group mice using the same schedule as main study group mice. Palpations for masses were performed beginning in week 13 for all mice. Satellite TK/calcitonin group mice and antibody group mice were examined daily for welfare purposes.

Body weights: Recorded once a week prior to starting treatment, then daily during treatment. Body weight of main study mice was reported once a week.

Food consumption: Quantity of food consumed by main study group mice (per cage) was recorded weekly until the end of week 13, then monthly afterward. For week 78/104 group mice, food consumption over a 4 week period (per cage) was recorded starting in week 83.

Water consumption: Water consumption was monitored by visual inspection, but it wasn't quantified.

Anti-NNC 90-1170 antibody: Up to 1 mL orbital sinus blood samples from isoflurane anesthetized antibody study group mice (10/sex/dose prior to initiating treatment, 5/sex/dose during treatment) was taken to determine if anti-NNC 90-1170 antibodies occurred after treatment. Mice were bled 3 days after their last dose in week 26 and 6 days after their last dose in weeks 52 and 78. Samples for antibody analysis were taken from week 104 satellite group mice bled 10 days after their last dose in week 97. Samples for antibody analysis from week 78 satellite group mice reassigned for termination in week 104 were bled 10 days after the last dose. After bleeding mice were sacrificed and necropsied. The assay is a radioimmunoassay precipitating immunoglobulin bound ^{125}I –liraglutide after overnight incubation of ^{125}I –liraglutide with mouse plasma.

In the absence of NNC 90-1170, the sensitivity of the anti-NNC 90-1170 antibody radioimmunoassay was 25 – 50 ng/mL. The assay sensitivity decreased to > 1 mcg/mL antibody at ≥ 10 nM NNC 90-1170 in plasma. To decrease interference of plasma NNC 90-1170 in treated mice, plasma samples were taken after dosing in week 26, 3 days after dosing in study week 52, and 6 days after the last dose in week 78. Since plasma levels > 10 nM NNC 90-1170 occurred in 3/10 mice in the 3 mg/kg/day group in week 78, the washout period was increased to 10 days after dosing in week 104. Residual plasma NNC 90-1170 in mice from the 3 mg/kg/day group might have interfered with the assay in weeks 26, 52, and 78.

Plasma calcitonin: Measured in weeks 26, 52, and 104 using a commercial immunoradiometric assay for rat calcitonin (report Appendix NN) with a 2 pg/ml lower detection limit (LOD). Although the sponsor claims the commercial immunoradiometric assay for rat calcitonin assay (bead immobilized anti-calcitonin monoclonal antibody and ^{125}I-labeled goat anti-calcitonin polyclonal antibody) was valid for measuring plasma mouse calcitonin, assay validation was not submitted in the NDA..

Hematology: Immediately prior to termination in week 104, orbital sinus blood for hematology was obtained from isoflurane anesthetized mice from week 104 main study group mice and week 78/104 group mice. Hematology parameters are shown in the table below. Blood smears were obtained, but due to the absence of hematology findings, they weren't examined.

Haematology Parameters
Haemoglobin
Red Blood Cell Count
Red Blood Cell Distribution Width
Haematocrit
White Blood Cell Count
Mean Cell Volume
Mean Cell Haemoglobin
Mean Cell Haemoglobin Concentration
Reticulocytes
Platelet Count
Differential White Blood Cell Count:
Neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils
Large Unclassified Cells

[N000 4.2.3.4.1.1 P23]

Gross Pathology: Mice surviving 104 weeks of treatment (main study group mice and week 78/104 group mice rescheduled for sacrifice in week 104) were asphyxiated with CO_2 and exsanquinated. All mice sacrificed on study were necropsied, except for antibody group mice sacrificed prior to initiating treatment. Mice found dead or sacrificed moribund were necropsied at the discretion of the pathologist, with the intent of determining a cause of death.

Toxicokinetics: At least 0.8 mL orbital sinus blood was obtained from isoflurane anesthetized TK satellite group mice prior to dosing and 1, 2, 4, 8, and 12 hours after dosing. During week 26, 52, and 104 (78 week group reassigned to week 104), plasma samples for toxicokinetics and calcitonin levels were obtained from 2 mice /sex/dose/time points (dose groups 1 – 5). In week 78, samples were obtained from week 104 satellite group mice reassigned to week 78 using 4 mice/sex/dose/time point for dose groups 1 and 5 or 2 mice/sex/dose/time point for dose groups 2,3, and 4. Sampling for week 78 group mice is shown in the table below.

Week 78 (allocated wk 104 animals)

Males

Timepoint (h)	Group 1	Group 2	Group 3	Group 4	Group 5
Predose (0)	4	2	2	2	4
1	4	2	2	2	4
2	4	2	2	2	4
4	4	2	2	2	4
6	4	2	2	2	4
12	4	2	2	2	4

Females

Timepoint (h)	Group 1	Group 2	Group 3	Group 4	Group 5
Predose (0)	4	2	2	2	4
1	4	2	2	2	4
2	4	2	2	2	4
4	4	2	2	2	4
6	4	2	2	2	4
12	4	2	2	2	4

[N000 4.2.3.4.1.1 P25]

Histopathology: Peer review: yes (X), no ( ) - internal peer review at Charles River Labs, external peer review by the sponsor.

Unless otherwise noted, tissues for microscopic examination were fixed in 10% neutral buffered formalin.

Organs/Tissues for Preservation and Evaluation

Tissues collected	Examined	Comments
Adrenal x 2	x	-
Aortic Arch	x	-
Blood Smear	-	From animals killed prematurely.
Brain	x	Forebrain, midbrain and cerebellum.
Dose Site(s)	x	-
Exorbital Lacrimal Glands	x	-
Eye x 2	x	Both eyes fixed in Davidson's fluid. The left eye processed and examined histologically.
Femoral Bone (including stifle joint)	x	-
Gastro-intestinal Tract:		Opened at necropsy and mucosa examined. Peyers patches sampled from small intestine.
Stomach	x	
Duodenum	x	
Ileum	x	
Jejunum	x	
Colon	x	
Caecum	x	
Rectum	x	
Harderian Gland x 2	x	Both fixed. One only processed and examined histologically.
Heart	x	-
Implant(s)	-	For identification purposes.
Kidney + Ureter x 2	x	-
Liver + Gall Bladder	x	-
Lung	x	Inflated after weighing; all lobes examined including mainstem bronchi.
Marrow Smear (Femur)	-	Smear air-dried and fixed in methanol.
Mesenteric Lymph Node	x	-
Nasal Cavity	-	Trans-tove section examined if clinical signs dictate.
Oesophagus	x	-
Optic Nerve x 2	x	Fixed in Davidson's fluid. The left optic nerve examined.
Ovary x 2	x	-
Pancreas	x	-
Parotid Salivary Gland	x	-
Pituitary	x	-
Prostate	x	-
Rib	x	Including costochondral junction.
Sciatic Nerve	x	-
Seminal Vesicles	x	-
Skin + Mammary Gland	x	-
Spinal Cord	x	Cervical, midthoracic and lumbar regions.
Spleen	x	-
Sternum	x	Including bone marrow.
Sublingual Salivary Gland x 2	x	Only one processed
Submandibular Lymph Node	x	-
Submaxillary (Mandibular) Salivary Gland x 2	x	Only one processed.
Testis + Epididymis x 2	x	-
Thigh Muscle	x	-
Thymus	x	-
Thyroid + Parathyroid x 2	x	Parathyroid examined if present on slide
Tongue	x	-
Trachea	x	-
Urinary Bladder	x	Contracted bladders distended with fixative; epithelial surface in animals which underwent histological evaluation examined after fixation.
Uterus with Cervix and Oviduct	x	-
Vagina	x	-

[N000 4.2.3.4.1.1 P28-29]

Results

Formulation: Dosing solutions were considered stable for up to 7 days stored at 2 – 4C and were considered within an acceptable an acceptable range during weeks 1, 39, and 103 on days 1 and 7. NNC 90-1170 was not detected in vehicle control samples.

	Dose Solution Concentration (mg/mL)	
Dose (mg/kg)	**Nominal**	**Actual Range**
0.03	0.006	0.002 – 0.006
0.2	0.04	0.030 – 0.043
1.0	0.20	0.183 – 0.204
3.0	0.60	0.560 – 0.598

Mortality:

Statistical analysis from the Dr. Min (Office of Biostatistics) showed there were treatment related effects on mortality (Tables 9A and 9B, below).

Table 9A: Intercurrent Mortality Comparisons
Male Mice in Combined Main and Week 78/104 Satellite Studies

Test:	P-Value Cox	P-Value Kruskal-Wallis
Dose Response	0.2903	0.5184
Homogeneity	0.5051	0.5900

Table 9B: Intercurrent Mortality Comparisons
Female Mice in Combined Main and Week 78/104 Satellite Studies

Test:	P-Value Cox	P-Value Kruskal-Wallis
Dose Response	0.1639	0.1404
Homogeneity	0.3284	0.3512

The sponsor's statistical analysis showed survival in main study group males was unaffected by treatment. Compared to the control group, mortality in females was decreased in 1.0 and 3.0 mg/kg/day groups.

		Main Study at Week 104				
Group		1	2	3	4	5
Dose Level (mg/kg/day)		0	0.03	0.2	1.0	3.0
Survivors/	M	25/50	29/50	28/50	31/50	19/50
No. in Group	F	14/50	11/50	19/50	19/50	19/50

[N000 4.2.3.4.1.1 P32]

Analysis of Survival Times – Main Study

Comparison	Test Statistic	P-Value
Males		
Control Group vs. 0.03 mg/kg/day	0.531	0.47
Control Group vs. 0.2 mg/kg/day	0.901	0.34
Control Group vs. 1.0 mg/kg/day	2.692	0.10
Control Group vs. 3.0 mg/kg/day	0.002	0.97
Females		
Control Group vs. 0.03 mg/kg/day	1.107	0.29
Control Group vs. 0.2 mg/kg/day	0.878	0.35
Control Group vs. 1.0 mg/kg/day	3.985	0.046
Control Group vs. 3.0 mg/kg/day	4.714	0.030

[N000 4.2.3.4.1.1 P33]

Survival in week 78 interim sacrifice group in week 104 (rescheduled for sacrifice in week 104 with continued treatment until sacrifice) is shown in the table below. Compared to concurrent controls, mortality increased in group 2 males and females (0.03 mg/kg/day).

		"Week 78" Toxicokinetic Study at Week 104				
Group		1	2	3	4	5
Dose Level (mg/kg/day)		0	0.03	0.2	1.0	3.0
Decedents /	M	15/29	12/17	8/17	10/17	15/29
No. in Group	F	19/29	14/17	13/17	12/17	20/29

[N000 4.2.3.4.1.1 P33]

Analysis of Survival Times – Wk 78 satellites

Comparison	Test Statistic	P-Value
Males		
Control Group vs. 0.03 mg/kg/day	4.108	0.043
Control Group vs. 0.2 mg/kg/day	0.210	0.65
Control Group vs. 1.0 mg/kg/day	2.272	0.13
Control Group vs. 3.0 mg/kg/day	0.191	0.66
Females		
Control Group vs. 0.03 mg/kg/day	4.117	0.042
Control Group vs. 0.2 mg/kg/day	1.784	0.18
Control Group vs. 1.0 mg/kg/day	0.165	0.68
Control Group vs. 3.0 mg/kg/day	0.701	0.40

[N000 4.2.3.4.1.1 P33]

Kaplan Meier survival curves for both main study and 78 week satellite group males (Figures 1 and 3) and females (Figures 2 and 4) are shown below. Considering survival up to 104 weeks in combined main study and week 78 satellite groups, the number of unscheduled deaths increased in females at 0 mg/kg/day (56/79), 0.03 mg/kg/day (51/79) and 3.0 mg/kg/day (56/79) groups.

Figure 1 Kaplan Meier Survival Curve: Main Study: Males

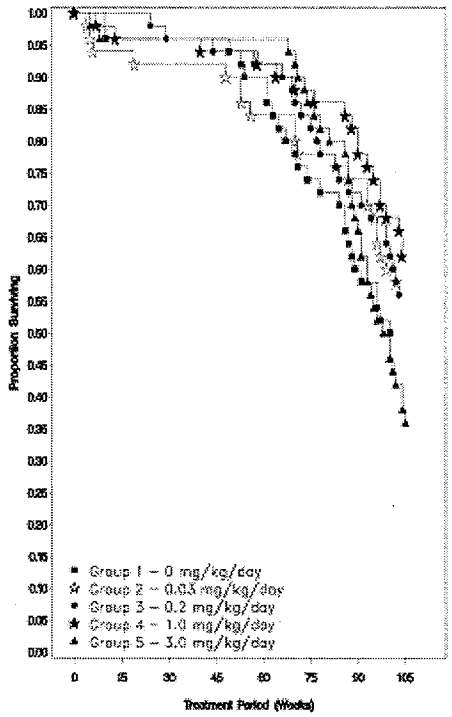
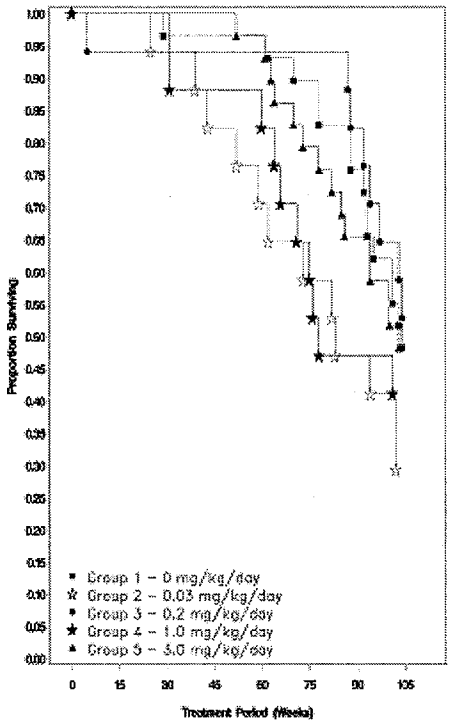


Figure 3 Kaplan Meier Survival Curve: Wk 78 Satellite Study: Males



[N000 4.2.3.4.1.1 P554, 556]

Figure 2 Kaplan Meier Survival Curve: Main Study: Females

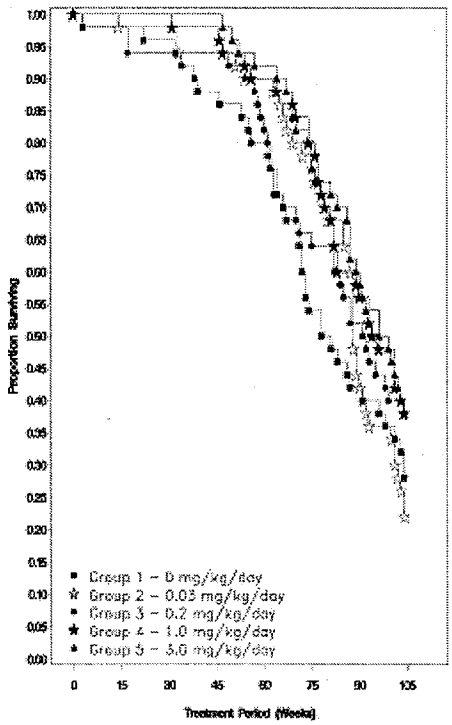
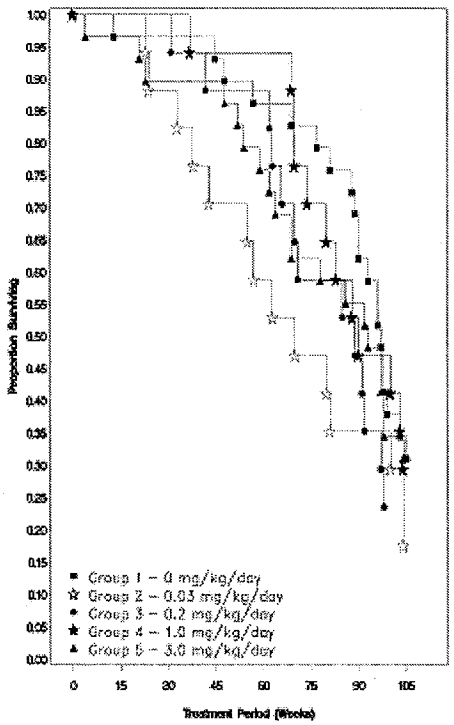


Figure 4 Kaplan Meier Survival Curve: Wk 78 Satellite Study: Females



[N000 4.2.3.4.1.1 P555, 557]

Although thyroid C-cell hyperplasia, adenomas, and carcinomas were treatment related, C-cell carcinoma was considered a caused of death for only one high dose group female. At 3.0 mg/kg/day in males, fibrosarcoma on the dorsal surface was a cause of death in 9/47 decedents (19.1%).

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS Males Grp 1 0 mg/kg /day	Grp 2 0.03 mg/kg /day	Grp 3 0.2 mg/kg /day	Grp 4 1.0 mg/kg /day	Grp 5 3.0 mg/kg /day	Females Grp 1 0 mg/kg /day	Grp 2 0.03 mg/kg /day	Grp 3 0.2 mg/kg /day	Grp 4 1.0 mg/kg /day	Grp 5 3.0 mg/kg /day
TISSUES NOT INCLUDED WITHIN BODY SYSTEMS											
CAUSE OF DEATH		(40)	(33)	(30)	(29)	(47)	(56)	(53)	(44)	(43)	(51)
FIBROSARCOMA [M], dorsal		0	3	1	1	9**	2	1	0	0	2
C-CELL CARCINOMA [M]		0	0	0	0	0	0	0	0	0	1
Dermatitis		4	4	4	5	2	4	6	3	1	2
Inflammation, acute, (genito-urinary system)		1	0	0	0	3	0	0	0	0	0
Cystitis		2	7	2	1	5	0	0	0	0	0
Arteritis/periarteritis		0	0	0	0	0	1	1	1	0	4
Cause of death undetermined		10	5	8	5	9	2	10*	7*	3	9*

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

[N000 4.2.3.4.1.1 P477-481]

Clinical signs:

There were no clinical signs considered treatment-related. Injection site scabbing was significantly higher in mice at 0.2 mg/kg/day compared to 1.0 or 3.0 mg/kg/day, but in the absence of a relation to dose, the increase was not biologically relevant.

Body weights:

By the end of the 104 treatment period, there were no significant differences in group mean body weight between control and NNC 90-1170 treated groups (Figures 5 & 6, and summary table below).

Figure 5 Group Mean Bodyweight: Main Study: Males

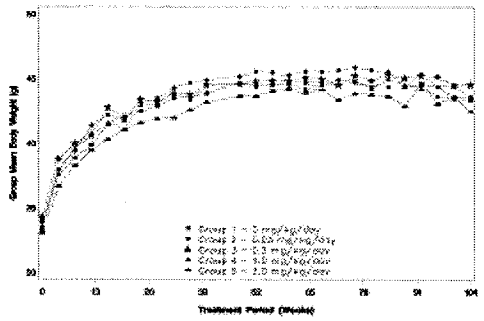
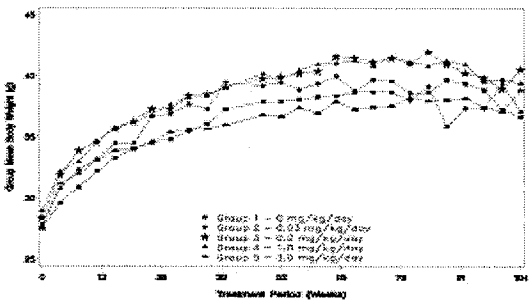


Figure 6 Group Mean Bodyweight Main Study: Females



[N000 4.2.3.4.1.1 P558-9]

Sex		Male					Female				
NNC 90-1170 (mg/kg/day)		0	0.03	0.2	1	3	0	0.03	0.2	1	3
Parameter											
Body weight	N, week 0	50	50	50	50	50	50	50	50	50	50
	g, week 0	33.5	33.9	34.3	33.4	33.1	27.6	29.0	28.8	28.6	27.8
	N, week 104	25	29	28	31	19	14	11	19	19	19
	g, week 104	43.7	42.5	43.8	44.1	43.7	37.4	37.3	40.2	38.9	39.1
	% difference from control, week 104	0.0	-2.7	0.2	0.9	0.0	0.0	-0.3	7.5	4.0	4.5
Body weight gain (week 0 to week 104)	g, (week 104 - week 0)	10.2	8.6	9.5	10.7	10.6	9.8	8.3	11.4	10.3	11.3
	% of pretest body weight	30.4	25.4	27.7	32.0	32.0	35.5	28.6	39.6	36.0	40.6
	% difference from control	0.0	-15.7	-6.9	4.9	3.9	0.0	-15.3	11.8	5.1	15.3

Group mean body weight in NNC 90-1170 treated groups tended to be lower than controls during the first week of treatment (see tables below) and this corresponded with reduced food consumption. Reduced body weight was transient; group mean body weight in treated groups was near of above control group be the end of the study.

Week 1 Group Mean Bodyweights (g) Males

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	33.5	33.9	34.0	34.4	35.0	34.8	35.1	35.1
Low	33.9	34.3	34.4	35.5	35.2	34.9	34.7	34.8
Intermediate 1	34.5	34.0	34.1	34.9	35.2	34.9	34.8	35.5
Intermediate 2	33.3	32.4	32.3	33.1	33.2	33.4	33.5	34.1
High	33.1	32.0	31.9	32.3	32.7	32.5	32.8	32.9

Week 1 Group Mean Bodyweights (g) Females

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	27.6	27.7	28.0	27.5	27.6	27.2	27.3	27.3
Low	27.9	28.7	29.4	28.5	28.6	28.5	28.9	28.9
Intermediate 1	28.4	28.2	28.3	28.3	28.3	29.0	29.1	29.0
Intermediate 2	29.0	28.0	28.1	28.4	29.2	29.1	29.2	29.4
High	27.5	26.2	26.4	26.7	27.2	27.1	27.3	27.4

[N000 4.2.3.4.1.1 P34]

Food consumption:

NNC 90-1170 treatment decreased food consumption during the first week, but food consumption returned to control group levels as the study progressed.

Food Consumption in Males (g/mouse/day, main study weeks -1 to 12)

Group (Dose Level mg/kg/day)		Pretrial (week)	Treatment Period (weeks)											
		-1	1	2	3	4	5	6	7	8	9	10	11	12
1 (0)	Number	50	50	50	50	50	50	50	50	50	50	48	48	48
	Mean	5.9	6.1	6.0	6.2	6.2	6.1	6.2	6.2	6.1	6.1	6.1	6.1	6.1
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
2 (0.03)	Number	50	50	50	50	49	48	47	47	47	47	47	47	47
	Mean	6.0	5.8	6.4	6.3	6.4	6.2	6.5	6.3	6.4	6.3	6.4	6.3	6.2
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.		aa	ac				cc		cc			bc	ac
3 (0.2)	Number	50	50	50	50	50	50	50	50	50	50	50	50	50
	Mean	6.1	5.3	6.0	6.4	6.2	6.1	6.5	6.2	6.3	6.3	6.1	6.3	6.6
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.		cs					cc		cc			cc	cc
4 (1.0)	Number	49	50	50	50	50	50	50	49	49	49	49	49	49
	Mean	5.9	4.9	6.2	6.0	6.4	6.4	6.4	6.5	6.4	6.4	6.6	6.5	6.6
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.		cs				ac	cc		cc		ac	cc	cc
5 (3.0)	Number	50	50	50	50	50	49	49	49	48	48	48	48	48
	Mean	6.0	4.5	5.7	5.7	6.0	6.0	6.3	6.1	6.1	5.9	6.1	6.0	6.2
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.		cs	ac	bc			cc		cc			cc	cc

[N000 4.2.3.4.1.1 P120, 122]

Food Consumption in Males (g/mouse/day, main study weeks -1 to 12)

Group (Dose Level mg/kg/day)		Pretrial (Week)	Treatment Period (weeks)											
		-1	1	2	3	4	5	6	7	8	9	10	11	12
1 (0)	Number	17	17	17	17	17	17	17	17	17	17	17	17	17
	Mean	5.0	4.9	5.1	5.2	5.3	5.2	5.4	5.4	5.3	5.5	5.5	5.7	5.5
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
2 (0.03)	Number	17	17	17	17	17	17	17	17	17	17	17	17	17
	Mean	4.9	4.8	5.0	5.3	5.2	5.1	5.4	5.3	5.3	5.7	5.6	5.7	5.6
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.			ac				cc		cc			bc	ac
3 (0.2)	Number	36	17	17	17	17	17	17	17	17	17	17	17	17
	Mean	4.9	4.4	5.4	5.4	5.4	5.3	5.5	5.4	5.6	5.5	5.8	5.8	5.7
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.		cs					cc		cc			cc	cc
4 (1.0)	Number	17	17	17	17	17	17	17	17	17	17	17	17	17
	Mean	5.0	4.1	5.0	5.4	5.3	5.3	5.5	5.3	5.5	5.5	5.6	5.6	5.7
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.		cs				ac	cc		cc		ac	cc	cc
5 (3.0)	Number	17	17	17	17	17	17	17	17	17	17	17	17	17
	Mean	5.0	3.7	4.8	5.2	5.4	5.2	5.4	5.1	5.3	5.5	5.5	5.4	5.5
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Prob.		cs	ac	bc			cc		cc			cc	cc

[N000 4.2.3.4.1.1 P125, 127]

Water consumption: Water consumption was considered unaffected by treatment.

Hematology:

RBCs were significantly decreased and MCH and MCV were significantly increased at ≥ 0.2 mg/kg/day in males and females. In males, decreased RBCs was dose-related and it was accompanied by non-significant decreases in Hb and Hct. RBC parameter changes occurred in the absence of statistically significant changes in reticulocytes, but there was a trend of increased reticulocytes in males.

Hematology, Week 104

Sex	Male					Female				
NNC 90-1170 Dose (mg/kg/day)	0	0.03	0.2	1	3	0	0.03	0.2	1	3
Parameter / N	21	28	24	29	18	13	9	18	17	17
	Absolute Value	% Difference from Control				Absolute Value	% Difference from Control			
RBC (X10^12/L)	9.05	-7.3	-8.4	-11.7	-13.7	8.10	-6.2	-7.5	-3.5	-4.1
Hb (g/dL)	13.0	-7.69	-3.85	-6.15	-10.77	11.8	-1.7	-0.8	2.5	2.5
Hct (%)	41	-5.9	-5.1	-6.3	-10.5	37	-1.4	-1.1	1.9	2.2
MCH (pg)	14.4	-0.7	4.9	6.9	3.5	14.6	5.5	7.5	6.8	6.8
MCV (fL)	45.4	1.5	3.7	6.6	3.7	45.8	5.9	7.9	5.5	7.2
Retic (%)	3.4	0.0	14.7	11.8	26.5	5.5	1.8	3.6	-25.5	3.6

Underlined values are statistically different from controls.

Anti-NNC 90-1170 antibodies:

Anti-NNC 90-1179 antibodies were not detected in plasma of any treated mice.

Plasma calcitonin:

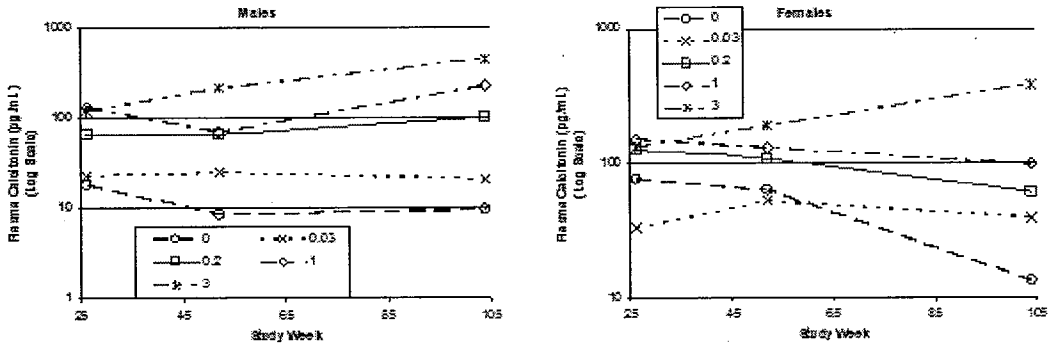
Group mean calcitonin levels were significantly higher than controls at ≥ 0.2 mg/kg/day NNC 90-1170 from week 26 to study termination and at ≥ 0.03 mg/kg/day from week 52 onward in males. In females, group mean calcitonin levels exceeded concurrent controls at ≥ 0.2 mg/kg/day in weeks 26 and 52 and at ≥ 0.03 mg/kg/day in week 104.

Plasma Calcitonin

NNC 90-1170 Dose (mg/kg/day)	Males						Females					
	Week 26		Week 52		Week 104		Week 26		Week 52		Week 104	
	pg/mL	N	pg/mL	N	pg/mL	N	pg/mL	N	pg/mL	N	pg/mL	N
0	17.9	16	8.58	15	9.67	12	76	16	63.3	15	13.3	3
0.03	22.6	17	25.05	14	20.92	4	33.4	16	52.3	16	39.1	4
0.2	65.1	17	66.43	17	102.16	8	125.6	16	107.4	13	61.4	2
1	129.2	15	70.3	13	228.5	11	152.9	17	129.5	12	98.6	2
3	119	15	211.4	15	453.9	5	133.7	16	191.4	14	383.5	7

Values statistically significantly different from control are underlined.

Plasma calcitonin increased > 2 fold between weeks 26 and 105 at 3 mg/kg/day in males and females, but not at lower doses (summary table above, graphs below). Compared to week 26, week 104 plasma calcitonin levels were the same of lower in control group males and at 0.03 mg/kg/day in males and at ≤ 1 mg/kg/day in females. Plasma calcitonin increased with treatment duration at 3 mg/kg/day in males and females.



Gross pathology:

The sponsor states there were masses in thyroid of 3 mice in the 3 mg/kg/day group and occasionally in the bone, heart, intestine, duodenum, and cecum of other mice.

Histopathology:

Peer review of tissue samples from the first 8 mice in 0, 1.0, and 3.0 mg/kg/day groups (both sexes) and thyroid and pancreas samples from all mice resulted in some differences of opinion of some tumor findings in liver, blood vessels, lungs, uterus, WBCs, and thyroid. Discrepancies between the study pathologist and reviewing pathologist, summarized in the tables below, were resolved by consensus diagnosis.

Original diagnosis	*Consensus diagnosis*	*Animal No.*
Hepatocellular carcinoma	Hepatocellular adenoma	96, 103, 114, 126, 172, 182, 215, 221, 427, 504, 613
Haemangiosarcoma	Haemangioma	202, 225, 291, 310, 343, 364, 372, 381, 405, 435, 441, 653, 697, 714
Bronchiolo-alveolar carcinoma	Bronchiolo-alveolar adenoma	76, 233, 502, 544, 616
Endometrial adenocarcinoma (uterus)	Adenomyosis (uterus)	276, 294, 395, 641, 645, 691
Lymphoma/leukaemia	Hyperplasia/inflammation	170, 235, 352, 405, 525, 704

A consensus by the Peer review Pathologist and the Study Pathologist was also reached on diagnoses relating to the evaluation of the Thyroid Gland:

Animal No.	*Reviewing Pathologist's opinion*	*Study Pathologist's opinion*	*Consensus*
75	Follicular cell adenoma, undifferentiated, with solid pattern	Follicular cell adenoma	Follicular cell adenoma with unusual features
158	C-cell adenoma	Follicular cell adenoma	C-cell adenoma
163	C-cell adenoma + C-cell hyperplasia	2 C-cell hyperplasia (on 1st section)	C-cell adenoma + C-cell hyperplasia
452	C-cell adenoma (autolysed section)	Follicular cell adenoma	C-cell adenoma
371	C-cell hyperplasia	-	C-cell hyperplasia
213	C-cell hyperplasia	-	C-cell hyperplasia
665	C-cell hyperplasia	-	C-cell hyperplasia

[N000 4.2.3.4.1.1 P1405]

Non-neoplastic:

Non-neoplastic histopathology findings related to NNC 90-1170 treatment occurred in the thyroid, liver, spleen, femoro-tibial joint, seminal vesicles, and thymus. The incidence of non-neoplastic microscopic pathology findings in males and females are summarized in the tables below.

The incidence of inflammatory cell infiltrate in the thyroid was greater than the control group at $\geq$ 0.03 mg/kg/day NNC 90-1170 in males and at 0.3 and 3 mg/kg/day in females. Thyroid c-cell hyperplasia occurred at $\geq$ 0.2 mg/kg/day in males and females. (Because it's considered a preneoplastic lesion, c-cell hyperplasia was included the in discussion of thyroid tumor findings in the "Neoplastic"section).

In liver, the incidence of pigmented Kupffer cells was above control group levels at $\geq$ 0.03 mg/kg/day in males and at $\geq$ 0.2 mg/kg/day in females. The increase was above control group levels at all doses in males and at $\geq$ 0.2 mg/kg/day in females. The incidence of centrilobular hypertrophy and diffuse centrilobular hepatocyte vacuolation was elevated above control group levels at all NNC 90-1170 doses in males, but the findings were not dose-related and it didn't occur in females. The incidence of microgranuloma was significantly increased in males at all NNC 90-1170 doses compared to controls, but the increase was only statistically significant in the 0.2 mg/kg/day group, so its relation to treatment was equivocal.

In spleen, the incidence of hemosiderin was above the control group levels at $\geq$ 0.03 mg/kg/day in females. In males, the finding was considered incidental because the incidence was low and it only occurred at 0.03 and 1 mg/kg/day. The incidence of lymphoid hyperplasia was increased above control group levels at all NNC 90-1170 doses in males, but the incidence was inversely related to dose and statistically significantly increased above the control group only at 0.03 mg/kg/day, so it was considered equivocal.

The incidence of degenerative disease of the femoro-tibial joint was higher than controls at $\geq$ 0.03 mg/kg/day in males and at 0.03, 1, and 3 mg/kg in females.

Lymphocytic infiltration in seminal vesicles occurred at a higher incidence than controls at all NNC 90-1170 doses reaching statistical significance in 0.03, 0.2, and 1 mg/kg/day groups, but not in the 3 mg/kg/day group. Inflammation occurred at 3 mg/kg/day.

Tubular cystic hyperplasia in the thymus was elevated above control group levels in all NNC 90-1170 groups in males and at $\geq$ 0.2 mg/kg/day in females.

Organ	Finding	Severity	Sex / NNC 90-1170 Dose (mg/kg/day) / Fate (Survivor or Decendent) / N	Males 0 S	Males 0 D	Males 0.03 S	Males 0.03 D	Males 0.2 S	Males 0.2 D	Males 1 S	Males 1 D	Males 3 S	Males 3 D
				39	40	34	33	37	30	38	29	32	47
				79		67		67		67		79	
Thyroid	inflammatory cell infiltrate			0	0	1	0/32	4	0/28	5*	1	5*	2
				0		1 (1.5%)		4 (6.2%)*		6 (9.0%)**		7 (8.8%)*	
Liver	pigmented Kupffer cells	minimal		1	0	4	0	1	2	4	1	4	0
		mild		1	1	1	2	2	0	4	0	0	1
		moderate						1		1		1	
		Total affected		2	1	5	2	4	2	9*	1	5	1
				3 (3.8%)		7 (10.4%)		6 (9.0%)		10 (14.9%)*		6 (7.6%)	
	centrilboular hypertrophy			4 (5.1%)		12 (17.9%)*		11 (16.4%)*		9 (13.4%)		13 (16.5%)*	
	diffuse centrilobular hepatocyte vacuolation			1	1	2	5	3	1	3	3	1	3
				2 (2.5%)		7 (10.4%)		4 (6.0%)		6 (9.0%)		4 (5.1%)	
	microgranuloma			1	0	2	0	9*	0	3	0	4	0
				1 (1.3%)		2 (3.0%)		9 (13.4%)**		3 (4.5%)		4 (5.1%)	
Spleen	lymphoid hyperplasia			1	3	9**	2	9**	0	8*	0	4	2
				4 (5.1%)		11 (16.4%)*		9 (13.4%)		8 (11.9%)		6 (7.6%)	
	hemosiderin	minimal		0	0	1	1	0	0	1	0	0	0
		mild									1		
		moderate											
		Total affected		0	0	1	1	0	0	1	1	0	0
				0		2 (3.0%)		0		2 (3.0%)		0	
Femoro-tibial joint	degenerative joint disease			15/38	15	24*	17/32	26*	13/29	25*	15/27	11	24
				30 (38.0%)		41 (62.1%)**		39 (59.1%)*		40 (61.5%)**		35 (44.3%)	
Seminal vesicle	inflammation			1	5	2	6	3/36	2	2/37	3	6*	5
				6 (7.6%)		8 (11.9%)		5 (7.6%)		5 (7.6%)		11 (13.9%)	
	lymphocytic infiltration			6	3	13*	0	16**/36	1	20**/37	0	10	4
				9 (11.4%)		13 (19.4%)		17 (25.8%)*		20 (30.3%)**		14 (17.7%)	
Thymus	tubular cystic hyperplasia			3 / 33	0/33	5/27	2/30	6/32	1/27	4/29	4*/24	3/31	3/35
				3 (4.5%)		7 (12.2%)		7 (12.3%)		8 (15.1%)		6 (9.1%)	

At the injection site, minimal to marked myofiber degeneration and myositis and minimal to severe dermatitis occurred in all dose groups lacking a relation to dose for both the incidence and severity. Therefore, there were no non-neoplastic injection site findings considered treatment-related. Statistically significant changes occurred for other findings including pelvic dilatation in kidney and inflammatory cell infiltrate in hearts of females at 1 mg/kg/day and ovarian cysts at 0.2 and 1 mg/kg/day, but the increases were considered incidental because they only occurred at 1 or 2 doses and lacked a dose-response. For ovarian cysts, the background incidence was high (62.3%) in controls and comparable to the 0.2 mg/kg/day (81.5%) and 1 mg/kg/day (79.1%) groups.

Organ	Finding	Severity	Sex	Females									
			NNC 90-1170 Dose (mg/kg/day)	0		0.03		0.2		1		3	
			Fate (Survivor or Decendent)	S	D	S	D	S	D	S	D	S	D
			N	23	56	14	53	23	44	24	43	28	51
				79		67		67		67		79	
Thyroid	inflammatory cell infiltrate			3/22	0/53	2	5*/52	2	2	1	2/42	4	4*/48
				3 (4.0%)		7 (10.6%)		4 (6.0%)		3 (4.5%)		8 (10.5%)	
Liver	pigmented Kupffer cells	minimal		3	2	4	4	6	6	9	5	9	9*
		mild		6	2	1	2	4	3	5	3	8	7
		moderate								2	3		2
		Total affected		9	4	5	6	10	9	16	11*	17	18**
				13 (16.5%)		11 (16.4%)		19 (28.4%)		27 (40.3%)**		35 (44.3%)**	
	centrilboular hypertrophy			1		1		2		1		1	
	diffuse centrilobular hepatocyte vacuolation			0	4	0	3	0	3	1	2	0	2
				4 (5.1%)		3 (4.5%)		3 (4.5%)		3 (4.5%)		2 (2.5%)	
	microgranuloma			8	1	4	0	2	0	2*	0	6	3
				9 (11.4%)		4 (6.0%)		2 (3.0%)		2 (3.0%)		9 (11.4%)	
Spleen	lymphoid hyperplasia			3	3/55	4	2	4	4	4	4	5	5
				6 (7.7%)		6 (9.0%)		8 (11.9%)		8 (11.9%)		10 (12.7%)	
	hemosiderin	minimal		3	0	7*	5*	5	0	4	7**	7	3
		mild		3	1		2	1	2		4	6	4
		moderate			1								2
		Total affected		6	2/55	7	7	6	2	4	11**	13	9*
				8 (10.3%)		14 (20.9%)		8 (11.9%)		15 (22.4%)		22 (27.8%)**	
Femoro-tibial joint	degenerative joint disease			5	9/55	2	12/52	3/22	7/42	1	16*/42	4	12
				14 (17.9%)		14 (21.2%)		10 (15.6%)		17 (25.8%)		16 (20.3%)	
Thymus	tubular cystic hyperplasia			0	2/52	0	0	2	1/39	2/23	0/38	3	2/45
				2 (2.7%)		0		3 (4.8%)		2 (3.3%)		5 (6.8%)	

Neoplastic:

Treatment-related neoplasms occurred in thyroid (c-cell adenomas in males and c-cell adenomas and carcinomas in females at ≥ 1 mg/kg/day) and dorsal skin and subcutis (fibrosarcomas at 3 mg/kg/day in males). In males, equivocal findings occurred in dorsal skin and subcutis (dose-related increased rhabdomyosarcomas), injection site (dose-related increased fibrosarcomas), adrenal (subcapsular cell tumor at 0.03 mg/kg only), and vasculature (combined hemangiomas and hemangiosarcomas from all sites at uterus at 0.2 mg/kg only). A complete list of tumor findings compiled from the sponsor's Tables 22 (Peto analysis of tumor incidence: Males) and 23 (Peto analysis of tumor incidence: Females) is Appendix 1. Historical control group data of tumor incidence in control groups of CD-1 mice from 2 year carcinogenicity studies at the [redacted] in years 2002 – 2004 are included as Appendix 2. The background incidence of skin tumors for subcutaneously dosed mice in 2 year carcinogenicity studies is Appendix 3. b(4)

Tabulated summaries of tumor types with p-values < 0.05 for either dose-response relationship or pair-wise comparisons determine from statistical analysis of combined main study and week 78/104 male or female groups are shown below. In several instances, statistical analysis performed by the sponsor differs from ours.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons Combined Main and Week 78/104 Satellite Studies in Male Mice

Organ Name	Tumor Name	0 mg/kg Cont N=57	0.03 mg/kg Low N=48	0.2 mg/kg Mod N=45	1.0 mg/kg Midhi N=39	3.0 mg/kg High N=62	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H	P_Value Ptt C vs. H
ADRENAL GLAND	SUBCAPSULAR CELL TUMOUR	4	11	5	4	5	0.854	0.013	0.368	0.421	0.526
ALL_SITES	HAEMANGIOSARCOMA+HAEMANGI	1	2	10	0	7	0.154	0.412	0.005	0.497	0.036
INJECTION/TREAT	FIBROSARCOMA [M]	0	1	1	0	4	0.019	0.402	0.442	.	0.064
INJECTION_SITE	FIBROMA+FIBROSARCOMA	1	1	1	0	4	0.048	0.686	0.686	0.482	0.187
LYMPH NODE (MES	HAEMANGIOMA [B]	0	0	2	0	3	0.047	.	0.192	.	0.129
SKIN AND SUBCUT	FIBROSARCOMA [M]	0	2	1	2	7	0.003	0.198	0.448	0.169	0.008
	RHABDOMYOSARCOMA [M]	0	0	2	1	4	0.018	.	0.198	0.487	0.066
THYROID	C-CELL ADENOMA+CARCINOMA	0	0	0	0	15	0.000	.	.	0.000	0.000
THYROID GLAND	C-CELL ADENOMA [B]	0	0	0	0	15	0.000	.	.	0.000	0.000

Adrenal subcapsular cell tumor was not described as benign, so it was considered to be an adenoma. Therefore, the p value for control group pair-wise comparison is not significant because $p > 0.01$.

Hemangioma or hemangiosarcoma from all sites (vascular tumor) is common, but the p value for control group pair-wise comparison is < 0.01, and therefore statistically significant.

Fibrosarcomas at the injection site in subcutaneously injected Sprague Dawley rats in 2 year studies is a rare tumor because the incidence was < 1% (1.03% in males (4/388) and 0% in females (0/380)). The dose-related trend is considered significant.

Since injection site fibroma only occurred in control group males, the combined incidence of injections site fibroma and fibrosarcoma aren't relevant to treatment-related affects.

Lymph node hemangioma was included as a statistically significant tumor (trend analysis $p < 0.05$) in the final draft of the statistics review. There were no instances indicated in the historical control data for lymphoreticular/hematopoietic tumors, but the historical control group rate for vascular hemangiomas is 1.8% and therefore, a common tumor. The dose-related trend $p > 0.005$ is not significant.

Skin and subcutis fibrosarcomas are common because the historical control group incidence is > 1% (3.4%) and rhabdomyosarcomas are rare because their background incidence is 0%.

Thyroid adenomas are rare tumors in mice because the historical control group incidence is < 1% (0%). Dose related increased thyroid c-cell adenoma is significant, but in the absence of c-cell carcinomas, the significant dose-related increased combined c-cell adenomas + carcinomas is irrelevant.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons Combined Main and Week 78/104 Satellite Studies in Female Mice

Organ Name	Tumor Name	0 mg Cont N=57	0.03 mg Low N=44	0.2 mg Med N=57	1.0 mg Mid Hi N=47	3.0 mg High N=58	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. MH	P_Value C vs. H
HARDERIAN GLAND	ADENOMA [B]	1	0	1	2	5	0.014	0.415	0.753	0.409	0.105
PITUITARY GLAND	ADENOMA, ANTERIOR LOBE [B	0	0	2	0	5	0.006	.	0.240	.	0.042
SKIN AND SUBCUT	SARCOMA (NOT OTHERWISE SP	1	0	1	0	5	0.010	0.400	0.747	0.466	0.150
THYROID GLAND	C-CELL ADENOMA+CARCINOMA	0	0	0	4	17	0.000	.	.	0.051	0.000
	C-CELL ADENOMA [B]	0	0	0	4	15	0.000	.	.	0.051	0.000
UTERUS	LEIOMYOMA+LEIOMYOSARCOMA	5	7	14	8	10	0.578	0.187	0.017	0.221	0.216

Harderian gland adenoma is a common tumor because the average background incidence is 1.7% in females. Since the p-value for trend analysis was > 0.005 (actual p-value 0.014), the dose-related trend of increased tumor incidence was not significant.

Anterior pituitary adenoma is a common tumor in females because the average background incidence was 3.3%. Since the p-value for trend analysis was > 0.005 (actual p-value 0.006) and the p-value for pair-wise comparison of the HD group with control was > 0.01 (actual p-value 0.042), the dose-related trend of increased tumor incidence and increased tumor incidence in the HD group were not significant.

Sarcoma of the skin and subcutis is a common tumor because the background incidence is 1.8% in females. Since the p-value for trend analysis was > 0.005 (actual p-value 0.010), the dose-related trend of increased tumor incidence was not significant.

Uterine leiomyoma is a common tumor because the background incidence is 3.8%. Since the p-value for pair-wise comparison of the mid-dose group with controls was > 0.01 (actual p-value 0.029), the increased tumor incidence was not significant.

Thyroid c-cell adenomas and carcinomas are rare tumors because the average background incidence is 0% (for either tumor). The dose related trend and increased incidence for adenomas and combined adenomas or carcinomas were significant.

Thyroid Tumors

Thyroid c-cell focal hyperplasia, adenomas, or carcinomas are rare histology findings in mice. Treatment-related C-cell neoplasms in males and females were considered a progression of focal c-cell hyperplasia. Although physiologic diffuse and/or focal c-cell hyperplasia and/or hypertrophy can be considered a normal physiologic response, when c-cell neoplasms occur in the same study, focal hyperplasia is considered a preneoplastic lesion. Its notable that diffuse c-cell hyperplasia didn't occur in this study.

NNC 90-1170 dose-dependently increased the incidence of focal thyroid c-cell hyperplasia at ≥ 0.2 mg/kg/day in males and females. Thyroid c-cell adenomas dose-dependently increased compared to controls at ≥ 1 mg/kg/day in males and females. C-cell carcinomas occurred in females at 3 mg/kg, but the increased incidence wasn't significant by pair-wise comparison to control or trend analysis.

Organ	Finding	Severity	Sex	Males									
			NNC 90-1170 Dose (mg/kg/day)	0		0.03		0.2		1		3	
			Fate (Survivor or Decendent)	S	D	S	D	S	D	S	D	S	D
			N	39	40	34	33	37	30	38	29	32	47
				79		67		67		67		79	
thyroid	Focal C-cell hyperplasia (preneoplastic)	minimal		0	0	0	0	0	0	3	2	6##	7#
		mild						1		1	0	3	4
		moderate								3	2	5#	3
		marked										1	1
		total afftected		0	0	0	0/32	1	0/28	7##	4#	15##	15##
				0		0		1 (1.5%)		11 (16.4%)###		30 (38.0%)###	
	C-cell adenoma			0	0	0	0	0	0	7**	2	10**	5
				0		0		0		9 (13.4%)***		15 (19.0%)***	
	C-cell carcinoma			0	0	0	0	0	0	0	0	0	0
				0		0		0		0		0	
	C-cell tumor			0		0		0		9 (13.4%)***		15 (19.0%)***	

According to the sponsor's statistical analysis of thyroid c-cell hyperplasia, statistically significant differences from control by Fisher analysis are denoted at $p < 0.05$ (#), $p < 0.01$ (##), or $p < 0.001$ (###). According to the sponsor's statistical analysis of thyroid c-cell adenomas, carcinomas, or total tumors, statistically significant differences from control by Peto analysis are denoted at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

Organ	Finding	Severity	Sex	Females									
			NNC 90-1170 Dose (mg/kg/day)	0		0.03		0.2		1		3	
			Fate (Survivor or Decendent)	S	D	S	D	S	D	S	D	S	D
			N	23	56	14	53	23	44	24	43	28	51
				79		67		67		67		79	
thyroid	Focal C-cell hyperplasia (preneoplastic)	minimal		0	0	0	0	3	3	0	1	3	7##
		mild							1	4	3	2	7##
		moderate								0	1	0	2
		marked								1		1	
		total afftected		0	0	0	0	3	4#	5	5/42#	6*	16/48##
				0		0		7 (10.4%)##		10 (15.2%)###		22 (28.9%)###	
	C-cell adenoma			0	0	0	0	0	0	1	3/42	9**	6/48**
				0		0		0		4 (6.0%)*		15 (19.7%)***	
	C-cell carcinoma			0	0	0		0	0	0	0	0	2/48
				0		0		0		0		2 (2.6%)	
	C-cell tumor			0		0		0		4 (6.0%)*		17 (22.4%)***	

According to the sponsor's statistical analysis of thyroid c-cell hyperplasia, statistically significant differences from control by Fisher analysis are denoted at $p < 0.05$ (#), $p < 0.01$ (##), or $p < 0.001$ (###). According to the sponsor's statistical analysis of thyroid c-cell adenomas, carcinomas, or total tumors, statistically significant differences from control by Peto analysis are denoted at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

Focal C-cell hyperplasia was considered a preneoplastic lesion because:

1. the incidence of both hyperplasia and c-cell tumors were dose related in both males and females.
2. hyperplasia occurs at lower doses than tumors.

3. the incidence of hyperplasia is greater than the incidence of tumors in all dose groups.
4. in 3 mg/kg/day group mice with c-cell adenomas (the only group analyzed), hyperplasia occurred in 9/16 males (56%) and 5/15 females (33%).
5. in decedents, a finding of c-cell hyperplasia preceded c-cell tumors by 17 weeks in both males (weeks 61 and 78) and females (weeks 47 and 64).

Thyroid C-cell Findings in 3 mg/kg/day NNC 90-1170 Treated Mice

Sex	Mouse #	Week of Death	Mode of Death[1]	Thyroid c-cell Finding				
				Focal Hyperplasia			Adenoma	Carcinoma
				min - mild	moderate	marked		
Males	203	105	T		X			
	204	105	T				X	
	208	102	SM		X			
	213	105	T		X			
	217	101	FD				X	
	218	87	SM	X				
	220	81	SM		X		X	
	221	105	T		X		X	
	222	105	T	X			X	
	223	105	T	X				
	224	105	T		X			
	226	105	T	X				
	227	70	SM	X				
	230	78	SM	X			X	
	232	86	SM		X			
	233	105	T	X				
	236	104	SM				X	
	240	76	SM	X				
	242	105	T				X	
	250	105	T				X	
	583	106	T	X				
	584	94	SM	X				
	585	106	T	X				
	586	106	T		X		X	
	588	82	FD	X				
	590	106	T				X	
	593	100	SM			X	X	
	594	100	SM		X		X	
	595	103	FD	X				
	597	106	T	X			X	
	599	106	T		X		X	
	600	61	SM	X				
	602	94	FD	X				
	603	73	SM	X				
	604	106	T	X				
	607	106	T				X	
Females	452	100	FD				X	
	455	105	T	X				
	457	69	SM	X				
	458	105	T				X	
	459	105	T				X	
	467	101	FD	X				
	473	99	SM	X				
	476	83	SM	X				
	478	81	SM	X				
	481	105	T	X			X	
	485	104	FD				X	
	487	86	FD	X				
	489	47	FD	X				
	490	67	SM	X				
	494	87	FD				X	
	496	96	FD		X			
	497	87	SM	X				
	500	75	SM	X				
	692	98	SM				X	
	695	106	T	X			X	
	697	106	T			X	X	
	698	98	SM	X				
	699	59	SM	X				
	702	97	SM		X		X	
	708	69	SM	X				
	709	106	T				X	
	726	106	T				X	
	713	106	T				X	
	714	92	SM					X
	715	106	T	X			X	
	716	104	SM	X			X	
	717	106	T	X				
	718	64	SM					X

[1]Modes of death were termination (at end of study, T), sacrificed moribund (SM), or found dead (FD).

Skin and Subcutis

Mice were treated by subcutaneous injections in the dorsal surface; between the scapula and on the rump. Sarcomas of the dorsal surface (includes fibrosarcoma, sarcoma (not otherwise specified), rhabdomyosarcoma and leiomyosarcoma) occurred in all groups, except 1 mg/kg/day females (see Table

3 below) with 12 of 49 occurring around the microchip implant. In males, the incidence of sarcoma on the dorsal surface was dose-related at ≥ 0.03 mg/kg/day (Peto analysis, $p < 0.001$) and significantly increased compared to controls at 3 mg/kg/day.

Table 3: Incidence of Sarcoma on the Dorsal Surface in Main and Extension animals (Peto statistical analysis)

	Animals/Dose									
	Males					Females				
Group Number	1	2	3	4	5	1	2	3	4	5
Dose of NNC 90-1170 (mg/kg/day)	0	0.03	0.2	1.0	3.0	0	0.03	0.2	1.0	3.0
Number examined	79	67	67	67	79	79	67	66	65	78
Sarcoma, dorsal surface	2	3	5	3	16***	6	4	2	0	8

* Statistically different from the Control: p<0.05; *** Statistically different from the Control: p<0.001

[N000 4.2.3.4.1.1 P39]

The following summary table for sarcomas in the skin and subcutis was compiled from the "Summary of Histological Findings Separated by Survivors and Decedents" (Tables 19 & 20, pages 374-375, 454).

In males, the incidence of fibrosarcoma was significantly higher than control at 3 mg/kg/day. Fibrosarcomas on the dorsal surface (combined dorsal and injection site) were identified as a cause of death in 9/47 (19.1%) high dose group male decedents. Dose-related increased incidences of injection site fibrosarcoma and dorsal skin and subcutis rhabdomyosarcoma were equivocal because although dose-related trend analysis was statistically significant, dose group pair-wise comparison with controls was not..

Organ	Finding	Sex	Males									
		NNC 90-1170 Dose (mg/kg/day)	0		0.03		0.2		1		3	
		Fate (Survivor or Decendent)	S	D	S	D	S	D	S	D	S	D
		N	39	40	34	33	37	30	38	29	32	47
		Severity	79		67		67		67		79	
Skin & subcutis (dorsal)	fibrosarcoma		0	0	0	2	0	1	1	1	1	6*
			0		2 (3.0%)		1 (1.5%)		2 (3.0%)		7 (8.8%)**	
	sarcoma (unspecified)		0	1	0	0	0	0	0	0	0	1
			1 (1.2%)		0		0		0		1 (1.2%)	
	rhabdomyosarcoma		0	0	0	0	0	2	0	1	0	4
			0		0		2 (3.0%		1 (1.5%)		4 (5.1%)	
	leiomyosarcoma		0	0	0	0	0	1	0	0	0	1
			0		0		1 (1.5%)		0		1 (1.2%)	
	fibrosarcoma (injection site)		0		1 (1.5%)		1 (1.5%)		0		4 (5.0%)	
	sarcoma (treated skin)		2 (2.5%)		3 (4.5%)		5 (7.5%)		3 (4.5%)		16 (20.2%)***	

According to the sponsor's statistical analysis, statistically significant differences from control by Peto analysis are denoted at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

There were no dose-related tumor findings in the skin or subcutis in females, but the background incidence of sarcoma was markedly elevated above the background incidence for 2 year carcinogenicity studies using subcutaneous injection (7.6% in control for this study compared to mean of < 1% for historical controls.).

Organ	Finding	Sex	Females				
		NNC 90-1170 Dose (mg/kg/day)	0	0.03	0.2	1	3
		Fate (Survivor or Decendent)	S D	S D	S D	S D	S D
		N	23 56	14 53	23 44	24 43	28 51
		Severity	79	67	67	67	79
Skin & subcutis (dorsal)	fibrosarcoma		0 1 1 (1.2%)	0 1 1 (1.5%)	0 1 1 (1.5%)	0 0 0	1 1 2 (2.3%)
	sarcoma (unspecified)		0 1 1 (1.2%)	0 0 0	0 1 1 (1.5%)	0 0 0	0 5 5 (6.3%)
	rhabdomyosarcoma		0 2 2 (2.4%	0 1 1 (1.5%)	0 1 1 (1.5%)	0 0 0	0 0 0
	leiomyosarcoma		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	fibrosarcoma (injection site)		1 (1.3%)	0	0	0	2 (2.6%)
	sarcoma (treated skin)		6 (7.6%)	4 (6.0%)	2 (3.0%)	0	8 (10.3%)

According to the sponsor's statistical analysis, statistically significant differences from control by Peto analysis are denoted at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

The following table shows the incidence of neoplasms around the microchip implant site.

Incidence of Neoplasms Identified Around the Implant (12 Rats Affected)

	Sex	Males					Females				
	NNC 90-1170 Dose (mg/kg/day)	0	0.03	0.2	1	3	0	0.03	0.2	1	3
	Finding N	79	67	67	67	79	79	67	67	67	79
Skin & subcutis (dorsal)	fibrosarcoma	0	0	0	2	1	1	0	1	0	1
	sarcoma (unspecified)	0	0	0	0	1	1	0	0	0	1
	rhabdomyosarcoma	0	0	0	0	0	0	1	0	0	0
	leiomyosarcoma	0	0	1	0	0	0	0	1	0	0
	total sarcomas	0	0	1	2	2	2	1	2	0	2

The incidence of dorsal skin and subcutis neoplasms, corrected for tumors occurring at the microchip identification implant site not attributable to NNC 90-1170 treatment, is shown in the table below. Total sarcomas increased at 3 mg/kg/day NNC 90-1170 in males and females with an apparent dose related increase in fibrosarcomas, injection site fibrosarcomas, and rhabdomyosarcomas in males and unspecified sarcomas in females.

Organ	Finding	Sex	Males					Females				
		NNC 90-1170 Dose (mg/kg/day)	0	0.03	0.2	1	3	0	0.03	0.2	1	3
		Fate (Survivor or Decendent)	S D	S D	S D	S D	S D	S D	S D	S D	S D	S D
		N	39 40	34 33	37 30	38 29	32 47	23 56	14 53	23 44	24 43	28 51
			79	67	67	67	79	79	67	67	67	79
Skin & subcutis (dorsal)	fibrosarcoma		0 0 0	0 2 2 (3.0%)	0 1 1 (1.5%)	0 0 0	1 5 6 (7.6%)	0 0 0	0 1 1 (1.5%)	0 0 0	0 0 0	1 0 1 (1.3%)
	sarcoma (unspecified)		0 1 1 (1.3%)	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 1 1 (1.5%)	0 0 0	0 4 4 (5.1%)
	rhabdomyosarcoma		0 0 0	0 0 0	0 2 2 (3.0%)	0 1 1 (1.5%)	0 4 4 (5.1%)	0 2 2 (2.5%)	0 0 0	0 1 1 (1.5%)	0 0 0	0 0 0
	leiomyosarcoma		0 0 0	0 0 0	0 0	0 0 0	0 1 1 (1.2%)	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	fibrosarcoma (injection site)		0	1 (1.5%)	1 (1.5%)	0	4 (5.1%)	1 (1.3%)	0	0	0	2 (2.6%)
	Total sarcomas		1 (1.3%)	3 (4.5%)	4 (6.0%)	1 (1.5%)	15 (19.0%)	3 (3.8%)	1 (1.5%)	2 (3.0%)	0	7 (8.9%)

Uterus

There were no uterine tumors considered treatment related, but t notable increases in uterine hemangiomas, leiomyomas, and leiomyosarcomas occurred. Although the sponsor's pair-wise comparison with control showed a significant increase in leiomyomas at 0.2 mg/kg/day ($p < 0.05$), it did not meet the significance criteria for a common tumor ($p < 0.01$). Uterine hemangiomas occurred in all NNC 90-1170 groups and the incidence of hemangioma was significantly higher than controls at 0.03, 0.2, and 1 mg/kg/day, but not at 3 mg/kg/day. The incidence exceeded the historical control group maximum of 2% in all dose groups. The incidence of leiomyoma exceeded the concurrent control and historical control range (2.0 – 6.0%) at 0.03, 0.2, and 3 mg/kg/day. The incidence of leiomyosarcomas exceeded the concurrent control and historical control range (0 – 2.5%) at 0.03, 0.2, and 1 mg/kg/day. The incidence of combined leiomyomas / leiomyosarcomas exceeded the concurrent controls group at $\geq$ 0.03, 0.2, and 1 mg/kg/day NNC 90-1170, but the increased incidence was not dose-dependent. According to statistical analysis from the Biostatistics reviewer (Dr. Min), dose-related trend or pair-wise comparison criteria were not met for any uterine tumor.

	Sex	Females									
	NNC 90-1170 Dose (mg/kg/day)	0		0.03		0.2		1		3	
	Fate (Survivor or Decendent)	S	D	S	D	S	D	S	D	S	D
Organ	Finding N	23	56	14	53	23	44	24	43	28	51
		79		67		67		67		79	
Uterus	hemangioma	0		2 (3.0%)*		3 (4.5%)*		3 (4.5%)*		3 (3.8%)	
	leiomyoma	3	2/55	1	4	5	6/43	1	3	7	3/50
		5 (6.4%)		5 (7.5%)		11 (16.7%)*		4 (6.0%)		10 (12.8%)	
	leiomyosarcoma	0		2 (3.0%)		3 (4.5%)		4 (6.0%)		0	
	Total leiomyomas / leiomyosarcomas	5 (6.4%)		7 (10.4%)		14 (21.2%)		8 (11.9%)		10 (12.8%)	

Statistically significantly different from control by Peto analysis: *p < 0.05, **p < 0.01

Other Neoplastic Findings

The tables below show the tumor incidence at each dose of NNC 90-1170 for statistically significantly increased tumors compared to concurrent controls or tumors that increased with dose, but without a significant increase compared to control at any dose.

In males, benign adrenal subcapsular tumors increased at 0.03 mg/kg/day, and although the incidence of 15.2% exceeded the control group historical maximum of 14.2%, the increase was not significant because the p-value for pair-wise comparison was > 0.01 (0.013), the criteria for a common tumor.

The combined incidence of hemangiomas / hemangiosarcomas was significantly increased ($p = 0.001$) at 0.2 mg/kg/day NNC 90-1170, therefore the relation to treatment was considered equivocal.

Interstitial cell adenomas of the testes were significantly decreased compared to controls at 0.2 mg/kg/day, but this finding was not considered relevant to human risk assessment.

Organ / Tissue	Finding	Sex / NNC 90-1170 Dose (mg/kg/day) / Fate (Survivor or Decendent) / N	Males 0 S	0 D	0.03 S	0.03 D	0.2 S	0.2 D	1 S	1 D	3 S	3 D
		N	39	40	34	33	37	30	38	29	32	47
			79		67		67		67		79	
Adrenal	subcapsular tumor		4	0	10/33	1	3	2	3	1/25	3/30	2
			4 (5.1%)		11 (15.2%)*		5 (7.5%)		4 (6.3%)		5 (6.5%)	
Vascular (all sites)	hemangioma / hemangiosarcoma		1 (1.3%)		2 (3.0%)		10 (14.9%)**		0		7 (8.9%)	
Testes	interstitial cell adenoma		9	2	3	0	0**	0	4/38	0	5	2
			11 (13.9%)		3 (4.5%)		0		4 (6.0%)		7 (8.9%)	

According to the sponsor's statistical analysis, statistically significant differences from control by Peto analysis are denoted at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

In females, the incidence of pituitary adenoma (6.4%) at 3 mg/kg/day exceeded the control group historical maximum of 5.9%, but because it's a common tumor, the dose-related increase was not considered statistically significant ($p = 0.006$, $p > 0.005$) and pair-wise comparison with the control group was not statistically significant ($p=0.042$, $p > 0.01$). Harderian gland adenoma increased with dose (Peto analysis, $p < 0.05$) the incidence of 6.3% at 3 mg/kg/day exceeded the control group historical control maximum of 4.0%. Because Harderian gland adenoma was considered a common tumor, the dose-related increased was not significant ($p=0.014$, $p > 0.005$).

Organ	Finding / Severity	Sex / NNC 90-1170 Dose (mg/kg/day) / Fate (Survivor or Decendent) / N	Females 0 S	0 D	0.03 S	0.03 D	0.2 S	0.2 D	1 S	1 D	3 S	3 D
		N	23	56	14	53	23	44	24	43	28	51
			79		67		67		67		79	
Pituitary	adenoma, anterior lobe		0	0	0	0	2 / 22	0	0	0	4/27	1
			0		0		2 (3.0%)		0		5 (6.4%)*	
	adenoma, intermediate lobe		0	0	0	0	0	0	1	1	1	0
			0		0		0		2 (3.0%)		1 (1.3%)	
Harderain gland	adenoma		1	0	0	0	0	1/43	1	1	3	2
			1 (1.3%)		**0**		**1 (1.5%)**		**2 (3.0%)**		**5 (6.3%)**	

According to the sponsor's statistical analysis, statistically significant differences from control by Peto analysis are denoted at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

Summary and Conclusions

A 104 week carcinogen bioassay of 0, 0.03, 0.2, 1, or 3 mg/kg/day NNC 90-1170 injected subcutaneously once a day in CD-1 mice included a main study group (50/sex/.dose), a 78-week interim sacrifice group (29/sex/control and high dose, 17/sex/low and intermediate doses), and satellite toxicokinetic / plasma calcitonin groups (51/sex/dose, 17/sex/sample week). Although mortality was unaffected by treatment, due to reduced survival in control group females, the 78 week intermittent sacrifice was canceled with dosing continued to week 104. The sponsor's tumor analysis combined results from both main study and week 78/104 groups. Toxicokinetic parameters were determined in weeks 26, 52, and 104 using an ELISA detecting the peptide moiety of NNC 90-1170. In general, Cmax and AUC_{0-24} increased linearly with dose. Estimated human exposure multiples based on AUC_{0-24} 816 nM.hr at the MRHD of 1.8 mg/day NNC 90-1170 and week 104 mouse AUC_{0-24} (average of male and female combined) were 0.23, 1.8, 10, and 45 for doses of 0.03, 0.2, 1, and 3 mg/kg/day NNC 90-1170 (exposures not corrected for higher plasma protein binding in mice).

At 0.03 mg/kg/day, NNC 90-1170 decreased group mean body weight gain 15 – 16 % compared to controls, but the decrease occurred in the absence of a dose response and without significantly decreasing group mean body weight or food consumption. There were no treatment-related effects on water consumption.

There was evidence of a mild hemolytic anemia in mice at ≥ 1 mg/kg/day NNC 90-1770 including decreased RBCs (males and females), increased reticulocytes (males), pigmented Kupffer cells (males and females), and hemosiderin in spleen (females). In males, there was a significant dose-related 8.4 – 13.7% decrease in RBCs at ≥ 0.2 mg/kg/day and a corresponding increase in reticulocytes, but 14.7 – 26.5% increased relative reticulocyte count did not reach statistical significance. RBCs were significantly decreased 7.5 – 4.1% compared to concurrent controls in females, but the decrease was not dose related.

Plasma calcitonin in mice was measured after 26, 52, and 104 weeks of treatment using a rat calcitonin IRMA assay, and specificity and sensitivity of the assay for mouse calcitonin was not reported. Using this assay, group mean plasma calcitonin was higher than controls at ≥ 0.2 mg/kg/day in males and females. Between weeks 26 and 104, plasma calcitonin levels increased > 2 fold at 3 mg/kg/day in females. Increased calcitonin is likely related to increased incidence of thyroid focal c-cell hyperplasia and c-cell tumors in the high dose group.

Anti-NNC 90-1170 antibodies were not detected in mice treated with up to 3 mg/kg/day NNC 90-1170 for 26, 52, 78, or 104 weeks. Although the potential interference from plasma NNC 90-1170 and the absence of a sustained pharmacodynamic effect preclude a definitive assessment of an antibody response to NNC 90-1170, the impact on the acceptability of the study is minimal because treatment-related tumors occurred. However, carcinogenic effects due to exaggerated pharmacology may not be fully assessed.

Treatment-related necropsy findings were a low incidence of masses in thyroid of 3 mice in the 3 mg/kg/day NNC 90-1170. Masses occasionally occurred in the bones, heart, intestine, duodenum, and cecum of other mice.

Treatment-related non-neoplastic histopathology findings occurred in thyroid, liver, spleen, femoro-tibial joint, and seminal vesicles. In the thyroid, inflammatory cell infiltrate occurred at 0.03 mg/kg/day NNC 90-1170 and focal c-cell hyperplasia, considered a precursor to thyroid c-cell tumors, occurred at ≥ 1 mg/kg/day in males and at ≥ 0.2 mg/kg/day in females. In liver, pigmented Kupffer cells (attributed to hemosiderin accumulation), centrilobular hypertrophy, and diffuse centrilobular hepatocyte vacuolation occurred at ≥ 0.03 mg/kg/day in males. Liver pigmented Kupffer cells occurred at ≥ 1 mg/kg/day in females. Hemosiderin accumulation in spleen occurred at ≥ 0.03 mg/kg/day in females. The incidence of degenerative disease in the femoro-tibial joint was above control group levels at ≥ 0.03 mg/kg/day in males and at 0.03, 1, and 3 mg/kg/day in females. Lymphocytic infiltration in the seminal vesicles occurred at ≥ 0.03 mg/kg/day and inflammation of the seminal vesicles was higher than controls at 3 mg/kg/day. Thymus tubular cystic hyperplasia occurred at ≥ 0.03 mg/kg/day in males and at ≥ 0.2 mg/kg/day in females.

NNC 90-1170 treatment-related neoplastic findings occurred in **thyroid c-cells** (males and females) and **dorsal skin and subcutis** (females). Equivocal findings occurred in **dorsal skin and subcutis** (males), **injection site on the dorsal surface** (males) and **vasculature** (males). Thyroid c-cell hyperplasia and tumors are rare spontaneous findings in mice.

NNC 90-1170 dose-dependently increased the incidence of **focal thyroid c-cell hyperplasia**, a preneoplastic lesion, and dose-dependently increased the incidence of **thyroid c-cell adenomas** at $\geq$ 1 mg/kg/day in males and females, and increased the incidence of combined **c-cell adenomas / carcinomas** at $\geq$ 1 mg/kg/day in females. Greater than 2 fold increased plasma calcitonin occurring between weeks 26 and 104 at 3 mg/kg/day in males and females was coincident with an increased incidence of thyroid c-cell focal hyperplasia and tumors.

A positive finding of **fibrosarcomas** of the **dorsal skin and subcutis** occurred at 3 mg/kg/day NNC 90-1170 in males. There were equivocal finding of dose-related **dorsal skin and subcutis rhabdomyosarcoma** and **injection site fibrosarcoma** in males. Incidences of dorsal skin and subcutis rhabdomyosarcomas and injection site fibrosarcoma in 3 mg/kg/day group males exceeded the historical control range for both tumors, but the increased incidence for either finding never reached statistical significance. The sponsor's analysis of tumor incidence data grouping **total dorsal surface sarcomas** was statistically significant for trend ($p < 0.001$) and pair-wise analysis compared to controls at 3 mg/kg/day NNC 90-1170 in males ($p < 0.001$).

An equivocal finding of increased combined incidence of **hemangiomas / hemangiosarcomas at all sites** in males at 0.2 mg/kg/day NNC 90-1170 (uHEM 45), but the increased incidence was not dose related.

APPENDIX 1 FOR MOUSE CARCINOGENICITY STUDY REVIEW: LIST OF TUMOR INCIDENCES

Compiled from 'Peto analysis of tumor incidence: Males' and 'Peto analysis of tumor incidence: Females' (Tables 22 & 23 in the sponsor's report)

Males, Peto analysis of tumor incidence

			Group (Dose Level in mg/kg/day)				
			1 (0)	2 (0.08)	3 (0.2)	4 (1.0)	5 (3.0)
Organ/Finding			(N=79)	(N=67)	(N=67)	(N=67)	(N=79)
LUNG	NEX		79	67	67	67	79
BRONCHIOLO-ALVEOLAR CARCINOMA [M]	NOBS		5	3	12	7	7
	I		7	3	9	4	5
	F		1	0	3	3	2
	P-VALUE		0.60	0.90	0.12	0.51	0.59
LUNG	NEX		79	67	67	67	79
BRONCHIOLO-ALVEOLAR ADENOMA [B]	NOBS		18	19	16	7	18
	I		18	19	16	7	18
	F		0	0	0	0	0
	P-VALUE		0.99	0.18	0.51	0.97	0.94
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA [M] not otherwise specified	NOBS		0	1	0	0	0
	I		0	0	0	0	0
	F		0	1	0	0	0
	P-VALUE	#	0.79	0.47	1.00	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA [M] lymphoblastic	NOBS		1	3	0	1	2
	I		0	0	0	0	0
	F		1	3	0	1	2
	P-VALUE	#	0.39	0.25	1.00	0.71	0.51
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA LYMPHOCYTIC [M]	NOBS		1	1	0	1	0
	I		0	1	0	0	0
	F		1	0	0	1	0
	P-VALUE	#	0.75	0.71	1.00	0.74	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA FOLLICULAR CENTRE CELL [M]	NOBS		7	1	2	4	8
	I		4	0	2	2	6
	F		3	1	0	3	2
	P-VALUE		0.032	0.97	0.94	0.79	0.34
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LEUKAEMIA GRANULOCYTIC [M]	NOBS		2	1	0	0	0
	I		0	1	0	0	0
	F		2	0	0	0	0
	P-VALUE	#	0.99	0.79	1.00	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
HISTIOCYTIC SARCOMA [M]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.59	1.00	0.49	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LEUKAEMIA [M]	NOBS		0	0	0	0	1
	I		0	0	0	0	0
	F		0	0	0	0	1
	P-VALUE	#	0.23	1.00	1.00	1.00	0.52
LYMPH NODE (MESENTERIC)	NEX		77	65	66	63	78
HAEMANGIOMA [B]	NOBS		0	0	2	0	3
	I		0	0	2	0	3
	F		0	0	0	0	0
	P-VALUE	#	0.049	1.00	0.25	1.00	0.14
SPLEEN	NEX		79	67	67	67	79
HAEMANGIOSARCOMA [M]	NOBS		0	0	1	0	1
	I		0	0	0	0	1
	F		0	0	1	0	0
	P-VALUE	#	0.19	1.00	0.51	1.00	0.45
THYROID GLAND	NEX		79	66	65	67	79
FOLLICULAR CELL CARCINOMA [M]	NOBS		0	0	0	0	1
	I		0	0	0	0	0
	F		0	0	0	0	1
	P-VALUE	#	0.22	1.00	1.00	1.00	0.50
THYROID GLAND	NEX		79	66	65	67	79
C-CELL ADENOMA [B]	NOBS		0	0	0	9	15
	I		0	0	0	9	15
	F		0	0	0	0	0
	P-VALUE		<0.001	1.00	1.00	<0.001	<0.001
THYROID GLAND	NEX		79	66	65	67	79
C-CELL TUMOUR	NOBS		0	0	0	9	15
	I		0	0	0	9	15
	F		0	0	0	0	0
	P-VALUE		<0.001	1.00	1.00	<0.001	<0.001
THYROID GLAND	NEX		79	66	65	67	79
FOLLICULAR CELL ADENOMA [B]	NOBS		1	1	0	2	1
	I		1	1	0	2	1
	F		0	0	0	0	0
	P-VALUE	#	0.38	0.67	1.00	0.49	0.79
ADRENAL GLAND	NEX		79	66	67	63	77
SUBCAPSULAR CELL TUMOUR [B]	NOBS		4	11	5	4	5
	I		4	11	5	4	5
	F		0	0	0	0	0
	P-VALUE		0.75	0.010	0.34	0.45	0.26

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
ADRENAL GLAND	NEX		79	66	67	63	77
PHAEOCHROMOCYTOMA [B]	NOBS		1	0	1	0	1
	I		0	0	1	0	1
	F		1	0	0	0	0
	P-VALUE	#	0.38	1.00	0.73	1.00	0.72
PITUITARY GLAND	NEX		76	66	66	66	77
ADENOMA ANTERIOR LOBE [B]	NOBS		1	0	1	1	0
	I		1	0	1	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.67	1.00	0.73	0.75	1.00
PANCREAS (ENDOCRINE)	NEX		79	67	66	67	78
ISLET CELL ADENOMA [B]	NOBS		1	0	1	0	0
	I		1	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.84	1.00	0.74	1.00	1.00
TESTIS	NEX		79	67	67	66	79
HAEMANGIOSARCOMA [M]	NOBS		0	0	0	0	1
	I		0	0	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.18	1.00	1.00	1.00	0.45
TESTIS	NEX		79	67	67	66	79
RETE TESTIS ADENOMA [B]	NOBS		1	1	0	2	0
	I		1	1	0	2	0
	F		0	0	0	0	0
	P-VALUE	#	0.66	0.72	1.00	0.48	1.00
TESTIS	NEX		79	67	67	66	79
INTERSTITIAL CELL ADENOMA [B]	NOBS		11	3	0	4	7
	I		11	3	0	4	7
	F		0	0	0	0	0
	P-VALUE		0.22	0.97	1.00	0.96	0.78
TESTIS	NEX		79	67	67	66	79
HAEMANGIOMA [B]	NOBS		0	0	3	0	0
	I		0	0	3	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.79	1.00	0.081	1.00	1.00
TESTIS	NEX		79	67	67	66	79
SEX CORD/STROMAL TUMOUR [B]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.78	0.47	1.00	1.00	1.00
EPIDIDYMIS	NEX		79	67	67	66	79
HISTIOCYTIC SARCOMA [M]	NOBS		1	0	0	0	1
	I		1	0	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.33	1.00	1.00	1.00	0.70
EPIDIDYMIS	NEX		79	67	67	66	79
INTERSTITIAL CELL ADENOMA [B]	NOBS		0	0	0	1	1
	I		0	0	0	1	1
	F		0	0	0	0	0
	P-VALUE	#	0.11	1.00	1.00	0.49	0.45
SEMINAL VESICLE	NEX		79	67	66	66	78
LEIOMYOSARCOMA [M]	NOBS		0	1	0	0	0
	I		0	0	0	0	0
	F		0	1	0	0	0
	P-VALUE	#	0.79	0.47	1.00	1.00	1.00
SEMINAL VESICLE	NEX		79	67	66	66	78
GRANULAR CELL TUMOUR [M]	NOBS		1	0	0	0	0
	I		1	0	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
KIDNEY	NEX		79	67	67	67	79
TUBULAR CELL CARCINOMA [M]	NOBS		0	0	2	0	0
	I		0	0	2	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.67	1.00	0.23	1.00	1.00
KIDNEY	NEX		79	67	67	67	79
TUBULAR CELL ADENOMA [B]	NOBS		3	0	2	2	0
	I		3	0	2	2	0
	F		0	0	0	0	0
	P-VALUE	#	0.89	1.00	0.80	0.79	1.00
STOMACH	NEX		79	66	67	67	79
ADENOMA [B]	NOBS		0	0	0	0	1
	I		0	0	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.18	1.00	1.00	1.00	0.45
DUODENUM	NEX		75	64	64	66	75
OSTEOSARCOMA [M]	NOBS		0	1	0	0	0
	I		0	0	0	0	0
	F		0	1	0	0	0
	P-VALUE	#	0.78	0.44	1.00	1.00	1.00

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
DUODENUM	NEX		75	64	64	66	75
ADENOMA [B]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.61	1.00	0.41	1.00	1.00
JEJUNUM	NEX		75	61	63	65	73
OSTEOSARCOMA [M]	NOBS		1	0	0	0	0
	I		0	0	0	0	0
	F		1	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
JEJUNUM	NEX		75	61	63	65	73
ADENOMA [B]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.39	1.00	1.00	0.49	1.00
CAECUM	NEX		76	65	66	67	77
LYMPHOMA PLASMACYTIC [M]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.59	1.00	0.49	1.00	1.00
CAECUM	NEX		76	65	66	67	77
ADENOCARCINOMA [M]	NOBS		0	1	0	1	0
	I		0	1	0	0	0
	F		0	0	0	1	0
	P-VALUE	#	0.56	0.47	1.00	0.47	1.00
COLON	NEX		78	64	67	67	77
ADENOCARCINOMA [M]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.42	1.00	1.00	0.29	1.00
LIVER	NEX		79	67	67	67	79
HEPATOCELLULAR CARCINOMA [M]	NOBS		4	2	1	2	5
	I		3	2	1	0	3
	F		2	0	0	2	0
	P-VALUE		0.51	0.29	0.52	0.37	0.78
LIVER	NEX		79	67	67	67	79
HAEMANGIOSARCOMA [M]	NOBS		1	1	2	0	0
	I		0	0	0	0	0
	F		1	1	1	0	0
	P-VALUE	#	0.92	0.72	0.47	1.00	1.00
LIVER	NEX		79	67	67	67	79
HEPATOCELLULAR ADENOMA [B]	NOBS		12	6	5	4	11
	I		12	6	5	4	11
	F		0	0	0	0	0
	P-VALUE		0.28	0.89	0.93	0.97	0.59
LIVER	NEX		79	67	67	67	79
ITO CELL TUMOUR [B]	NOBS		0	1	1	0	0
	I		0	1	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.74	0.47	0.35	1.00	1.00
LIVER	NEX		79	67	67	67	79
HAEMANGIOMA [B]	NOBS		0	1	0	0	1
	I		0	1	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.25	0.47	1.00	1.00	0.45
GALL BLADDER	NEX		72	59	58	65	68
ADENOMA [B]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.78	0.47	1.00	1.00	1.00
PANCREAS (EXOCRINE)	NEX		79	67	67	67	79
MESOTHELIOMA [M]	NOBS		0	0	0	1	0
	I		0	0	0	0	0
	F		0	0	0	1	0
	P-VALUE	#	0.41	1.00	1.00	0.45	1.00
EYE	NEX		77	65	65	67	77
AMELANOTIC MELANOMA [M]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.39	1.00	1.00	0.49	1.00
HARDERIAN GLAND	NEX		78	65	67	67	78
ADENOCARCINOMA [M]	NOBS		0	0	3	0	0
	I		0	0	3	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.81	1.00	0.12	1.00	1.00
HARDERIAN GLAND	NEX		78	65	67	67	78
ADENOMA [B]	NOBS		4	3	1	1	2
	I		4	2	1	1	2
	F		0	1	0	0	0
	P-VALUE		0.69	0.58	0.91	0.91	0.77
SPINAL CORD	NEX		77	67	67	67	78
MENINGEAL SARCOMA [M]	NOBS		0	0	1	0	0
	I		0	0	0	0	0
	F		0	0	1	0	0
	P-VALUE	#	0.61	1.00	0.49	1.00	1.00
SPINAL CORD	NEX		77	67	67	67	78
ASTROCYTOMA [B]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.79	0.47	1.00	1.00	1.00

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
STERNUM	NEX		79	67	67	67	79
MAST CELL TUMOUR [B]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.39	1.00	1.00	0.49	1.00
FEMUR	NEX		78	66	66	63	79
CHONDROMA [B]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	1	0	0	0
	P-VALUE	#	0.81	0.48	1.00	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
MALIGNANT SCHWANNOMA [M]	NOBS		0	0	1	0	0
	P-VALUE	#	0.61	1.00	0.48	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
FIBROSARCOMA [M]	NOBS		0	3	3	2	7
	P-VALUE		<0.001	0.054	0.13	0.070	0.004
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
NEUROFIBROSARCOMA [M]	NOBS		0	0	0	0	1
	P-VALUE	#	0.23	1.00	1.00	1.00	0.52
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
RHABDOMYOSARCOMA [M]	NOBS		0	0	2	3	4
	P-VALUE	#	0.013	1.00	0.24	0.49	0.061
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
LEIOMYOSARCOMA [M]	NOBS		0	0	1	0	1
	P-VALUE	#	0.22	1.00	0.48	1.00	0.48
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
HAEMANGIOSARCOMA [M]	NOBS		0	0	1	0	1
	P-VALUE	#	0.22	1.00	0.51	1.00	0.51
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
SARCOMA (NOT OTHERWISE SPECIFIED) [M]	NOBS		1	0	0	0	1
	P-VALUE	#	0.37	1.00	1.00	1.00	0.75
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
SQUAMOUS-CELL CARCINOMA [M]	NOBS		0	1	0	0	0
	P-VALUE	#	0.79	0.47	1.00	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
SQUAMOUS-CELL PAPILLOMA [B]	NOBS		0	0	1	0	0
	P-VALUE	#	0.60	1.00	0.45	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
KERATOACANTHOMA [B]	NOBS		0	0	1	0	0
	P-VALUE	#	0.61	1.00	0.48	1.00	1.00
INJECTION/TREATMENT SITE	NEX		79	67	67	67	79
FIBROSARCOMA [M]	NOBS		0	3	1	0	4
	P-VALUE	#	0.013	0.46	0.49	1.00	0.064
INJECTION/TREATMENT SITE	NEX		79	67	67	67	79
RHABDOMYOSARCOMA [M]	NOBS		1	0	0	0	1
	P-VALUE	#	0.38	1.00	1.00	1.00	0.75
INJECTION/TREATMENT SITE	NEX		79	67	67	67	79
SARCOMA (NOT OTHERWISE SPECIFIED) [M]	NOBS		1	0	1	0	0
	P-VALUE	#	0.84	1.00	0.74	1.00	1.00
INJECTION/TREATMENT SITE	NEX		79	67	67	67	79
SQUAMOUS-CELL PAPILLOMA [B]	NOBS		0	0	0	0	1
	P-VALUE	#	0.22	1.00	1.00	1.00	0.50
INJECTION/TREATMENT SITE	NEX		79	67	67	67	79
FIBROMA [B]	NOBS		1	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
SKIN (TREATED)	NEX		79	67	67	67	79
SARCOMA [M] dorsal surface	NOBS		2	3	3	3	16
	P-VALUE		<0.001	0.25	0.10	0.30	<0.001

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

[N000 4.2.3.4.1.1 complied from Table 22 P 482 – 514]

Females, Peto analysis of tumor incidence

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
LUNG	NEX		79	67	67	67	79
BRONCHIOLO-ALVEOLAR CARCINOMA [M]	NOBS		3	1	6	2	5
	I		3	1	3	1	4
	F		0	0	3	1	1
	P-VALUE		0.92	0.73	0.13	0.59	0.26
LUNG	NEX		79	67	67	67	79
BRONCHIOLO-ALVEOLAR ADENOMA [B]	NOBS		11	8	11	5	4
	I		11	8	11	5	4
	F		0	0	0	0	0
	P-VALUE		0.99	0.62	0.45	0.93	0.98
LUNG	NEX		79	67	67	67	79
MESOTHELIOMA [M]	NOBS		0	1	0	0	0
	I		0	0	0	0	0
	F		0	1	0	0	0
	P-VALUE	#	0.79	0.46	1.00	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA [M] not otherwise specified	NOBS		1	1	0	0	1
	I		0	0	0	0	0
	F		1	1	0	0	1
	P-VALUE	#	0.52	0.70	1.00	1.00	0.78
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA [M] leukaemic	NOBS		1	0	0	0	0
	I		0	0	0	0	0
	F		1	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA [M] lymphoblastic	NOBS		5	4	5	4	3
	I		1	0	0	1	1
	F		4	4	5	3	2
	P-VALUE		0.85	0.52	0.33	0.59	0.78
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA LYMPHOCYTIC [M]	NOBS		0	0	0	1	1
	I		0	0	0	1	0
	F		0	0	0	0	1
	P-VALUE	#	0.16	1.00	1.00	0.51	0.54
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA PLASMACYTIC [M]	NOBS		0	0	1	2	0
	I		0	0	1	1	0
	F		0	0	1	1	0
	P-VALUE	#	0.60	1.00	0.50	0.21	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LYMPHOMA FOLLICULAR CENTRE CELL [M]	NOBS		13	8	14	7	11
	I		4	3	8	2	5
	F		9	5	6	5	6
	P-VALUE		0.84	0.66	0.33	0.93	0.77
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
LEUKAEMIA GRANULOCYTIC [M]	NOBS		0	0	1	1	0
	I		0	0	0	0	0
	F		0	0	1	1	0
	P-VALUE	#	0.52	1.00	0.45	0.48	1.00
HAEMOPOIETIC SYSTEM	NEX		79	67	67	67	79
HISTIOCYTIC SARCOMA [M]	NOBS		5	6	4	4	1
	I		1	1	0	0	0
	F		4	5	4	4	1
	P-VALUE		0.98	0.21	0.53	0.60	0.96
LYMPH NODE (MESENTERIC)	NEX		75	65	63	63	74
OSTEOSARCOMA [M]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.37	1.00	1.00	0.51	1.00
LYMPH NODE (MESENTERIC)	NEX		75	65	63	63	74
HAEMANGIOMA [B]	NOBS		3	2	1	0	0
	I		2	1	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.97	0.82	0.85	1.00	1.00
SPLEEN	NEX		78	67	67	67	79
STROMAL SARCOMA [M]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.57	1.00	0.33	1.00	1.00
SPLEEN	NEX		78	67	67	67	79
HAEMANGIOSARCOMA [M]	NOBS		0	0	1	0	1
	I		0	0	1	0	0
	F		0	0	0	0	1
	P-VALUE	#	0.25	1.00	0.50	1.00	0.54
SPLEEN	NEX		78	67	67	67	79
HAEMANGIOMA [B]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.55	1.00	0.55	1.00	1.00
HEART	NEX		79	67	67	67	79
MESOTHELIOMA [M]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.46	1.00	1.00	0.51	1.00

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
THYROID GLAND	NEX		75	66	67	66	76
FOLLICULAR CELL CARCINOMA [M]	NOBS		0	0	0	0	0
	I		0	0	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
THYROID GLAND	NEX		75	66	67	66	76
C-CELL CARCINOMA [M]	NOBS		0	0	0	0	2
	I		0	0	0	0	1
	F		0	0	0	0	1
	P-VALUE	#	0.043	1.00	1.00	1.00	0.30
THYROID GLAND	NEX		75	66	67	66	76
C-CELL ADENOMA [B]	NOBS		0	0	0	4	15
	I		0	0	0	4	15
	F		0	0	0	0	0
	P-VALUE		<0.001	1.00	1.00	0.028	<0.001
THYROID GLAND	NEX		75	66	67	66	76
C-CELL TUMOUR	NOBS		0	0	0	4	17
	I		0	0	0	4	16
	F		0	0	0	0	1
	P-VALUE		<0.001	1.00	1.00	0.028	<0.001
ADRENAL GLAND	NEX		79	67	67	66	78
SUBCAPSULAR CELL TUMOUR [B]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.36	1.00	1.00	0.40	1.00
ADRENAL GLAND	NEX		79	67	67	66	78
CORTICAL CARCINOMA [M]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.67	1.00	0.50	1.00	1.00
ADRENAL GLAND	NEX		79	67	67	66	78
PHAEOCHROMOCYTOMA [M]	NOBS		1	0	1	0	0
	I		1	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.89	1.00	0.76	1.00	1.00
ADRENAL GLAND	NEX		79	67	67	66	78
CORTICAL ADENOMA [B]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.49	1.00	0.38	1.00	1.00
ADRENAL GLAND	NEX		79	67	67	66	78
PHAEOCHROMOCYTOMA [B]	NOBS		4	0	0	1	0
	I		4	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.94	1.00	1.00	0.96	1.00
PITUITARY GLAND	NEX		77	67	66	65	78
ADENOMA ANTERIOR LOBE [B]	NOBS		0	0	2	0	5
	I		0	0	2	0	5
	F		0	0	0	0	0
	P-VALUE	#	0.006	1.00	0.23	1.00	0.037
PITUITARY GLAND	NEX		77	67	66	65	78
ADENOMA INTERMEDIATE LOBE [B]	NOBS		0	0	0	2	1
	I		0	0	0	2	1
	F		0	0	0	0	0
	P-VALUE	#	0.16	1.00	1.00	0.22	0.54
OVARY	NEX		77	67	65	67	76
SARCOMA (NOT OTHERWISE SPECIFIED) [M]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.46	1.00	1.00	0.51	1.00
OVARY	NEX		77	67	65	67	76
TUBULOSTROMAL ADENOMA [B]	NOBS		1	0	1	1	3
	I		1	0	1	1	3
	F		0	0	0	0	0
	P-VALUE	#	0.073	1.00	0.76	0.77	0.38
OVARY	NEX		77	67	65	67	76
LEIOMYOMA [B]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.67	1.00	0.50	1.00	1.00
OVARY	NEX		77	67	65	67	76
SERTOLI CELL TUMOUR [B]	NOBS		0	0	0	1	1
	I		0	0	0	1	1
	F		0	0	0	0	0
	P-VALUE	#	0.16	1.00	1.00	0.51	0.54
OVARY	NEX		77	67	65	67	76
HAEMANGIOMA [B]	NOBS		0	2	0	0	0
	I		0	1	0	0	0
	F		0	1	0	0	0
	P-VALUE	#	0.89	0.28	1.00	1.00	1.00

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
OVARY	NEX		77	67	65	67	76
SEX CORD/STROMAL TUMOUR [B]	NOBS		1	0	1	0	0
	I		1	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.89	1.00	0.76	1.00	1.00
OVARY	NEX		77	67	65	67	76
GRANULOSA CELL TUMOUR [B]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.56	1.00	0.57	1.00	1.00
OVARY	NEX		77	67	65	67	76
DECIDUOMA [B]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.86	0.68	1.00	1.00	1.00
OVARY	NEX		77	67	65	67	76
LUTEOMA [B]	NOBS		1	1	2	1	2
	I		1	1	2	1	2
	F		0	0	0	0	0
	P-VALUE	#	0.41	0.62	0.50	0.77	0.56
OVARY	NEX		77	67	65	67	76
CYSTADENOMA [B]	NOBS		1	1	1	0	1
	I		1	1	1	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.59	0.70	0.67	1.00	0.76
UTERUS	NEX		78	67	66	67	78
ENDOMETRIAL CARCINOMA [M]	NOBS		0	0	1	3	0
	I		0	0	1	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.66	1.00	0.50	0.12	1.00
UTERUS	NEX		78	67	66	67	78
ENDOMETRIAL STROMAL SARCOMA [M]	NOBS		1	0	0	2	0
	I		1	0	0	1	0
	F		0	0	0	1	0
	P-VALUE	#	0.62	1.00	1.00	0.46	1.00
UTERUS	NEX		78	67	66	67	78
LEIOMYOSARCOMA [M]	NOBS		0	2	3	4	0
	I		0	1	3	4	0
	F		0	1	0	0	0
	P-VALUE	#	0.88	0.21	0.12	0.058	1.00
UTERUS	NEX		78	67	66	67	78
HAEMANGIOSARCOMA [M]	NOBS		2	1	2	1	1
	I		1	0	1	1	1
	F		1	1	1	0	0
	P-VALUE	#	0.75	0.78	0.61	0.87	0.90
UTERUS	NEX		78	67	66	67	78
HISTIOCYTIC SARCOMA [M]	NOBS		0	2	1	1	3
	I		0	2	1	1	3
	F		0	0	0	0	0
	P-VALUE	#	0.12	0.21	0.46	0.46	0.24
UTERUS	NEX		78	67	66	67	78
MALIGNANT SCHWANNOMA [M]	NOBS		0	0	0	0	1
	I		0	0	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.23	1.00	1.00	1.00	0.45
UTERUS	NEX		78	67	66	67	78
ENDOMETRIAL ADENOMA [B]	NOBS		0	0	0	0	1
	I		0	0	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.25	1.00	1.00	1.00	0.35
UTERUS	NEX		78	67	66	67	78
STROMAL POLYP [B]	NOBS		9	3	6	2	5
	I		9	2	6	2	5
	F		0	1	0	0	0
	P-VALUE		0.81	0.91	0.70	0.97	0.89
UTERUS	NEX		78	67	66	67	78
LEIOMYOMA [B]	NOBS		5	5	11	4	10
	I		5	5	11	4	10
	F		0	0	0	0	0
	P-VALUE		0.27	0.37	0.027	0.63	0.34
UTERUS	NEX		78	67	66	67	78
GRANULAR CELL TUMOUR [B]	NOBS		0	1	0	0	1
	I		0	1	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.31	0.38	1.00	1.00	0.48
UTERUS	NEX		78	67	66	67	78
SCHWANNOMA [B]	NOBS		0	0	1	0	1
	I		0	0	1	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.20	1.00	0.46	1.00	0.45
UTERUS	NEX		78	67	66	67	78
HAEMANGIOMA [B]	NOBS		0	2	3	3	3
	I		0	2	3	2	3
	F		0	0	0	1	0
	P-VALUE		0.31	0.033	0.024	0.042	0.057
UTERUS	NEX		78	67	66	67	78
DECIDUOMA [B]	NOBS		1	1	2	0	0
	I		1	1	2	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.94	0.62	0.48	1.00	1.00

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
VAGINA	NEX		77	67	63	66	78
STROMAL SARCOMA [M]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	1	0	0	0
	P-VALUE	#	0.80	0.48	1.00	1.00	1.00
URINARY BLADDER	NEX		77	63	64	64	74
MESENCHYMAL TUMOUR [B]	NOBS		0	0	0	0	1
	I		0	0	0	0	1
	F		0	0	0	0	0
	P-VALUE	#	0.25	1.00	1.00	1.00	0.55
STOMACH	NEX		77	67	66	66	76
SQUAMOUS-CELL PAPILLOMA [B]	NOBS		1	0	0	1	1
	I		1	0	0	1	1
	F		0	0	0	0	0
	P-VALUE	#	0.36	1.00	1.00	0.74	0.80
DUODENUM	NEX		72	64	63	63	73
HAEMANGIOMA [B]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.88	0.73	1.00	1.00	1.00
DUODENUM	NEX		72	64	63	63	73
ADENOMA [B]	NOBS		0	0	0	1	0
	I		0	0	0	0	0
	F		0	0	0	1	0
	P-VALUE	#	0.44	1.00	1.00	0.51	1.00
CAECUM	NEX		79	64	64	62	73
LEIOMYOSARCOMA [M]	NOBS		0	0	0	1	0
	I		0	0	0	0	0
	F		0	0	0	1	0
	P-VALUE	#	0.43	1.00	1.00	0.48	1.00
CAECUM	NEX		79	64	64	62	73
LEIOMYOMA [B]	NOBS		0	2	0	0	0
	I		0	2	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.87	0.16	1.00	1.00	1.00
CAECUM	NEX		79	64	64	62	73
PLASMACYTOMA [B]	NOBS		0	1	0	0	0
	I		0	1	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.79	0.38	1.00	1.00	1.00
COLON	NEX		79	66	61	63	76
ADENOCARCINOMA [M]	NOBS		1	0	0	1	0
	I		0	0	0	0	0
	F		0	0	0	1	0
	P-VALUE	#	0.70	1.00	1.00	0.73	1.00
COLON	NEX		79	66	65	63	76
ADENOMA [B]	NOBS		1	0	0	0	0
	I		1	0	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
LIVER	NEX		79	67	67	67	79
HEPATOCELLULAR CARCINOMA [M]	NOBS		0	1	1	1	1
	I		0	1	1	1	1
	F		0	0	0	0	0
	P-VALUE	#	0.32	0.66	0.50	0.55	0.48
LIVER	NEX		79	67	67	67	79
HEPATOCELLULAR ADENOMA [B]	NOBS		2	0	0	3	0
	I		2	0	0	3	0
	F		0	0	0	0	0
	P-VALUE	#	0.80	1.00	1.00	0.52	1.00
LIVER	NEX		79	67	67	67	79
HAEMANGIOMA [B]	NOBS		0	1	2	0	0
	I		0	1	2	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.85	0.38	0.27	1.00	1.00
PANCREAS (EXOCRINE)	NEX		79	67	66	66	76
HAEMANGIOMA [B]	NOBS		1	0	0	0	0
	I		1	0	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
HARDERIAN GLAND	NEX		79	67	66	67	79
ADENOMA [B]	NOBS		1	0	1	2	5
	I		1	0	1	2	5
	F		0	0	0	0	0
	P-VALUE	#	0.012	1.00	0.74	0.48	0.14
BRAIN	NEX		79	67	67	67	79
MALIGNANT ASTROCYTOMA [M]	NOBS		1	0	0	0	0
	I		1	0	0	0	0
	F		0	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
BRAIN	NEX		79	67	67	67	79
MENINGIOMA [B]	NOBS		0	0	1	0	0
	I		0	0	0	0	0
	F		0	0	1	0	0
	P-VALUE	#	0.61	1.00	0.48	1.00	1.00
BRAIN	NEX		79	67	67	67	79
LIPOMA [B]	NOBS		0	0	0	1	0
	I		0	0	0	1	0
	F		0	0	0	0	0
	P-VALUE	#	0.48	1.00	1.00	0.51	1.00
SPINAL CORD	NEX		79	66	67	66	78
MENINGEAL SARCOMA [M]	NOBS		0	0	0	2	0
	I		0	0	0	2	0
	F		0	0	0	0	0
	P-VALUE	#	0.48	1.00	1.00	0.26	1.00

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			Group (Dose Level in mg/kg/day) 1 (0) (N=79)	2 (0.03) (N=67)	3 (0.2) (N=67)	4 (1.0) (N=67)	5 (3.0) (N=79)
SKELETAL MUSCLE	NEX		79	67	67	66	79
RHABDOMYOSARCOMA [M]	NOBS		1	0	0	0	0
	I		0	0	0	0	0
	F		1	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
SKELETAL MUSCLE	NEX		79	67	67	66	79
LIPOSARCOMA [M]	NOBS		0	0	0	0	1
	I		0	0	0	0	0
	F		0	0	0	0	1
	P-VALUE	#	0.25	1.00	1.00	1.00	0.33
FEMUR	NEX		78	66	64	66	79
OSTEOMA [B]	NOBS		0	0	1	0	0
	I		0	0	1	0	0
	F		0	0	0	0	0
	P-VALUE	#	0.50	1.00	0.38	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
MALIGNANT FIBROUS HISTIOCYTOMA [M]	NOBS		1	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
FIBROSARCOMA [M]	NOBS		1	1	1	0	2
	P-VALUE	#	0.31	0.68	0.70	1.00	0.54
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
RHABDOMYOSARCOMA [M]	NOBS		2	1	1	0	0
	P-VALUE	#	0.97	0.83	0.83	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
LIPOSARCOMA [M]	NOBS		0	2	0	0	0
	P-VALUE	#	0.88	0.22	1.00	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
SARCOMA (NOT OTHERWISE SPECIFIED) [M]	NOBS		1	0	1	0	5
	P-VALUE	#	0.007	1.00	0.70	1.00	0.34
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
BASAL CELL CARCINOMA [M]	NOBS		2	0	0	0	1
	P-VALUE	#	0.57	1.00	1.00	1.00	0.89
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
SQUAMOUS-CELL CARCINOMA [M]	NOBS		1	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
SKIN AND SUBCUTIS	NEX		79	67	67	67	79
SQUAMOUS-CELL PAPILLOMA [B]	NOBS		0	0	0	1	0
	P-VALUE	#	0.44	1.00	1.00	0.50	1.00
MAMMARY GLAND	NEX		76	64	63	66	76
ADENOCARCINOMA [M]	NOBS		2	2	4	2	3
	P-VALUE		0.46	0.23	0.070	0.27	0.19
MAMMARY GLAND	NEX		76	64	63	66	76
ADENOACANTHOMA [M]	NOBS		0	1	0	1	1
	P-VALUE	#	0.28	0.41	1.00	0.49	0.54
MAMMARY GLAND	NEX		76	64	63	66	76
FIBROADENOMA [B]	NOBS		1	0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00	1.00
MAMMARY GLAND	NEX		76	64	63	66	76
ADENOMA [B]	NOBS		0	0	0	0	1
	P-VALUE	#	0.24	1.00	1.00	1.00	0.53
INJECTION/TREATMENT SITE	NEX		78	67	66	65	78
FIBROSARCOMA [M]	NOBS		1	0	0	0	2
	P-VALUE	#	0.14	1.00	1.00	1.00	0.54
INJECTION/TREATMENT SITE	NEX		78	67	66	65	78
SARCOMA (NOT OTHERWISE SPECIFIED) [M]	NOBS		1	1	0	0	0
	P-VALUE	#	0.95	0.69	1.00	1.00	1.00
INJECTION/TREATMENT SITE	NEX		78	67	66	65	78
KERATOACANTHOMA [B]	NOBS		0	0	0	1	0
	P-VALUE	#	0.43	1.00	1.00	0.49	1.00
SKIN (TREATED)	NEX		79	67	66	65	78
SARCOMA [M] dorsal surface	NOBS		6	4	2	0	8
	P-VALUE		0.15	0.61	0.87	0.99	0.37

[M] denotes a malignant tumour, [B] denotes a benign tumour
NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

[N000 4.2.3.4.1.1 complied from Table 23P515 - 583]

APPENDIX 2 FOR MOUSE CARCINOGENICITY STUDY REVIEW: HISTORICAL CONTROL GROUP DATA OF TUMOR INCIDENCE IN CD-1 MICE FROM 2 YEAR CARCINOGENICITY STUDIES

[redacted], from 2002 to 2004.

b(4)

ENDOCRINE SYSTEM

ADRENAL (SUBCAPSULAR)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	25	1 adenoma	0	50	46	0	0
658	119	42	5 adenoma	0	119	88	2 adenoma	0
832	47	21	5 adenoma	0	49	40	1 adenoma	0
874	94	38	9 adenoma	0	96	72	7 adenoma	0
937	120	58	17 adenoma	1 carcinoma	117	106	4 adenoma	2 carcinoma
988	109	38	13 adenoma	0	108	97	4 adenoma	0
TOTAL	539	222	50	1	539	449	18	2
RATE		41.2	9.3	0.2		83.3	3.3	0.4
RANGE		35-50%	2.0-14.2%	0.0-0.8%		73.9-92.0	0.0-7.3	0.0-1.7

ADRENAL (MEDULLA)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	4	1 Phaeochromocytoma	0	50	0	2	0
658	119	6	0	0	119	3	1 Phaeochromocytoma	1 Phaeochromocytoma
832	47	1	0	0	49	0	0	0
874	94	2	0	0	96	2	1 Phaeochromocytoma	1 Phaeochromocytoma
937	120	0	0	0	117	1	0	0
988	109	1	0	0	108	0	1 Phaeochromocytoma	0
TOTAL	539	14	1	0	539	6	5	2
RATE		2.6%	0.2%	0.0%		1.1%	0.9%	0.4%
RANGE		0-6%	0-2%	0%		0-2.5%	0-4%	0-1%

TABLE 1 (CONTINUED)

CARDIOVASCULAR SYSTEM

AORTA

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	49	0	0	0
658	120	0	0	0	119	0	0	0
832	49	0	0	0	49	0	0	0
874	99	0	0	0	99	0	0	0
937	120	0	0	0	120	0	0	0
988	109	0	0	0	105	0	0	0
TOTAL	547	0	0	0	541	0	0	0
RATE		0.0	0.0	0.0		0.0	0.0	0.0
RANGE		0.0	0.0	0.0		0.0	0.0	0.0

NERVOUS SYSTEM AND ORGANS OF SPECIAL SENSE

BRAIN

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	50	0	0	1 malignant astrocytoma
658	120	0	1 meningioma	0	119	0	0	0
832	50	0	0	0	50	0	0	0
874	100	0	0	0	100	0	0	0
937	119	0	0	0	120	0	1 choroid plexus papilloma	1 malignant astrocytoma
988	110	0	0	1 oligodendroglioma	109	0	0	0
TOTAL	549	0	1	1	548	0	1	2
RATE		0.0	0.2	0.2		0.0	0.2	0.4
RANGE		0.0	0.0-0.8	0.0-0.8		0.0	0.0-0.8	0.0-2.0

TABLE 1 (ALIMENTARY SYSTEM CONTINUED)

CAECUM

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	49	0	0	0	50	0	0	0
658	119	0	0	1 adenocarcinoma	116	0	0	0
832	44	0	0	0	46	0	0	0
874	96	0	0	0	99	0	0	0
937	108	0	0	0	111	0	0	0
988	107	0	0	0	107	0	0	0
TOTAL	523	0	0	1	529	0	0	0
RATE		0.0	0.0	0.2		0.0	0.0	0.0
RANGE		0.0	0.0	0.0-0.8		0.0	0.0	0.0

COLON

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	50	0	0	1
658	120	1	0	0	116	0	0	0
832	44	0	0	0	47	0	0	0
874	96	0	0	0	99	0	0	0
937	114	0	0	0	117	1	0	0
988	107	0	0	0	109	0	0	0
TOTAL	531	1	0	0	538	1	0	1
RATE		0.2	0.0	0.0		0.2	0.0	0.2
RANGE		0.0-0.8	0.0	0.0		0.0-0.9	0.0	0.0-2.0

DUODENUM

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	48	0	0	0	47	0	0	0
658	119	2	0	1 adenocarcinoma	116	1	0	0
832	44	0	0	0	43	0	0	0
874	97	0	0	0	97	0	0	0
937	107	0	0	0	109	0	1 adenoma	0
988	108	0	0	0	106	0	0	0
TOTAL	523	2	0	1	518	1	1	0
RATE		0.4	0.0	0.2		0.2	0.2	0.0
RANGE		0.0-1.7	0.0	0.0-0.8		0.0-0.9	0.0-0.9	0.0

EPIDIDYMIS

Study No.	Male			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	1 histiocytic sarcoma
658	120	0	1 interstial adenoma	1 haemangiosarcoma
832	50	0	0	0
874	100	0	1 interstitial adenoma	0
937	120	0	0	0
988	110	0	0	0
TOTAL	550	0	2	2
RATE		0.0	0.4	0.4
RANGE		0.0	0.0-1.0	0.0-2.0

EYE

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	49	0	0	0	49	0	0	0
658	120	0	0	0	115	0	0	0
832	46	0	0	0	41	0	0	0
874	98	0	0	0	97	0	0	0
937	112	0	0	0	108	0	0	0
988	108	0	0	2 adenocarcinoma	105	0	0	0
TOTAL	533	0	0	2	515	0	0	0
RATE		0.0	0.0	0.4		0.0	0.0	0.0
RANGE		0.0	0.0	0.0-1.9		0.0	0.0	0.0

TABLE 1 (ALIMENTARY SYSTEM CONTINUED)

OESOPHAGUS

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	48	0	0	0	50	0	0	0
658	120	11	0	0	118	0	0	0
832	50	0	0	0	49	0	0	0
874	99	0	0	0	100	0	0	0
937	120	0	2 adenomatous polyp	0	120	0	0	0
988	109	0	1 squamous-cell papilloma	0	109	0	0	0
TOTAL	548	11	3	0	546	0	0	0
RATE		2.0	0.5	0.0		0.0	0.0	0.0
RANGE		0.0-9.2	0.0-1.7	0.0		0.0	0.0	0.0

GALL BLADDER

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	48	0	0	0	47	1	0	0
349	97	0	0	0	100	0	0	0
658	110	0	0	0	107	0	0	0
832	42	0	0	0	38	0	0	0
874	96	0	2 adenoma - papillary	0	97	0	0	0
937	108	9	3 adenoma	0	102	5	0	0
988	103	0	0	0	102	0	0	0
TOTAL	604	9	5	0	593	6	0	0
RATE		1.5	0.8	0.0		1.0	0.0	0.0
RANGE		0.0-8.3	0.0-2.8	0.0		0.0-4.9	0.0	0.0

HARDERIAN GLAND

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	49	0	2 adenoma	0	274	50	2 adenoma	1
658	120	0	5 adenoma	1 adenocarcinoma	658	120	3 adenoma	1 adenocarcinoma
832	49	0	4 adenoma	0	832	50	0	2
874	14	0	0	0	874	26	0	1
937	120	1	11 adenoma	0	937	120	5 adenoma	3
988	109	0	0	0	988	110	0	0
TOTAL	461	1	22	1	476	10	8 adenoma	2
RATE		0.2	4.8	0.2		2.1	1.7	0.4
RANGE		0.0-0.8	0.0-9.2	0.0-0.8		0.0-4.2	0.0-4.0	0.0-1.7

HEART

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	0	0	50	0	0	0
658	120	0	0	0	120	1	0	0
832	50	0	0	0	50	0	0	0
874	100	0	0	0	99	0	0	0
937	120	0	0	0	120	0	0	0
988	110	0	0	0	110	0	0	1 haemangiosarcoma
TOTAL	550	0	0	0	549	1	0	1
RATE		0.0	0.0	0.0		0.2	0.0	0.2
RANGE		0.0	0.0	0.0		0.0	0.0	0.0-0.9

ILEUM

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	48	0	0	0	48	0	0	0
658	117	0	0	0	112	0	0	0
832	46	0	0	0	44	0	0	0
874	97	0	0	0	98	0	0	0
937	112	0	0	0	107	0	0	0
988	106	0	0	0	105	0	0	0
TOTAL	526	0	0	0	514	0	0	0
RATE		0.0	0.0	0.0		0.0	0.0	0.0
RANGE		0.0	0.0	0.0		0.0	0.0	0.0

INJECTION/TREATMENT SITE

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	1 haemangio-pericytoma	1 rhabdomyo-sarcoma	49	0	0	0
832	49	0	0	0	47	0	0	1 haemangio-sarcoma
TOTAL	99	0	1	1	96	0	0	1
RATE		0.0	1.0	1.0		0.0	0.0	1.0

RANGE NOT APPLICABLE

JEJUNUM

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	49	0	0	0	47	1	0	0
658	115	0	0	0	115	0	0	0
832	43	0	0	0	42	0	0	0
874	97	0	0	0	95	0	0	0
937	111	0	0	0	109	0	0	0
988	109	0	0	0	105	0	0	0
TOTAL	524	0	0	0	513	1	0	0
RATE		0.0	0.0	0.0		0.2	0.0	0.0
RANGE		0.0	0.0	0.0		0.0-2.1	0.0	0.0

URINARY SYSTEM

KIDNEY

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	2 adenoma	0	50	0	0	0
658	120	0	2 adenoma	0	120	0	0	0
832	49	1	0	1	49	0	0	0
874	99	0	1 adenoma	1 carcinoma 1 haemangio-sarcoma	100	1	0	0
937	120	0	0	1	120	0	0	1 carcinoma
988	110	0	2 adenoma	1	110	0	0	0
TOTAL	548	1	7	5	549	1	0	1
RATE		0.2	1.3	0.9		0.2	0.0	0.2
RANGE		0.0-2.0	0.0-4.0	0.0-2.0		0.0-1.0	0.0	0.0-0.8

LIVER (HEPATOCYTE)

Study Number	Male Number Examined	Eosinophilic / clear cell foci	Basophilic foci	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Eosinophilic / clear cell foci	Basophilic foci	Benign Neoplasia	Malignant Neoplasia
274	50	2	0	7 adenoma	3 carcinoma	50	0	0	1 adenoma	0
349	110	3	3	5 adenoma	2 carcinoma	110	1	0	0	0
658	120	6	1	18 adenoma	3 carcinoma	120	2	1	5 adenoma	1 carcinoma
832	48	8	6	10 adenoma	2 carcinoma	47	2	2	1 adenoma	0
874	100	2	2	4 adenoma	1 carcinoma	100	0	0	0	0
937	120	4	8	12 adenoma	2 carcinoma	120	3	4	3 adenoma	0
988	110	1	1	15 adenoma	7 carcinoma	109	0	0	0	0
TOTAL	658	26	21	71	20	656	8	7	10	1
RATE		4.0	3.2	10.8	3.0		1.2	1.1	1.5	0.2
RANGE		0.9-16.7	0.0-12.5	4.0-20.8	1.0-6.0		0.0	0.0	0.0	0.0

TABLE 1 (ALIMENTARY SYSTEM CONTINUED)

LIVER (BILIARY)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	50	0	0	0
349	110	0	0	0	110	0	0	0
658	120	0	0	0	120	0	0	0
832	48	0	0	0	47	0	0	0
874	100	0	0	0	100	0	0	0
937	120	0	0	0	120	0	0	0
988	110	0	0	0	109	0	0	0
TOTAL	658	0	0	0	656	0	0	0
RATE		0.0	0.0	0.0		0.0	0.0	0.0
RANGE		0.0	0.0	0.0		0.0	0.0	0.0

RESPIRATORY SYSTEM

LUNG

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	2	10 adenoma	3 carcinoma	50	1	3 adenoma	2 carcinoma
658	120	5	24 adenoma	11 carcinoma	119	1	8 adenoma	13 carcinoma
832	50	3	19 adenoma	5 carcinoma	49	2	4 adenoma	4 carcinoma
874	100	5	9 adenoma	12 carcinoma	100	5	14 adenoma	6 carcinoma
937	119	6	29 adenoma	9 carcinoma	120	3	15 adenoma	2 carcinoma
988	110	6	19 adenoma	13 carcinoma	110	4	6 adenoma	7 carcinoma
TOTAL	549	27	110	53	548	16	50	34
RATE		4.9	20.0	9.7		2.9	9.1	6.2
RANGE		4.0-6.0	9.0-38.0	6.0-12.0		0.8-5.0	5.5-14.0	1.7-10.9

LYMPHORETICULAR / HAEMOPOIETIC SYSTEM

LYMPHORETICULAR / HAEMOPOIETIC

Study No.	Male Number Examined	Male Lymphoma	Male Leukaemia	Male Histiocytic sarcoma	Female Number Examined	Female Lymphoma	Female Leukaemia	Female Histiocytic sarcoma
274	50	6	1	0	50	11	0	4
658	120	6	2	3	120	20	0	8
832	49	5	0	0	50	23	2	0
874	99	13	0	1	100	34	0	8
937	120	20	3	1	119	27	3	12
988	110	20	0	3	110	20	0	4
TOTAL	548	70	6	8	549	135	5	36
RATE		12.8	1.1	1.5		24.6	0.9	6.6
RANGE		5.0-18.2	0.0-2.5	0.0-2.7		16.7-46.0	0.0-4.0	0.0-10.1

MAMMARY GLAND

Study No.	Male* Number Examined	Male* Focal Hyperplasia	Male* Benign Neoplasia	Male* Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274					49	0	0	1 adenocarcinoma
658					116	0	0	1 adenoacanthoma 4 adenocarcinoma
832					50	0	1 fibroadenoma	0
874					96	0	0	1 adenosquamous carcinoma 2 carcinoma
937					110	1	1 fibroadenoma 1 adenoma	1 mixed malignant tumour
988					107	0	0	6 adenocarcinoma
TOTAL					528	1	3	16
RATE						0.2	0.6	3.0
RANGE						0.0-0.9	0.0-2.0	0.0-4.3

*** Not a protocol organ**

OPTIC NERVE

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	43	0	0	0	42	0	0	0
658	108	0	0	0	100	0	0	0
832	42	0	0	0	33	0	0	0
874	86	0	0	0	79	0	0	0
937	116	0	0	0	116	0	0	0
988	104	0	5 adenoma	0	104	0	1 adenoma	0
TOTAL	499	0	5	0	474	0	1	0
RATE		0.0	1.0	0.0		0.0	0.2	0.0
RANGE		0.0	0.0-4.8	0.0		0.0	0.0-1.0	0.0

FEMALE GENITAL SYSTEM

OVARY

Study No.	Gonadal stroma				Epithelium			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	1 tubulostromal adenoma 1 luteoma	0	50	0	0	0
658	119	0	1 sex cord/stromal tumour 2 granulosa/thecal cell tumour 4 tubulostromal adenoma	2 granulosa cell tumour	119	0	1 cystadenoma	0
832	48	0	1 granulosa cell tumour 1 luteoma	0	48	0	0	0
874	98	0	1 tubulostromal adenoma 1 luteoma 1 sertoli cell tumour 1 teratoma	0	99	0	0	0
937	120	0	2 granulosa cell tumour 2 tubulostromal adenoma 3 luteoma 4 granulosa cell tumour	0	120	0	9 cystadenoma	0
988	110	0	3 luteoma	0	110	0	4	0
TOTAL	647	0	29	2	647	0	14	0
RATE		0.0	5.3	0.4		0.0	2.8	0.0
RANGE		0.0	2.7-7.5	0.0-1.7		0.0	0.0-7.5	0.0

PANCREAS (ENDOCRINE)

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	50	0	0	0
658	119	0	0	0	118	0	0	1 carcinoma
832	47	0	1 adenoma	0	48	0	1 adenoma	0
874	98	0	0	0	100	0	0	0
937	119	0	2 adenoma	0	118	0	1 adenoma	0
988	110	0	2 adenoma	0	109	0	0	0
TOTAL	543	0	5	0	544	0	2	1
RATE		0.0	0.9	0.0		0.0	0.4	0.2
RANGE		0.0	0.0-2.1	0.0		0.0	0.0-2.0	0.0-0.8

PANCREAS (EXOCRINE)

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	50	0	0	0
658	119	0	0	0	118	0	0	0
832	47	0	0	0	48	0	0	0
874	98	0	0	0	100	0	0	0
937	120	1	0	0	120	1	0	0
988	110	0	0	0	109	0	0	0
TOTAL	544	1	0	0	545	1	0	0
RATE		0.2	0.0	0.0		0.2	0.0	0.0
RANGE		0.0-0.8	0.0	0.0		0.0-0.8	0.0	0.0

TABLE 1 (ENDOCRINE SYSTEM CONTINUED)

PARATHYROID

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	37	0	0	0	274	34	1	0
658	91	0	0	0	658	84	0	0
832	42	1	0	0	832	39	0	0
874	80	0	0	0	874	79	2	0
937	108	0	0	0	937	107	0	0
988	101	0	0	0	988	103	0	0
TOTAL	459	1	0	0	446	3	0	0
RATE		0.2	0.0	0.0		0.7	0.0	0.0
RANGE		0.0-2.4	0.0	0.0		0.0-2.9	0.0	0.0

PITUITARY (INTERMEDIATE)

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	47	0	0	0	48	0	0	0
658	117	0	0	0	118	0	0	0
832	46	1	0	0	49	0	0	0
874	97	1	0	0	97	3	0	0
937	118	0	0	0	119	0	0	0
988	110	0	0	0	109	1	0	0
TOTAL	535	2	0	0	540	4	0	0
RATE		0.4	0.0	0.0		0.7	0.0	0.0
RANGE		0.0-2.2	0.0	0.0		0.0-3.1	0.0	0.0

PITUITARY (ANTERIOR)

Study Number.	Male				Female			
	Number Examined	Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	47	0	1 adenoma	0	48	1	0	0
658	117	1	0	0	116	2	6 adenoma	0
832	46	3	0	0	49	6	1 adenoma	0
874	97	3	1 adenoma	0	97	0	1 adenoma	0
937	118	2	1 adenoma	0	119	6	7 adenoma	0
988	110	0	0	0	109	5	3 adenoma	0
TOTAL	535	9	3	0	540	22	18	0
RATE		1.7	0.6	0.0		4.1	3.3	0.0
RANGE		0.0-6.5	0.0-2.1	0.0		0.0-12.2	0.0-5.9	0.0

Where the site of the tumour was not recorded it is assumed that it was in the anterior pituitary

PROSTATE

Study No.	Male			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	49	2	0	0
658	114	0	0	0
832	45	0	0	0
874	98	1	0	0
937	120	0	0	0
988	105	0	1 adenoma	1 haemangiosarcoma
TOTAL	531	3	1	1
RATE		0.6	0.2	0.2
RANGE		0.0-4.1	0.0-1.0	0.0-1.0

RECTUM

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	49	0	0	0	50	0	0	0
658	119	1	0	0	117	0	0	0
832	46	0	0	0	47	0	0	0
874	98	0	0	0	98	0	0	0
937	117	0	0	0	116	0	0	0
988	107	0	0	0	106	0	0	0
TOTAL	536	1	0	0	534	0	0	0
RATE		0.2	0.0	0.0		0.0	0.0	0.0
RANGE		0.0-0.8	0.0	0.0		0.0	0.0	0.0

TABLE 1 (ALIMENTARY SYSTEM CONTINUED)

SALIVARY GLAND

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	50	0	0	0
658	120	0	0	0	119	0	0	0
832	49	0	0	0	48	0	0	0
874	100	0	0	0	98	0	0	0
937	119	0	0	0	118	0	0	0
988	109	0	0	0	109	0	0	0
TOTAL	547	0	0	0	542	0	0	0
RATE		0.0	0.0	0.0		0.0	0.0	0.0
RANGE		0.0	0.0	0.0		0.0	0.0	0.0

SCIATIC NERVE

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	0	0	48	0	0	0
658	119	0	0	0	116	0	0	0
832	50	0	0	0	49	0	0	0
874	99	0	0	0	96	0	0	0
937	120	0	0	0	119	0	0	0
988	108	0	0	0	109	0	0	0
TOTAL	546	0	0	0	537	0	0	0
RATE		0.0	0.0	0.0		0.0	0.0	0.0
RANGE		0.0	0.0	0.0		0.0	0.0	0.0

SEMINAL VESICLE

Study No.	Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia
274	50	0	0	1 granular cell tumour
658	120	0	1adenoma	1 adenocarcinoma (coagulating gland)
832	47	0	1 adenoma 1 granular cell tumour	0
874	100	0	0	0
937	118	1	0	0
988	110	0	0	0
TOTAL	545	1	3	2
RATE		0.2	0.6	0.4
RANGE		0.0-0.8	0.0-4.3	0.0-2.0

SKELETAL MUSCLE

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	0	0	50	0	0	1 rhabdomyosarcoma 1 liposarcoma
658	120	0	0	0	120	0	0	0
832	50	0	0	0	50	0	0	0
874	100	0	0	0	100	0	0	0
937	120	0	0	0	120	0	0	0
988	110	0	0	0	110	0	0	1 haemangiosarcoma
TOTAL	550	0	0	0	550	0	0	3
RATE		0.0	0.0	0.0		0.0	0.0	0.5
RANGE		0.0	0.0	0.0		0.0	0.0	0.0-4.0

INTEGUMENTARY SYSTEM

SKIN / SUBCUTIS (EPITHELIUM)

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	1 sebaceous cell adenoma	0	50	0	0	1 sebaceous cell carcinoma 1 basal cell carcinoma
658	120	0	1 basal cell adenoma	0	119	0	1 squamous cell papilloma 1 basal cell adenoma	0
832	50	0	2 sebaceous cell adenoma	0	50	0	1 keratoacanthoma 1 squamous cell papilloma	0
874	100	0	1 basal cell adenoma	0	100	0	0	1 basal cell carcinoma
937	120	0	0	0	120	0	0	1 squamous cell carcinoma
988	110	0	0	0	108	0	0	1 trichoepithelioma 2 basal cell carcinoma
TOTAL	550	0	5	0	547	0	4	7
RATE		0.0	0.9	0.0		0.0	0.7	1.3
RANGE		0.0	0.0-4.0	0.0		0.0	0.0-4.0	0.0-4.0

SKIN / SUBCUTIS (MESODERM)

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	0	1 myxosarcoma 1 sarcoma	50	0	0	1 sarcoma 1 malignant fibrous histiocytoma 2 rhabdomyosarcoma
658	120	0	0	4 fibrosarcoma 1 sarcoma	119	0	0	1 fibrosarcoma 1 osteosarcoma 4 sarcoma
832	50	0	1 dermal fibroma	0	50	0	0	1 liposarcoma 5 fibrosarcoma
874	100	0	0	5 fibrosarcoma	100	0	0	2 haemangiosarcoma 1 malignant schwannoma 1 malignant fibrous histiocytoma
937	120	0	0	9 fibrosarcoma	120	0	1 fibroma	3 sarcoma 1 fibrosarcoma 1 osteosarcoma 3 rhabdomyosarcoma
988	110	0	1 mast cell tumour	1 fibrosarcoma	108	0	0	2 sarcoma 1 malignant schwannoma 1 fibrosarcoma
TOTAL	550	0	2	22	547	0	1	32
RATE		0.0	0.4	4.0		0.0	0.2	5.9
RANGE		0.0	0.0-2.0	0.0-7.5		0.0	0.0-0.8	2.0-9.0

SPINAL CORD

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	48	0	0	0	50	0	0	0
658	120	0	0	0	120	0	0	0
832	50	0	0	0	48	0	0	0
874	100	0	0	0	100	0	0	0
937	120	0	0	0	120	0	0	0
988	110	0	1 ganglioneuroma	0	110	0	0	0
TOTAL	548	0	1	0	548	0	0	0
RATE		0.0	0.2	0.0		0.0	0.0	0.0
RANGE		0.0	0.0-0.9	0.0		0.0	0.0	0.0

SPLEEN

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	0	1	50	0	0	0
658	120	0	0	2	119	0	0	1
832	47	0	0	1	47	0	0	0
874	98	0	0	3	100	0	0	1
937	120	0	1	0	119	0	1	0
988	110	0	0	1	110	0	0	0
TOTAL	545	0	1	8	545	0	1	2
RATE		0.0	0.2	1.5		0.0	0.2	0.4
RANGE		0.0	0.0-0.8	0.0-3.1		0.0	0.0-0.8	0.0-1.0

TABLE 1 (MUSCULOSKELETAL SYSTEM CONTINUED)

STERNUM

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	0	0	50	0	0	0
658	120	0	0	0	120	0	0	0
832	49	0	0	0	49	0	0	0
874	100	0	0	0	99	0	0	1 haemangiosarcoma
937	120	0	1 osteoma	0	120	0	0	0
988	110	1	0	0	110	1	0	0
TOTAL	549	1	1	0	548	1	0	1
RATE		0.2	0.2	0.0		0.2	0.0	0.2
RANGE		0.0-0.9	0.0-0.8	0.0		0.0-0.9	0.0	0.0-1.0

TABLE 1 (ALIMENTARY SYSTEM CONTINUED)

STOMACH

Study No	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	50	0	1 squamous cell papilloma	0
658	120	0	0	0	120	0	0	0
832	46	0	0	0	48	0	0	0
874	100	4	2 adenoma (glandular stomach)	0	100	2	0	0
937	114	0	0	1 squamous cell carcinoma	118	0	0	0
988	108	0	1 adenoma	0	109	0	0	0
TOTAL	538	4	3	1	545	2	1	0
RATE		0.7	0.6	0.2		0.4	0.2	0.0
RANGE		0.0-4.0	0.0-2.0	0.0-0.9		0.0-2.0	0.0-2.0	0.0

MALE GENITAL SYSTEM

TESTIS

Study No.	Total	Interstitial cell Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	9 adenoma	0
658	120	2	13 adenoma	0
832	50	0	2 adenoma	0
874	100	1	0	1 carcinoma
937	119	0	2 adenoma 1 haemangioma	1 carcinoma
988	110	1	4 adenoma 2 haemangioma	0
TOTAL	549	4	33	2
RATE		0.7	6.0	0.4
RANGE		0.0-1.7	0.0-18.0	0.0-1.0

THYROID (FOLLICULAR CELL)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	47	0	0	0
658	120	0	1 adenoma	0	118	0	1 adenoma	1 carcinoma
832	46	0	0	0	49	1	0	0
874	99	0	0	0	100	8	1 adenoma	0
937	119	1	0	1 carcinoma	118	2	0	0
988	108	0	0	0	109	0	0	0
TOTAL	542	1	1	1	541	11	2	1
RATE		0.2	0.2	0.2		2.0	0.4	0.2
RANGE		0.0-0.8	0.0-0.8	0.0-0.8		0.0-8.0	0.0-1.0	0.0-0.8

THYROID (C-CELL)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	0	47	0	0	0
658	120	0	0	0	118	0	0	0
832	46	0	0	0	49	0	0	0
874	99	0	0	0	100	0	0	0
937	119	0	0	0	118	1	0	0
988	108	0	0	0	109	0	0	0
TOTAL	542	0	0	0	541	1	0	0
RATE		0.0	0.0	0.0		0.2	0.0	0.0
RANGE		0.0	0.0	0.0		0.9	0.0	0.0

THYMUS

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	44	1	0	0	47	10	0	0
658	109	0	0	0	113	0	1	0
832	36	0	0	0	49	0	0	0
874	86	0	0	0	93	0	0	0
937	97	0	1	0	105	0	0	1
988	98	0	0	0	109	0	2	0
TOTAL	470	1	1	0	516	10	3	1
RATE		0.2	0.2	0.0		1.9	0.6	0.2
RANGE		0.0-2.3	0.0-1.0	0.0		0.0-21.3	0.0-1.8	0.0-1.0

TABLE 1

INCIDENCES OF NEOPLASTIC AND FOCAL HYPERPLASTIC LESIONS IN UNTREATED Crl: CD 1 MICE IN 104 WEEK STUDIES 2002-2004 at [redacted] b(4)

Study 274 and 832 s.c. dosing. Other studies p.o. dosing. All animals are micro chipped for identification

ALIMENTARY SYSTEM

TONGUE

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	50	0	0	0	50	0	0	0
658	120	0	1 squamous cell papilloma	0	119	0	0	0
832	50	0	0	0	49	0	0	0
874	100	0	0	1 squamous cell carcinoma	100	0	0	0
937	120	0	1 squamous cell papilloma	0	119	0	0	0
988	109	0	0	1 squamous cell carcinoma	110	0	0	0
TOTAL	549	0	2	2	547	0	0	0
RATE		0.0	0.4	0.4		0.0	0.0	0.0
RANGE		0.0	0.0-0.8	0.0-1.0		0.0	0.0	0.0

TRACHEA

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	49	0	0	0	50	0	0	0
658	120	0	0	0	120	0	0	0
832	47	0	0	0	48	0	0	0
874	100	0	0	0	100	0	0	0
937	120	0	0	0	120	0	0	0
988	109	0	0	0	108	0	0	0
TOTAL	545	0	0	0	546	0	0	0
RATE		0.0	0.0	0.0		0.0	0.0	0.0
RANGE		0.0	0.0	0.0		0.0	0.0	0.0

URINARY BLADDER

Study No.	Male Number Examined	Male Focal Hyperplasia	Male Benign Neoplasia	Male Malignant Neoplasia	Female Number Examined	Female Focal Hyperplasia	Female Benign Neoplasia	Female Malignant Neoplasia
274	49	0	0	0	48	0	0	0
658	117	0	0	0	116	0	0	0
832	46	0	0	0	47	0	0	0
874	99	0	0	0	99	0	0	0
937	119	0	0	0	116	0	0	0
988	109	0	0	0	105	0	0	0
TOTAL	539	0	0	0	531	0	0	0
RATE		0.0	0.0	0.0		0.0	0.0	0.0
RANGE		0.0	0.0	0.0		0.0	0.0	0.0

UTERUS

Study No.	Endometrium Total	Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Smooth muscle Total	Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Stroma Total	Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	50	0	0	2 carcinoma	50	0	3 leiomyoma	0	50	0	1 stromal polyp 1 deciduoma	2 haemangiosarcoma
658	120	0	1 adenoma	0	120	0	5 leiomyoma	1 leiomyosarcoma	120	0	12 stromal polyp	2 malignant schwannoma 1 stromal sarcoma
832	49	0	0	0	49	0	1 leiomyoma	0	49	0	2 stromal polyp 1 haemangioma	1 haemangiosarcoma
874	100	0	3 adenoma	0	100	0	2 leiomyoma	1 leiomyosarcoma	100	1	4 stromal polyp 1 deciduoma 1 granular cell tumour	2 stromal sarcoma 1 haemangiosarcoma
937	120	0	13 polyp 1 adenoma	0	120	0	7 leiomyoma	3 leiomyosarcoma	120	0	1 stromal polyp 1 haemangioma	3 haemangiosarcoma 1 malignant schwannoma
988	110	0	0	2 adenocarcinoma	110	0	3 leiomyoma	2 leiomyosarcoma	110	0	9 stromal polyp 1 deciduoma	3 stromal sarcoma 1 sarcoma (NOS) 1 haemangiosarcoma
TOTAL	549	0	16	4	549	0	21	7	549	1	38	18
RATE		0.0	2.9	0.7		0.0	3.8	1.3		0.2	6.9	3.3
RANGE		0.0	0.0-13.0	0.0-4.0		0.0	2.0-6.0	0.0-2.5		0.0	1.7	2.0

VAGINA

Study No.	Number Examined	Epithelium Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
274	49	0	0	0
658	117	0	1 stromal polyp	0
832	50	0	0	0
874	94	0	0	0
937	118	0	1 squamous cell papilloma 1 leiomyoma	0
988	107	0	0	0
TOTAL	535	0	3	0
RATE		0.0	0.6	0.0
RANGE		0.0	0.0	0.0

VASCULAR SYSTEM

Study No.	Male Number Examined	Focal Hyperplasia	Haemangioma	Haemangio sarcoma	Female Number Examined	Focal Hyperplasia	Haemangioma	Haemangio sarcoma
274	50	3	0	0	50	10	0	4
349*	110	0	0	5	110	0	0	0
658	120	14	2	7	110	17	0	4
832	50	6	2	6	120	2	2	2
874	100	0	0	5	50	0	1	5
937	120	0	3	2	100	0	6	6
TOTAL	660	25	12	26	120	8	0	3
RATE		3.8	1.8	3.9		0.0%	1.8%	3.6%
RANGE		0.0-12.0	0.0-4.5	0.0-12.0		0%	0-8%	0-13.3%

* Liver only study

APPENDIX 3 FOR MOUSE CARCINOGENICITY STUDY REVIEW: BACKGROUND INCIDENCE OF SKIN TUMORS FOR SUBCUTANEOUSLY DOSED RATS AND MICE IN 2 YEAR CARCINOGENICITY STUDIES.

SUBCUTANEOUS ROUTE

INTEGUMENTARY SYSTEM

SKIN OR INJECTION SITE

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
332 SD rat ski epi	100	0	1 basal cell adenoma 2 sebaceous cell adenoma 2 squamous-cell papilloma 5 keratoacanthoma	0	100	0	2 keratoacanthoma	1 basal cell carcinoma
332 SD rat ski mes	100	0	9 fibroma 7 dermal fibroma 1 fibrolipoma 3 lipoma	4 fibrosarcoma	100	0	1 fibroma 1 fibrolipoma 1 lipoma	1 liposarcoma
332 SD rat inj epi	94	0	1 keratoacanthoma	0	90	0	0	0
332 SD rat inj mes	94	0	1 dermal fibroma 1 lipoma	1 osteosarcoma 1 haemangiosarcoma	90	0	0	0
611 HW rat ski epi	50	0	1 basal cell adenoma 1 keratoacanthoma	0	50	0	1 keratoacanthoma	0
611 HW rat ski mes	50	0	1 fibroma	1 fibrosarcoma 1 sarcoma NOS 1 myxosarcoma	50	0	0	0
611 HW rat inj epi	50	0	0	0	50	0	0	0
611 HW rat inj mes	50	0	0	0	50	0	0	0
632 mouse ski epi	50	0	2 sebaceous cell adenoma	0	50	0	1 basal cell adenoma 1 keratoacanthoma	0
632 mouse ski mes	50	0	1 dermal fibroma	0	50	0	0	1 liposarcoma
632 mouse inj epi	49	0	0	0	47	0	0	0
632 mouse inj mes	49	0	0	0	47	0	0	1 haemangiosarcoma

332: 1998 Sprague Dawley rat; 611: 2002 Han Wistar; 632 2002 CD-1 mouse

Appendix B: Rat Carcinogenicity Study Review

Study title: NC 90-1170: 104-week carcinogenicity study in rats with subcutaneous administration

NNC 90-1170 Tumor Findings in Rats

Result	Organ/Tissue	Neoplasm	Historical Incidence	Parameter	Sex	Male				Female			
					NNC 90-1170 Dose (mg/kg/day)	0	0.075	0.25	0.75	0	0.075	0.25	0.75
						Trend analysis				Trend analysis			
Positive	Thyroid	c-cell adenoma	> 1%	incidence (%)		12.0	16.3	42.0	46.0	10.0	26.5	32.7	56.0
				p-value		0.000	0.431	0.002	0.000	0.000	0.021[a]	0.005	0.000
		c-cell carcinoma	< 1%	incidence (%)		2.0	8.2	6.0	14.0	0.0	0.0	4.1	6.0
				p-value		0.020	0.187	0.330	0.027	0.028	-	0.240	0.125
		c-cell adenoma + carcinoma	Not reported	incidence (%)		14.0	22.4	42.0	56.0	10.0	26.5	36.7	58.0
				p-value		0.000	0.227	0.000	0.000	0.000	0.021[a]	0.001	0.000

Underlined values considered positive based on trend analysis p-value for rare ($p < 0.025$) or common ($p < 0.005$) tumors, p-value for pairwise comparison to the control group for rare ($p < 0.05$) or common ($p < 0.01$) tumors, and the incidence in the historical control group.

[a]Although control group comparison p-value is above 0.01, the upper limit p-value for a false positive result for a common tumors, the finding was considered positive because trend analysis p-value was < 0.001, the p-value for pair-wise comparison with the control group was < 0.05, and incidence was above the sponsor reported historical control group range (1.3 - 16%).

Key study findings:

- NNC 90-1170 was a non-genotoxic carcinogen in male and female rats with treatment-related neoplasms occurring in **thyroid c-cells** at ≥ 0.25 mg/kg/day in males (human exposure multiple (HEM) ≥ 2.2) and at ≥ 0.075 mg/kg/day in females (HEM ≥ 0.5). Rat thyroid c-cell neoplasms were considered a progression of focal hyperplasia.
- Increased incidence or severity of **focal thyroid c-cell hyperplasia** occurred at ≥ 0.075 mg/kg/day in males and females (HEM ≥ 0.5).
- NNC 90-1170 dose-dependently increased the incidence of **thyroid c-cell adenomas** at ≥ 0.25 mg/kg/day in males (HEM ≥ 2.2) and at ≥ 0.075 mg/kg/day in females (HEM ≥ 0.5), **c-cell carcinomas** at 0.75 mg/kg/day in males, and **combined c-cell adenomas or carcinomas** at ≥ 0.25 mg/kg/day.
- The incidence of **c-cell carcinomas**, a rare tumor in rats, was above the historical control range at ≥ 0.075 mg/kg/day NNC 90-1170 in males (HEM ≥ 0.5) and at ≥ 0.25 mg/kg/day in females (HEM ≥ 2.2).
- Methodological / Protocol issues:
  - Toxicokinetic blood samples were obtained from main study group rats after the first dose and in study weeks 52 and 104.
  - Anti-NNC 90-1170 antibodies were not monitored during the study, however a pharmacodynamic effect of decreased body weight gain occurred during the study indicating if antibodies were formed, they weren't neutralizing.
  - Because the MRHD was increased from 0.6 mg/day NNC 90-1170 to 1.8 mg/day (AUC_{0-24} 814 nM.hr) during development, the AUC ratio for the highest dose of 0.75 mg/kg/day in the rat carcinogenicity study (AUC_{0-24} 6,225 nM.hr) was 7.6

Adequacy of the carcinogenicity study and appropriateness of the test model:

Sprague Dawley rats are pharmacologically responsive to subcutaneously administered NNC 90-1170 with reduced body weight gain and lower body weight compared to controls in all NNC 90-1170 treated groups and decreased food consumption, mainly in the high dose groups, observed throughout the 2 year study. Protein binding of NNC 90-1170 is slightly lower in rats than in humans. Metabolism of NNC 90-1170 was inadequately characterized in humans and rats. There are no major metabolites of lipid-labeled ^{3}H-[Pal]-liraglutide in humans, but metabolism of ^{3}H-[Pal]-liraglutide was similar in vivo and in vitro in rats and humans. In vitro metabolism of peptide-labeled ^{3}H-[tyr]-liraglutide is similar in mice and humans, but in vivo metabolism was not characterized in either species.

Evaluation of tumor findings:

In a 2 year carcinogenicity study of NNC 90-1170 in Sprague Dawley rats, treatment-related tumors in thyroid were c-cell adenomas at $\geq$ 0.25 mg/kg/day in males and at $\geq$ 0.075 mg/kg/day in females, c-cell carcinomas at 0.75 mg/kg/day in males, and combined c-cell adenomas and carcinomas at $\geq$ 0.25 mg/kg/day in males and at > 0.075 mg/kg/day in females.

CAC concurrence:

- The Committee concurred the study was acceptable, based on tumor findings in males and females.
- The Committee concurred that thyroid C-cell adenomas and adenomas or carcinomas (combined) were drug related. Liraglutide significantly increased the incidence of thyroid C-cell adenomas in males and females at $\geq$ 0.25 mg/kg, C-cell carcinoma in males at 0.75 mg/kg, and combined C-cell adenomas or carcinomas in males and females at $\geq$ 0.25 mg/kg.

Study no.: 200240 (sponsor), 455371 ——

Submission, Module, and page #: N000 4.2.3.4.1.1, pages 1 - 1631

Conducting laboratory and location: ——————————————— , b(4)

——————

Date of study initiation: 23 April 2001

Study ending date: 11 August 2005

GLP compliance: Yes (OECD compliance claimed)

QA report: yes (X) no ( )

Drug, lot #, and % purity: NNC 90-1170 lots shown in the table below. Purity of 97.7- 98.4 % reported for lots 317010, 317011, 317012, LLDP006,LLDP008, MLDP013 (certificate of analysis in Appendix B, page 278).

Batch Numbers

Batch Number	Concentration (mg.ml^{-1})	Date of Manufacture	Expiry Date
317010	5	14 Jun 2000	14 Dec 2001
317011	5	20 Jun 2000	20 Jun 2002
317012	5	22 Jun 2000	22 Mar 2002
LLDP006	2	27 Sept 2001	29 Mar 2003
LLDP008	5	04 Oct 2001	04 April 2003
MLDP013	5	06 Jun 2002	06 Dec 2003

[N000 4.2.3.4.1.1 P17]

Test Item Composition

Constituents:	Amount (per ml)
NNC 90-1170	5 mg
Phenol	5 mg

[N000 4.2.3.4.1.1 P17]

Methods

Doses: 0 (vehicle), 0.075, 0.25, 0.75 mg/kg/day NNC 90-1170

Basis of dose selection (MTD, MFD, AUC etc.): MTD in males (> 10% decreased body weight gain at ≥ 0.1 mg/kg in a 13 week dose range-finding study, AUC ratio > 25 in females (criteria not met based on toxicokinetic analysis in the 2 year study and human exposure at the MRHD of 1.8 mg/day).
Species/strain: Sprague Dawley rats (Crl:CD®(SD) ICR BR)
Number/sex/group (main study): 50 /sex/dose main study.

Group	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	
			Males	Females
1	Control	0	1-50	201-250
2	Low Dose	0.075	51-100	251-300
3	Intermediate Dose	0.25	101-150	301-350
4	High Dose	0.75	151-200	351-400

[N000 4.2.3.4.1.1 P19]

Route, formulation, volume: subcutaneous injection (dorsal area, 4 sites), 2 - 5 mg/mL NNC 90-117 solution diluted in vehicle (vehicle composition shown in summary table below), 1.0 mL/kg

Vehicle composition

Constituents:	Amount (per ml)
Disodium monohydrogenphosphate, Dihydrate	0.71 mg
Monosodium dihydrogenphosphate, Dihydrate	0.62 mg
Mannitol	36.9 mg
Phenol	5 mg
Water for injection	Add 1 ml
pH 7.4	pH was adjusted with NaOH

[N000 4.2.3.4.1.1 P16]

Frequency of dosing: once a day
Satellite groups used for toxicokinetics or special groups: None.
Tail vein blood from 2/sex/dose main study group rats was collected prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours after dosing after the first dose and during weeks 53 and 104.
Age and weight: ~ 6 weeks old at the beginning of the study with males weighing 150 -218 g, and females weighing 120 - 174 g.
Animal housing: Five rats/sex/dose were housed in solid bottom cages with sterilized pine wood shavings (analysis revealed no significant contaminants), a food hopper, and 2 polycarbonate water bottles with stainless steel nozzles (page 18).
Restriction paradigm for dietary restriction studies: None.
Drug stability/homogeneity: Drug dosing solution samples were taken immediately after preparation in weeks 1, 4, 8, 12, 24, 39, 52, 65, 78, 91, and 104 were taken. To assess stability, additional samples were taken on the last day of weekly preparation in weeks 100 and 104. Drug concentrations were measured by size-exclusion HPLC.
Dual controls employed: None.
Interim sacrifices: None.
Deviations from original study protocol:
Protocol deviations in animal housing environmental controls and dosing formulations, but the deviations did not affect the integrity of the study.

Observation times
Mortality: Twice a day.
Clinical signs: Main study rats checked for clinical signs during the workday with detailed examination performed once a week. Palpations for masses were performed once a week beginning in week 13.
Body weights: Recorded daily during the first 2 weeks of treatment, then weekly until study termination. Rats in deteriorating condition or losing weight were weighed more frequently.
Food consumption: Recorded once a week until week 13, then once every 4 weeks.
Water consumption: Water consumption was monitored by visual inspection, but it wasn't quantified.

Ophthalmoscopy: Rats examined by indirect ophthalmoscopy (anterior, lenticular, fundic areas) after instillation of a mydriatic (1% tropicamide) prior to dosing and in study weeks 51 and 102.
Hematology: Approximately 0.5 mL tail vein blood was taken from all surviving rats in study weeks 52, 78, and 103 for differential WBCs. Blood smears were prepared for future evaluation, if it was deemed necessary.
Toxicokinetics: At least 0.5 mL tail vein blood was obtained from 2 rats/sex/NNC 90-1170 dose/time point prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours after dosing on day 1 and in study weeks 53 and 104. Vehicle control group samples were taken in week 104. Plasma NNC 90-1170 was quantified using an ELISA (SOP 878-LP-08005, anti-NNC 90-1170 monoclonal antibody 1 coupled to the microtiter plate, and the second biotin-labeled monoclonal antibody targeted to a different NNC 90-1179 epitope). Because this assay requires diluting the rat plasma samples at least 25-fold in human serum, the lower limit of quantification of NNC 90-1170 in rat plasma is 450 pM (lowest limit of 18 pM X 25 fold dilution).
Gross Pathology: Rats surviving 104 week of treatment were anesthetized with CO_2 and exsanquinated. All rats sacrificed moribund or found dead were necropsied.
Histopathology: Peer review: yes (X), no ( ) - internal peer review at [redacted] evaluating a tissue samples from males and females in each dose group and thyroid glands from all control and low dose rats and all tumors from all rats. b(4)

Unless otherwise noted, tissues for microscopic examination were fixed in 10% neutral buffered formalin.

Tissues Collected	Weighed	Examined	Comments
Abnormal Tissue		X	Included local lymph nodes as remote.
Adrenal x 2	X	X	-
Aortic Arch		X	-
Blood Smear			Prepared from animals killed prematurely.
Brain	X	X	Forebrain, midbrain and cerebellum
Eye x 2		X	Fixed in Davidson's fluid. Only one was processed and examined histologically.
Femur – Bone Marrow		X	-
Gastrointestinal Tract:			
Stomach		X	Opened at necropsy and mucosa examined.
Duodenum		X	
Jejunum		X	
Ileum		X	
Caecum		X	
Colon		X	
Rectum		X	
Harderian Gland x 2		X	Both fixed. Only one was processed and examined histologically.
Heart	X	X	-
Implant			For identification purposes.
Injection Site		X	Four in total, 2 processed.
Kidneys 2	X	X	-
Liver	X	X	-
Lung	X	X	Inflated after weighing; all lobes examined including mainstem bronchi.
Mesenteric Lymph Node		X	-
Nasal Cavity			Examined if signs dictated.
Oesophagus		X	-
Optic Nerve x 2		X	Fixed in Davidson's fluid. Only one was examined.
Ovary x 2	X	X	-

Tissues Collected	Weighed	Examined	Comments
Pancreas		X	-
Pituitary	X	X	-
Prostate	X	X	-
Rib			Preserved, including costochondral junction
Sciatic Nerve		X	-
Seminal Vesicles		X	-
Skin + Mammary Gland		X	-
Spinal Cord		X	Sections from cervical, midthoracic and lumbar regions.
Spleen	X	X	-
Sternum		X	Included bone marrow.
Submandibular Lymph Node		X	-
Submaxillary Salivary Gland	X	X	-
Testis + Epididymis x 2	X	X	-
Thigh Muscle		X	-
Thymus	X	X	-
Thyroid Gland and Parathyroid Gland x 2	X	X	Weighed after fixation.
Tongue		X	-
Trachea		X	-
Urinary Bladder		X	Distended bladders distended with fixative; epithelial surface in animals which underwent histological evaluation was examined after fixation.
Uterus	X	X	-
Vagina		X	-

[N000 4.2.3.4.1.1 P25-26]

Results
Formulation: Concentrations of NNC 90-1170 in dosing solution samples were within an acceptable range of 84 – 101% for all samples.

Mortality:
There were no treatment-related effects on mortality.

Males

Group	Number in Group	Number Killed Prematurely	Number Found Dead	Number Surviving up to Scheduled Necropsy
1	50	21	0	29
2	50	22	4	24
3	50	17	4	29
4	50	22	3	26

Females

Group	Number in Group	Number Killed Prematurely	Number Found Dead	Number Surviving up to Scheduled Necropsy
1	50	21	1	28
2	50	24	1	25
3	50	27	2	21
4	50	18	3	29

[N000 4.2.3.4.1.1 P36]

Figure 4 Kaplan Meier Survival Graphs: Females

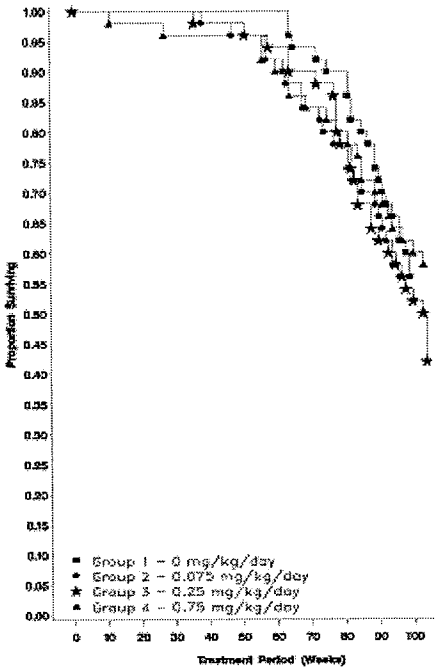
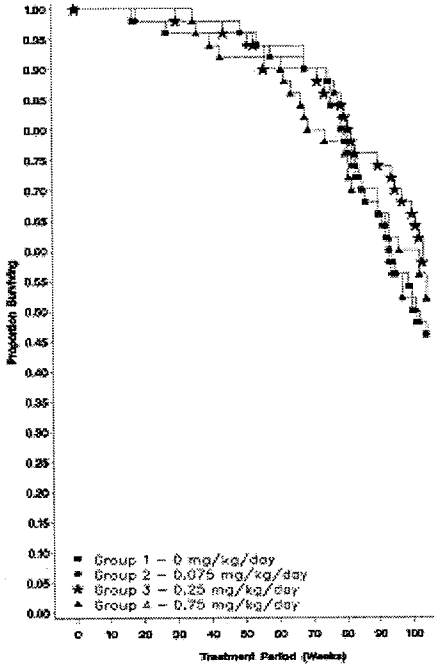


Figure 3 Kaplan Meier Survival Graphs: Males



[N000 4.2.3.4.1.1 P276-277]

Clinical signs:

Clinical signs considered treatment related due an increased incidence or number of observations occurring mainly during the second year were hunched posture and piloerection in females at ≥ 0.25 mg/kg/day and staining of the fur (near the injection site) at all doses in males and females.

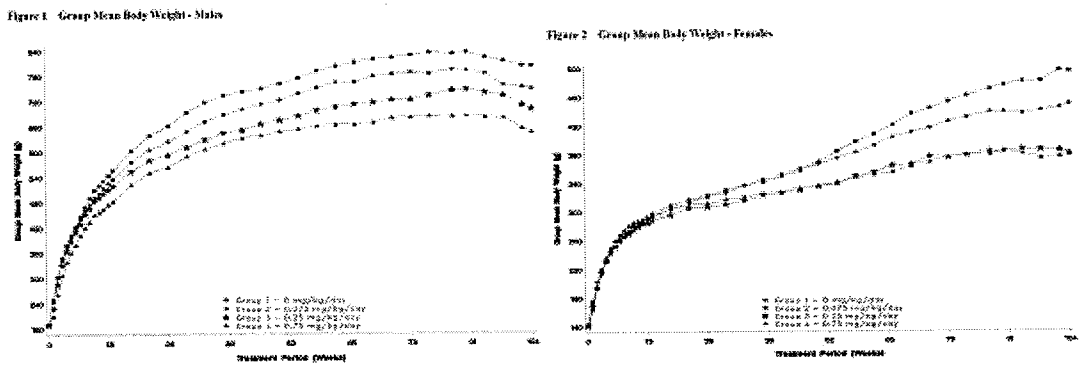
Clinical Sign	Group/Sex/Dose Level (mg.kg^{-1}.day^{-1})							
	1M	2M	3M	4M	1F	2F	3F	4F
	0	0.075	0.25	0.75	0	0.075	0.25	0.75
Hunched Posture								
Incidence[a]	12	12	18	20	23	18	32	26
Observations[b]	168	75	180	148	195	105	419	352
Staining on fur								
Incidence[a]	28	39	41	36	39	43	44	47
Observations[b]	549	1015	1328	1275	1231	2565	2917	3120
Piloerection								
Incidence[a]	15	17	18	19	24	26	36	39
Observations[b]	156	104	177	165	182	173	517	617

a = out of 50 per sex
b = number of occasions observed

[N000 4.2.3.4.1.1 P37]

Body weights:

By the end of the 104 treatment period, there were substantial differences in group mean body weight between control and NNC 90-1170 treated groups (Figures 5 & 6, and summary table below). Group mean body weight gain dose-dependently decreased compared to controls at ≥ 0.075 mg/kg/day in males and females.



[N000 4.2.3.4.1.1 P274-275]

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.075	0.25	0.75	0	0.075	0.25	0.75
Parameter									
Body weight	N, week 0	50	50	50	50	50	50	50	50
	g (group mean), week 0	189.7	189.5	190.4	209.7	143.2	143.0	141.4	143.4
	N, week 104	23	24	29	26	28	25	21	29
	g (group mean), week 104	800.6	745.5	699.4	643.7	494.5	448.9	380.1	376.2
	% difference from control, week 104	0.0	-6.9	-12.6	-19.6	0.0	-9.2	-23.1	-23.9
Body weight gain (week 0 to week 104)	g, (week 104 - week 0)	610.9	556.0	509.0	434.0	351.3	305.9	238.7	232.8
	% of pretest body weight	322.0	293.4	267.3	207.0	245.3	213.9	168.8	162.3
	% difference from control	0.0	-9.0	-16.7	-29.0	0.0	-12.9	-32.1	-33.7

Food consumption:

Compared to the vehicle control group, NNC 90-1170 dose-dependently decreased group mean food consumption during the first week at all doses in males and at ≥ 0.25 mg/kg/day in females (see summary table below). Food consumption decreased in NNC 90-1170 treated groups in the first week in males and females. In males, food consumption decreased at ≥ 0.25 mg/kg/day from week 5 to the end of the study. After the first week, group mean food consumption in NNC 90-117 treated females was similar to controls. From week 13 onward, food consumption decreased with increased treatment duration at ≥ 0.25 mg/kg/day NNC 90-1170 in males and at 0.75 mg/kg/day in females.

Week	Group/Sex/Dose Level ($mg.kg^{-1}.day^{-1}$)							
	1M	2M	3M	4M	1F	2F	3F	4F
	0	0.075	0.25	0.75	0	0.075	0.25	0.75
1	-	94%	76%	63%	-	103%	89%	78%
13	-	99%	96%	93%	-	102%	103%	104%
26	-	97%	94%	90%	-	100%	98%	98%
50	-	95%	89%	89%	-	100%	96%	93%
78	-	97%	91%	88%	-	106%	93%	97%
104	-	96%	88%	84%	-	97%	100%	92%

Percentage of Control Group Mean

[N000 4.2.3.4.1.1 P39]

Water consumption:

Water consumption was considered unaffected by treatment.

Ophthalmoscopy:

There were no treatment-related effects.

Hematology:

Compared to concurrent controls, lymphocytes increased in males at 0.75 mg/kg/day in weeks 52 and 78, but not in week 103. In females, lymphocytes increased at 0.75 mg/kg/day in week 52 and in week 78, but not at any dose in week 103. A statistically significant effect of treatment (comparing lymphocyte counts in NNC 90-1170 treated groups to control) occurred in both males and females in weeks 52 and 78, but not in week 103.

Lymphocytes (x 10^9/L)

Sex	Male					Female				
NNC 90-1170 Dose (mg/kg/day) / Study Week	0	0.075	0.25	0.75	Group Effect P-value	0	0.075	0.25	0.75	Group Effect P-value
52	7.17	7.14	6.96	7.42	0.044	3.93	4.24	4.41	4.82	0.044
78	5.52	6.02	5.56	6.08	0.010	3.32	3.66	3.76	3.66	0.010
103	4.57	**10.10**[a]	4.74	5.04	> 0.05	3.19	3.36	3.40	3.84	> 0.05

Group mean values statistically significantly different from contol are underlined.

[a]Male 59 had elevated WBC consisting of WBC 161.3, lymphocyte 129.6, basophils 10.3, and large unstained cells 25.5 and this rat was diagnosed with malignant lymphocytic lymphoma.

Statistically significantly decreased large unclassified cells occurred in week 52 in males (61, 69, and 53% of controls at 0.075, 0.25, and 0.75 mg/kg/day, respectively), but the decrease wasn't dose-related and it didn't occur in weeks 78 or 103, so it's relation to treatment was equivocal.

Organ weight:

Group mean relative weight of thyroid dose-dependently increased up to 2 fold in males and up to 1.8 fold in females at ≥ 0.075 mg/kg/day.

	Sex	Males				Females			
NNC 90-1170 Dose (mg/kg/day)		0	0.075	0.25	0.75	0	0.075	0.25	0.75
Parameter	N / Units	23	24	29	26	28	25	21	29
Body weight	g	797	744	695	642	487	441	373	374
Thyroid	mg	37.1	44.6	47.1	60.2	30.9	33.6	30.9	42.7
	mg % of bw	4.7	6.0	6.8	9.4	6.3	7.6	8.3	11.4
	Relative weight, fold change from control	1.0	1.3	1.5	2.0	1.0	1.2	1.3	1.8

Gross pathology:

A low incidence of masses in the abdominal cavity was higher than controls at 0.75 mg/kg/day in males.

A high incidence of staining of the skin, presumably near the injection site, was above control group levels at ≥ 0.075 mg/kg/day in both sexes.

Enlarged thyroid gland at ≥ 0.25 mg/kg/day in males and females correlated with increased relative weight of thyroid and histopathology findings of C-cell hyperplasia / adenoma / carcinoma.

NECROPSY FINDINGS	GROUP DOSE	GROUP TOTALS Males Grp 1 0 mg/kg /day	Grp 2 0.075 mg/kg /day	Grp 3 0.25 mg/kg /day	Grp 4 0.75 mg/kg /day	Females Grp 1 0 mg/kg /day	Grp 2 0.075 mg/kg /day	Grp 3 0.25 mg/kg /day	Grp 4 0.75 mg/kg /day
GENERAL COMMENTS									
Number of animals necropsied		50	50	50	50	50	50	50	50
ABDOMINAL CAVITY									
Mass(es)		1	1		3				
SKIN AND SUBCUTIS									
Hair loss		4	7	5	3	16	17	25	18
Swollen		1	1	2					
Bruising				1					
Staining		21	30	34	34	23	32	39	43
THYROID GLAND									
Mass(es), one/both			1		1				1
Dark, both					1				
Pale focus, right/left					1				1
Pale, right				1					
Dark focus, right/left				2		1			
Enlarged, one/both				5	7			1	2

The absence of a numeral indicates that the lesion specified was not identified

[N000 4.2.3.4.1.1 compiled from P70 - 92]

Histopathology:

Treatment-related histopathology findings occurred in thyroid gland (pre-neoplastic and neoplastic) and kidney (non-neoplastic).

Non-neoplastic:

In the thyroid, the incidence of mild to marked focal C-cell hyperplasia was considered higher than control group levels at $\geq$ 0.25 mg/kg/day. The total incidence of focal c-cell hyperplasia was only significantly higher than controls in high dose group males ($p < 0.05$) and mid-dose group females ($p < 0.01$). While the incidence of focal C-cell hyperplasia correlates with an increased incidence of C-cell tumors, the incidence and severity of diffuse C-cell hyperplasia does not. Proliferative C-cell lesions correlated with increased relative weight and macroscopic enlargement of the thyroid. Because focal c-cell hyperplasia is considered a preneoplastic tumor, incidences of diffuse and focal c-cell hyperplasia are shown in a table of neoplastic findings in the thyroid (see "Neoplastic" subsection below).

The incidence of mineralization in kidneys was significantly higher than controls in males at $\geq$ 0.075 mg/kg/day, but in females, the incidence of in NNC 90-1170 treated groups was similar to the elevated control group levels. The background incidence of kidney mineralization was 25 fold higher in female controls compared to males.

A statistically significant increased incidence of adrenal gland focal cortical cell hypertrophy with degeneration occurred at 0.25 mg/kg/day in males, but the increased incidence was not dose related and didn't occur in females, so its relevance to NNC 90-1170 toxicity was considered equivocal.

In liver, the incidence of basophilic cell focus was higher than controls in all dose groups in males and at $\geq$ 0.25 mg/kg/day in females. There was a higher incidence in NNC 90-1170 treated females compared to males, but the incidence in the female control group was 10 fold higher than in males. The increased incidence didn't reach statistical significance at any dose in males or females, so the liver finding was considered equivocal.

The incidence of focal / multifocal alveolar macrophage accumulation was considered higher than controls at 0.75 mg/kg/day, but the increase never reached statistical significance. Therefore, in the absence of corroborating findings, it was not considered relevant to NNC 90-1170 toxicity.

HISTOLOGICAL FINDINGS	GROUP TOTALS							
	MALES				FEMALES			
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 2 0.075 mg/kg /day	Grp 3 0.25 mg/kg /day	Grp 4 0.75 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 0.075 mg/kg /day	Grp 3 0.25 mg/kg /day	Grp 4 0.75 mg/kg /day
LUNG	(50)	(49)	(50)	(50)	(50)	(49)	(50)	(50)
Alveolar macrophage accumulation, focal/multifocal	15	14	15	20	12	16	16	21
THYROID GLAND	(50)	(49)	(50)	(50)	(50)	(49)	(49)	(50)
FOLLICULAR CELL ADENOMA [B]	0	2	1	2	1	0	2	0
FOLLICULAR CELL CARCINOMA [M]	0	1	1	0	0	0	0	0
Focal C-cell hyperplasia,								
minimal	3	0	2	3	6	1	2	4
mild	6	7	8	9	7	7	14	11
moderate	2	3	6	4	1	5	6	4
marked	0	4	4	8**	0	1	5*	5
Total Incidence	11	14	20	24*	14	14	27**	24
Diffuse C-cell hyperplasia,	0	1	0	0	0	0	0	0
minimal	1	0	0	0	2	0	0	0
mild	2	2	1	3	3	2	2	2
moderate	0	2	1	2	0	4	1	0
marked	0	1	1	2	1	2	0	1
Total Incidence	3	6	3	7	6	8	3	3
C-CELL ADENOMA [B],	6	8	21**	23***	5	13*	16**	28***
C-CELL CARCINOMA [M],	1	4	3	7	0	0	2	3
ADRENAL GLAND	(49)	(50)	(49)	(50)	(50)	(50)	(50)	(50)
Focal cortical cell hypertrophy, with degeneration,	3	9	14**	8	22	18	14	15
Focal cortical cell hypertrophy,	11	10	10	11	4	4	9	6
KIDNEY	(49)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Diffuse transitional cell hyperplasia,								
minimal	1	1	1	0	0	0	0	0
mild	11	6	12	6	9	6	11	6
moderate	0	1	6*	5	3	3	4	3
marked	0	1	0	0	0	0	2	0
Total Incidence	12	9	19	11	12	9	17	9
Mineralisation	1	14***	9*	8*	25	26	24	15
LIVER	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Basophilic cell focus, homogenous								
mild	1	0	1	1	0	2	3	5
moderate	0	0	0	0	2	0	1	0
marked	0	2	1	1	4	2	3	5
severe	0	0	0	0	0	1	0	0
Total Incidence	1	6	4	7	10	8	12	15

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

[N000 4.2.3.4.1.1 compiled from P174 - 228]

Neoplastic:

Treatment-related neoplastic findings occurred in thyroid (C-cell adenoma and carcinoma). The sponsor's statistical analysis of tumor incidence is Appendix 1 and historical control data from rats in 2 year carcinogenicity studies at [redacted] (1994 – 2001) is Appendix 2. Statistical analysis of tumor incidence from Dr. Min in CDER's Office of Biostatistics is included in a summary table below (refer to Dr. Min's Statistical Review of study results for this NDA). Tabulated summaries of tumor types with p-values < 0.05 for either dose-response relationship or pair-wise comparisons determine from statistical analysis of male or female groups are shown below. In several instances, statistical analysis performed by the sponsor differs from ours, but any differences were not material to determining which neoplasms were considered treatment-related.

b(4)

Tumor Types with P-Values ? 0.05 for Dose Response Relationship or Pairwise Comparisons

Sex	Organ Name	Tumor Name	Cont N=42	Low N=45	Med N=43	High N=43	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male	Thyroid Gland	C-CELL ADENOMA [B]	6	8	21	23	0.000	0.431	0.002	0.000
		C-CELL CARCINOMA [M]	1	4	3	7	0.020	0.187	0.330	0.027
		C-CELL TUMOR	7	11	21	28	0.000	0.227	0.003	0.000
Sex	Organ Name	Tumor Name	Cont N=47	Low N=47	Med N=48	High N=49	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Female	Pituitary Gland	CARCINOMA, ANTERIOR	1	0	1	5	0.009	0.472	0.740	0.106
	Thyroid Gland	C-CELL TUMOR	5	13	18	29	0.000	0.021	0.001	0.000
		C-CELL ADENOMA [B]	5	13	16	28	0.000	0.021	0.005	0.000
		C-CELL CARCINOMA [M]	0	0	2	3	0.028	.	0.240	0.125

Note: C-cell tumor=C-cell adenoma + C-cell carcinoma.

The incidence and/or severity of focal thyroid c-cell hyperplasia, a preneoplastic lesion, was higher than control group levels at ≥ 0.075 mg/kg/day liraglutide in males and females. The total incidence of focal c-cell hyperplasia was greater than the historical control mean incidences of 7.8% in males (0 – 14.3%) and 9.9% in females (0 – 20%) in all dose groups, including controls.

In thyroid, the dose-related increased incidence of C-cell adenoma significantly exceeded concurrent controls and the historical control range at ≥ 0.25 mg/kg/day in males and at ≥ 0.075 mg/kg/day in females. The historical control mean incidence of c-cell adenomas in Sprague Dawley rats at the ——→(1994 – 2001, see Appendix 2) was 10.8% (range 4 – 21.1%) in males and 8.2% (range 1.3 – 16%) in females. The incidence of c-cell adenomas exceeded the historical range of the control group at ≥ 0.25 mg/kg/day liraglutide in males and at ≥ 0.075 mg/kg/day in females. b(4)

The incidence of C-cell carcinoma , a rare tumor in rats, increased above concurrent and historical control group levels at ≥ 0.075 mg/kg/day in males (historical control range 0 – 2.1%) and at ≥ 0.25 mg/kg/day in females(range 0 – 4%), but the increase was only statistically significant by pair-wise comparison to control at 0.75 mg/kg/day in males($p < 0.05$). Although C-cell carcinoma was only significantly increased in high dose males compared to controls, there was a significant linear trend ($p < 0.05$) for the finding in both males and females.

Organ	Finding	Severity	Sex	Males								Females							
			NNC 90-1170 Dose (mg/kg/day)	0		0.075		0.25		0.75		0		0.075		0.25		0.75	
			Fate (Survivor or Decendent)	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D
		N		23	27	24	25	29	21	26	24	28	22	25	24	21	28	29	21
				50		49		50		50		50		49		49		50	
Thyroid	Diffuse C-cell hyperplasia	minimal		0	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0
		mild		1	1	2	0	1		1	2	3	0	2	0	0	2	1	1
		moderate				2	1	1		1	1		0	3	1	1		0	
		marked					1	1		1	1		1	1	1			1	
		total afftected		1	2	5	2	3	0	3	4	5	1	6	2	1	2	2	1
				3 (6.0%)		7 (14.3%)		3 (6.0%)		7 (14.0%)		6 (12.0%)		8 (16.3%)		3 (6.1%)		3 (6.0%)	
	Focal C-cell hyperplasia (preneoplastic)	minimal		2	1	0	0	1	1	3	0	3	3	0	1	1	1	2	2
		mild		3	3	3	4	4	4	8	1	6	1	3	4	7	7	6	5
		moderate		1	1	3	0	5	1	2	2	1		4	1	2	4	3	1
		marked				2	2	3	1	4	4*				1	3	2	3	2
		total afftected		6	5	8	6	13	7	17**	7	10	4	7	7	13	14*	14	10
				11 (22%)		14 (28.6%)		20 (40.0%)		24 (48.0%)*		14 (28%)		14 (28.6%)		27 (55.1%)**		24 (48.0%)	
	C-cell adenoma			5	1	5	3	11	10***	12	11***	4	1	10	3	9*	7	19***	9**
				6 (12.0%)		8 (16.3%)		21 (42.0%)**		23 (46.0%)***		5 (10.0%)		13 (26.5%)*		16 (32.7%)**		28 (56.0%)***	
	C-cell carcinoma			0	1	2	2	2	1	4	3	0	0	0	0	1	1	3	0
				1 (2.0%)		4 (8.2%)		3 (6.0%)		7 (14.0%)**		0		0		2 (4.1%)		3 (6.0%)	
	C-cell adenoma or carcinoma			7 (14.0%)		11 (22.4%)		21 (42.0%)***		28 (56.0%)***		5 (10%)		13 (26.5%)*		18 (36.7%)**		29 (58.0%)***	

Statistically significantly different from control by Peto analysis: *p < 0.05, **p < 0.01, ***p < 0.001

Carcinoma of the anterior pituitary in females, a common tumor (mean historical control incidence of 4.2%), was not considered treatment-related because the incidence was not statistically significantly higher than control group at any dose ($p > 0.01$ for pair-wise comparison with control), the highest incidence of 4.2% occurring at 0.75 mg/kg/day was within the historical control group range of 0 – 14.3%, and trend analysis yielded a p-value of 0.008 (p-value > 0.005, cutoff p-value established for a statistically significant dose-related trend for common tumors).

Organ	Finding	Severity	Sex	Males								Females							
			NNC 90-1170 Dose (mg/kg/day)	0		0.075		0.25		0.75		0		0.075		0.25		0.75	
			Fate (Survivor or Decendent)	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D
		N		23	27	24	25	28	20	26	24	28	22	25	24	21	28	29	21
				50		49		48		50		50		49		49		50	
Pituitary	adenoma, anterior lob			11	16	12	10	12	14	11	14	16	18	10	17	11	24	18	10*
				27 (54.0%)		22 (44.9%)		26 (54.1%)		25 (50.0%)		34 (68.0%)		27 (55.1%)		35 (71.4%)		28 (56.0%)	
	carcinoma, anterior lobe			0	0	0	1	0	0	0	1	0	1	0	0	1	0	2	3
				0		1 (2.0%)		0		1 (2.0%)		1 (2.0%)		0		1 (2.0%)		5 (10.0%)	

Statistically significantly different from control by Peto analysis: *p < 0.05, **p < 0.01

Sponsor's statistic analysis (PETO) of tumor findings

Organ	Tumour Type		P-VALUE[1]	P-VALUE[2]	P-VALUE[3]	P-VALUE[4]
Thyroid Gland	C-cell Carcinoma [M]	Males	0.013	0.058	0.17	0.009
		Females	0.027	1.00	0.27	0.12
	C-cell Adenoma [B]	Males	<0.001	0.28	<0.001	<0.001
		Females	<0.001	0.016	0.001	<0.001
	C-cell Tumour	Males	<0.001	0.13	<0.001	<0.001
		Females	<0.001	0.016	<0.001	<0.001
Pituitary Gland	Carcinoma anterior lobe [M], locally invasive	Males	0.30	0.48	1.00	0.47
		Females	0.008	1.00	0.69	0.099

([1]) Linear trend
([2]) Group 2 vs Group 1
([3]) Group 3 vs Group 1
([4]) Group 4 vs Group 1

[N000 4.2.3.4.1.1 P42]

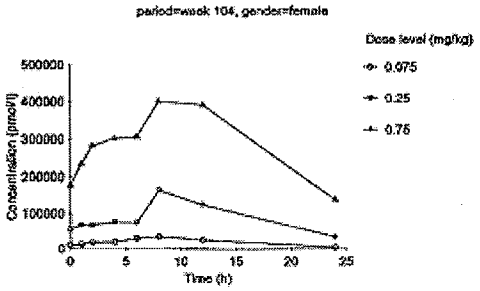
Toxicokinetics:

NNC 90-1170 plasma toxicokinetics were determined after dosing male and female rats with 0, 0.075, 0.2, or 0.75 mg/kg/day NNC 90-1170 on day 1 and weeks 52 and 104. Plasma NNC 90-1170 was analyzed using an ELISA assay with a 450 pM lower limit of quantification. The ELISA assay may not differentiate between intact and delipidated NNC 90-1170. Predose and control group plasma levels (week 104) of NNC 90-1170 were below the level of detection at all sample times. Toxicokinetic parameters are summarized in the table below.

NNC 90-1179 Dose (mg/kg/day)	Sample Time	Plasma NNC 90-1170 Cmax (nM) M	F	Average	AUC_{0-24} (nM.hr) M	F	Average	Tmax (hr) Range (M + F)
0.075	Day 1	33	31	32	299	347	323	3.1 - 6.1
	Week 52	24	41	33	332	511	422	4.8 - 6.4
	Week 104	26	36	31	361	485	423	5.3 - 7.1
0.25	Day 1	115	109	112	1,270	1,290	1,280	3.7 - 4.2
	Week 52	104	154	129	1,400	2,200	1,800	8 - 10
	Week 104	137	164	151	1,480	2,090	1,785	7.1 - 11.7
0.75	Day 1	361	338	350	4,950	4,830	4,890	4 - 4.8
	Week 52	287	453	370	4,680	7,110	5,895	6.9 - 9.6
	Week 104	394	401	398	5,580	6,870	6,225	7.0 - 9.8

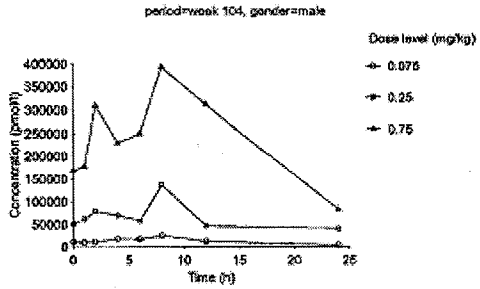
Plasma concentration versus time profiles after dosing male and female mice in week 104 are shown in the graphs below.

Figure 5 Mean (arithmetic) plasma concentration–time profiles following 104 weeks dosing of liraglutide in female rats.



[N000 4.2.3.4.1.1 P1600]

Figure 6 Mean (arithmetic) plasma concentration–time profiles following 104 weeks dosing of liraglutide in male rats.



[N000 4.2.3.4.1.1 P1600]

Plasma NNC 90-1170 peaked 3.1 to 11.7 hours after dosing. In general, both Cmax and AUC_{0-24} increased linearly with dose. There were no substantial sex differences in plasma exposures. Between study day 1 and week 104, NNC 90-1170 did not accumulated in plasma.

Summary and Conclusions

In a 104 week carcinogen bioassay of 0, 0.075, 0.25, or 0.75 mg/kg/day liraglutide injected subcutaneously once a day in Sprague Dawley rats, survival was unaffected by treatment. Toxicokinetic parameters were determined on day 1, week 52, and week 104 using an ELISA detecting the peptide moiety of liraglutide. In general, Cmax and AUC_{0-24} increased linearly with dose. Estimated human exposure multiples based on AUC_{0-24} 816 nM.hr at the MRHD of 1.8 mg/day liraglutide and week 104 rat AUC_{0-24} (average of male and female combined) were 0.5, 2.2, and 7.6 for doses of 0.075, 0.25, and 0.75 mg/kg/day liraglutide.

Liraglutide effects on food consumption, body weight gain, and body weight were consistent with its pharmacologic effect. At $\geq$ 0.075 mg/kg/day, liraglutide dose dependently decreased group mean body weight compared to controls, 6.9 – 19.6% in males and 9.2 – 23.9% in females, decreased body weight gain 9.0 – 29.0% in males and 12.9 – 33.7% in females, and decreased food consumption at $\geq$ 0.25 mg/kg/day in males and at 0.75 mg/kg/day in females. The effect on food consumption was more pronounced in the first week at all doses in males and at $\geq$ 0.25 mg/kg/day in females, and subsided with continued treatment at lower doses. Despite the relatively large decrease in body weight gain and lower body weight compared to controls at higher doses, survival wasn't affected.

There were no treatment-related effects on water consumption, hematology parameters, or ophthalmoscopy parameters.

Macroscopic pathology findings were a low incidence of masses in the abdominal cavity at 0.75 mg/kg/day NNC 90-1170 in males and enlarged thyroid at $\geq$ 0.25 mg/kg/day in males and females. Enlarged thyroid was consistent with dose-dependent increased relative thyroid weight, up to 2 fold in males and up to 1.8 fold in females, at $\geq$ 0.075 mg/kg/day and focal c-cell hyperplasia / adenomas / carcinomas at $\geq$ 0.075 mg/kg/day in males and females.

Treatment-related non-neoplastic histopathology findings occurred in thyroid. **Focal thyroid c-cell hyperplasia**, considered a precursor to thyroid c-cell tumors, occurred at $\geq$ 0.075 mg/kg/day in males and at $\geq$ 0.25 mg/kg/day in females.

Treatment-related neoplastic findings occurred in **thyroid c-cells (males and females)**. Thyroid c-cell tumors were considered a progression from focal hyperplasia to benign adenomas to malignant carcinomas. NNC 90-1170 dose-dependently increased the incidence of **thyroid c-cell adenomas** at $\geq$ 0.25 mg/kg/day in males (HEM 2.2) and at $\geq$ 0.075 mg/kg/day in females (HEM 0.5), increased the incidence of **c-cell carcinomas** at $\geq$ 0.75 mg/kg/day in males (HEM 7.6), and increased total combined c-cell adenomas or carcinomas at $\geq$ 0.025 mg/kg/day NNC 90-1170 in males (HEM 2.2) and at $\geq$ 0.075 mg/kg/day in females (HEM 0.5). Although the increased incidence of c-cell carcinomas was not statistically significant at any dose by pair-wise comparison with control except in high dose males, the incidence was above the concurrent controls and the historical control range at $\geq$ 0.075 mg/kg/day in males and at $\geq$ 0.25 mg/kg/day in females.

APPENDIX 1 RAT CARCINOGENICITY STUDY: LIST OF TUMOR INCIDENCES

Compiled from 'Peto analysis of tumor incidence: Males' and 'Peto analysis of tumor incidence: Females' (Tables 22 & 23 in the sponsor's report)

Males, Peto analysis of tumor incidence

Organ/Finding		0 mg/kg/day (N=50)	0.075 mg/kg/day (N=50)	0.25 mg/kg/day (N=50)	0.75 mg/kg/day (N=50)
HAEMOPOIETIC SYSTEM	NEX	50	50	50	50
LYMPHOMA FOLLICULAR CENTRE CELL [U]	NOBS	0	1	0	0
	I	0	1	0	0
	F	0	0	0	0
	P-VALUE #	0.77	0.33	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX	50	50	50	50
LEUKAEMIA LARGE GRANULAR CELL [U]	NOBS	1	0	0	0
	I	0	0	0	0
	F	1	0	0	0
	P-VALUE #	1.00	1.00	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX	50	50	50	50
LEUKAEMIA GRANULOCYTIC [M]	NOBS	1	0	0	0
	I	0	0	0	0
	F	1	0	0	0
	P-VALUE #	1.00	1.00	1.00	1.00
HAEMOPOIETIC SYSTEM	NEX	50	50	50	50
HISTIOCYTIC SARCOMA [M]	NOBS	2	3	1	0
	I	1	0	0	0
	F	1	3	1	0
	P-VALUE #	0.97	0.50	0.91	1.00
HAEMOPOIETIC SYSTEM	NEX	50	50	50	50
LYMPHOMA LYMPHOCYTIC [M]	NOBS	0	1	2	1
	I	0	1	1	0
	F	0	0	1	1
	P-VALUE #	0.34	0.51	0.31	0.51
LYMPH NODE [MESENTERIC]	NEX	50	50	49	50
HAEMANGIOMA [B]	NOBS	0	1	1	1
	I	0	1	1	1
	F	0	0	0	0
	P-VALUE #	0.37	0.51	0.35	0.67
LYMPH NODE [MESENTERIC]	NEX	50	50	49	50
HAEMANGIOSARCOMA [M]	NOBS	1	1	1	0
	I	1	1	1	0
	F	0	0	0	0
	P-VALUE #	0.79	0.75	0.76	1.00
THYMUS	NEX	48	48	47	48
THYMOMA [M]	NOBS	0	1	0	0
	I	0	1	0	0
	F	0	0	0	0
	P-VALUE #	0.73	0.52	1.00	1.00
THYMUS	NEX	48	48	47	48
THYMOMA [B]	NOBS	0	0	0	1
	I	0	0	0	1
	F	0	0	0	0
	P-VALUE #	0.26	1.00	1.00	0.66
HEART	NEX	50	50	49	50
MESOTHELIOMA [M] pericardial	NOBS	0	0	1	0
	I	0	0	1	0
	F	0	0	0	0
	P-VALUE #	0.52	1.00	0.45	1.00
HEART	NEX	50	50	49	50
HAEMANGIOSARCOMA [M]	NOBS	1	0	0	0
	I	1	0	0	0
	F	0	0	0	0
	P-VALUE #	1.00	1.00	1.00	1.00
THYROID GLAND	NEX	50	49	50	50
FOLLICULAR CELL CARCINOMA [B] unilateral	NOBS	0	1	1	0
	I	0	1	1	0
	F	0	0	0	0
	P-VALUE #	0.62	0.44	0.45	1.00
THYROID GLAND	NEX	50	49	50	50
FOLLICULAR CELL ADENOMA [B] unilateral	NOBS	0	2	1	2
	I	0	2	1	2
	F	0	0	0	0
	P-VALUE #	0.17	0.20	0.47	0.23
THYROID GLAND	NEX	50	49	50	50
FOLLICULAR CELL TUMOUR	NOBS	0	3	2	2
	I	0	3	2	2
	F	0	0	0	0
	P-VALUE #	0.09	0.11	0.21	0.23

NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			0 mg/kg/day (N=50)	0.075 mg/kg/day (N=50)	0.25 mg/kg/day (N=50)	0.75 mg/kg/day (N=50)
THYROID GLAND	NEX		50	49	50	50
C-CELL CARCINOMA [M]	NOBS		1	4	3	7
	I		1	4	3	7
	F		0	0	0	0
	P-VALUE		0.013	0.058	0.17	0.009
THYROID GLAND	NEX		50	49	50	50
C-CELL ADENOMA [B]	NOBS		6	8	21	23
	I		6	8	21	23
	F		0	0	0	0
	P-VALUE		<0.001	0.28	<0.001	<0.001
THYROID GLAND	NEX		50	49	50	50
C-CELL TUMOUR	NOBS		7	11	21	28
	I		7	11	21	28
	F		0	0	0	0
	P-VALUE		<0.001	0.13	<0.001	<0.001
PARATHYROID GLAND	NEX		46	45	46	46
ADENOMA [B] unilateral	NOBS		1	2	2	1
	I		1	2	2	1
	F		0	0	0	0
	P-VALUE	#	0.53	0.54	0.57	0.81
ADRENAL GLAND	NEX		49	50	49	50
CORTICAL ADENOMA [B] unilateral	NOBS		0	1	0	0
	I		0	1	0	0
	F		0	0	0	0
	P-VALUE	#	0.71	0.44	1.00	1.00
ADRENAL GLAND	NEX		49	50	49	50
PHAEOCHROMOCYTOMA [B]	NOBS		6	6	6	6
	I		6	6	6	6
	F		0	0	0	0
	P-VALUE		0.56	0.50	0.65	0.57
PITUITARY GLAND	NEX		50	49	48	50
CARCINOMA ANTERIOR LOBE [M] locally invasive	NOBS		0	1	0	1
	I		0	0	0	0
	F		0	1	0	1
	P-VALUE	#	0.30	0.48	1.00	0.47
PITUITARY GLAND	NEX		50	49	48	50
ADENOMA ANTERIOR LOBE [B]	NOBS		27	22	28	25
	I		14	16	19	13
	F		13	6	7	12
	P-VALUE		0.47	0.85	0.73	0.64
PANCREAS (ENDOCRINE)	NEX		50	50	49	50
ISLET CELL ADENOMA [B]	NOBS		6	1	2	2
	I		6	1	2	2
	F		0	0	0	0
	P-VALUE		0.82	0.98	0.94	0.91
TESTIS	NEX		50	50	49	50
ADENOCARCINOMA [M] metastasizing	NOBS		1	0	0	0
	I		0	0	0	0
	F		1	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
TESTIS	NEX		50	50	49	50
MESOTHELIOMA [M]	NOBS		0	2	0	0
	I		0	2	0	0
	F		0	0	0	0
	P-VALUE	#	0.83	0.23	1.00	1.00
TESTIS	NEX		50	50	49	50
INTERSTITIAL CELL ADENOMA [B]	NOBS		5	3	2	5
	I		5	3	2	5
	F		0	0	0	0
	P-VALUE		0.38	0.78	0.92	0.68
TESTIS	NEX		50	50	49	50
HAEMANGIOSARCOMA [M] unilateral	NOBS		1	0	0	0
	I		1	0	0	0
	F		0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
SEMINAL VESICLE	NEX		50	50	49	50
ADENOMA [B]	NOBS		0	1	0	0
	I		0	1	0	0
	F		0	0	0	0
	P-VALUE	#	0.77	0.51	1.00	1.00

NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			0 mg/kg/day (N=50)	0.075 mg/kg/day (N=50)	0.25 mg/kg/day (N=50)	0.75 mg/kg/day (N=50)
KIDNEY	NEX		49	50	50	50
TUBULAR CELL CARCINOMA [U] unilateral	NOBS		0	0	1	0
	I		0	0	0	0
	F		0	0	1	0
	P-VALUE	#	0.54	1.00	0.55	1.00
KIDNEY	NEX		49	50	50	50
TUBULAR CELL ADENOMA [B] unilateral	NOBS		0	0	0	1
	I		0	0	0	1
	F		0	0	0	0
	P-VALUE	#	0.25	1.00	1.00	0.52
KIDNEY	NEX		49	50	50	50
LIPOMA [B] unilateral	NOBS		1	0	0	0
	I		1	0	0	0
	F		0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
KIDNEY	NEX		49	50	50	50
LIPOSARCOMA [N] unilateral	NOBS		2	0	0	0
	I		2	0	0	0
	F		0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
STOMACH	NEX		49	50	49	50
SQUAMOUS-CELL CARCINOMA [N]	NOBS		0	0	1	0
	I		0	0	1	0
	F		0	0	0	0
	P-VALUE	#	0.53	1.00	0.55	1.00
JEJUNUM	NEX		45	47	47	50
LEIOMYOSARCOMA [N] locally invasive	NOBS		1	0	0	0
	I		0	0	0	0
	F		1	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
LIVER	NEX		50	50	50	50
HEPATOCELLULAR CARCINOMA [M]	NOBS		0	0	0	1
	I		0	0	0	1
	F		0	0	0	0
	P-VALUE	#	0.25	1.00	1.00	0.52
LIVER	NEX		50	50	50	50
HEPATOCELLULAR ADENOMA [B]	NOBS		1	0	0	1
	I		1	0	0	1
	F		0	0	0	0
	P-VALUE	#	0.45	1.00	1.00	0.76
SALIVARY GLAND (SUBMAXILLARY)	NEX		50	50	49	50
CARCINOMA [M] ductular	NOBS		0	1	0	0
	I		0	0	0	0
	F		0	1	0	0
	P-VALUE	#	0.75	0.51	1.00	1.00
PANCREAS (EXOCRINE)	NEX		50	50	49	50
ACINAR CELL ADENOMA [B]	NOBS		1	1	0	0
	I		1	1	0	0
	F		0	0	0	0
	P-VALUE	#	0.95	0.77	1.00	1.00
BRAIN	NEX		50	50	49	50
GLIOMA [M]	NOBS		0	1	0	0
	I		0	1	0	0
	F		0	0	0	0
	P-VALUE	#	0.77	0.51	1.00	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
FIBROSARCOMA [M]	NOBS		2	1	2	0
	P-VALUE	#	0.88	0.88	0.71	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
MALIGNANT SCHWANNOMA [M]	NOBS		0	0	1	1
	P-VALUE	#	0.18	1.00	0.49	0.50
SKIN AND SUBCUTIS	NEX		50	50	49	50
DERMAL FIBROMA [B]	NOBS		9	11	3	10
	P-VALUE		0.44	0.35	0.97	0.40
SKIN AND SUBCUTIS	NEX		50	50	49	50
NEURAL CREST TUMOUR [N]	NOBS		0	1	0	1
	P-VALUE	#	0.32	0.51	1.00	0.49

NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding			0 mg/kg/day (N=50)	0.075 mg/kg/day (N=50)	0.25 mg/kg/day (N=50)	0.75 mg/kg/day (N=50)
SKIN AND SUBCUTIS	NEX		50	50	49	50
BASAL CELL CARCINOMA [M]	NOBS		0	1	1	0
	P-VALUE	#	0.53	0.51	0.52	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
BASAL CELL ADENOMA [B]	NOBS		0	2	0	2
	P-VALUE	#	0.20	0.25	1.00	0.27
SKIN AND SUBCUTIS	NEX		50	50	49	50
SQUAMOUS-CELL PAPILLOMA [B]	NOBS		1	1	3	0
	P-VALUE	#	0.00	0.75	0.35	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
SQUAMOUS-CELL CARCINOMA [M]	NOBS		0	1	0	0
	P-VALUE	#	0.75	0.50	1.00	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
SEBACEOUS CELL ADENOMA [B]	NOBS		1	0	1	1
	P-VALUE	#	0.38	1.00	0.74	0.75
SKIN AND SUBCUTIS	NEX		50	50	49	50
SARCOMA (NOT OTHERWISE SPECIFIED) [M]	NOBS		0	1	0	0
	P-VALUE	#	0.74	0.49	1.00	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
FIBROMA [B]	NOBS		6	5	4	2
	P-VALUE		0.94	0.63	0.80	0.93
SKIN AND SUBCUTIS	NEX		50	50	49	50
LIPOMA [B]	NOBS		2	3	5	0
	P-VALUE	#	0.92	0.49	0.24	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
MYXOSARCOMA [M]	NOBS		0	0	1	0
	P-VALUE	#	0.55	1.00	0.56	1.00
SKIN AND SUBCUTIS	NEX		50	50	49	50
KERATOACANTHOMA [B]	NOBS		3	6	5	2
	P-VALUE		0.84	0.15	0.26	0.68
SKIN AND SUBCUTIS	NEX		50	50	42	50
HAEMANGIOSARCOMA [M]	NOBS		0	1	0	0
	P-VALUE	#	0.75	0.51	1.00	1.00
MAMMARY GLAND	NEX		43	48	47	48
FIBROADENOMA [B]	NOBS		1	3	0	0
	P-VALUE	#	0.97	0.34	1.00	1.00
MAMMARY GLAND	NEX		43	48	47	48
CARCINOMA [M]	NOBS		0	1	0	0
	P-VALUE	#	0.77	0.54	1.00	1.00

NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

[N000 4.2.3.4.1.1 compiled from P230-251]

Females

Organ/Finding			0 mg/kg/day (N=50)	0.075 mg/kg/day (N=50)	0.25 mg/kg/day (N=50)	0.75 mg/kg/day (N=50)
HAEMOPOIETIC SYSTEM	NEX		50	50	50	50
LEUKAEMIA GRANULOCYTIC [M]	NOBS		0	0	0	1
	I		0	0	0	0
	F		0	0	0	1
	P-VALUE	#	0.25	1.00	1.00	0.49
HAEMOPOIETIC SYSTEM	NEX		50	50	50	50
HISTIOCYTIC SARCOMA [M]	NOBS		0	0	0	1
	I		0	0	0	0
	F		0	0	0	1
	P-VALUE	#	0.26	1.00	1.00	0.49
HAEMOPOIETIC SYSTEM	NEX		50	50	50	50
LYMPHOMA LYMPHOCYTIC [M]	NOBS		1	1	1	1
	I		1	0	0	0
	F		0	1	1	1
	P-VALUE	#	0.51	0.74	0.70	0.74
HAEMOPOIETIC SYSTEM	NEX		50	50	50	50
LYMPHOMA [M]	NOBS		1	0	0	0
	I		1	0	0	0
	F		0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
LYMPH NODE (MESENTERIC)	NEX		49	50	49	49
HAEMANGIOMA [B]	NOBS		1	0	0	0
	I		1	0	0	0
	F		0	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
THYROID GLAND	NEX		50	49	49	50
FOLLICULAR CELL ADENOMA [B] unilateral	NOBS		1	0	2	0
	I		1	0	2	0
	F		0	0	0	0
	P-VALUE	#	0.70	1.00	0.39	1.00
THYROID GLAND	NEX		50	49	49	50
FOLLICULAR CELL TUMOUR	NOBS		1	0	2	0
	I		1	0	2	0
	F		0	0	0	0
	P-VALUE	#	0.70	1.00	0.39	1.00
THYROID GLAND	NEX		50	49	49	50
C-CELL CARCINOMA [M]	NOBS		0	0	2	3
	I		0	0	2	3
	F		0	0	0	0
	P-VALUE	#	0.027	1.00	0.27	0.12
THYROID GLAND	NEX		50	49	49	50
C-CELL ADENOMA [B]	NOBS		5	13	16	28
	I		5	13	16	28
	F		0	0	0	0
	P-VALUE		<0.001	0.018	0.001	<0.001
THYROID GLAND	NEX		50	49	49	50
C-CELL TUMOUR	NOBS		5	13	18	29
	I		5	13	18	29
	F		0	0	0	0
	P-VALUE		<0.001	0.016	<0.001	<0.001
PARATHYROID GLAND	NEX		49	46	50	48
ADENOMA [B] unilateral	NOBS		1	0	1	2
	I		1	0	1	2
	F		0	0	0	0
	P-VALUE	#	0.46	1.00	0.69	0.41
ADRENAL GLAND	NEX		50	50	50	50
SUBCAPSULAR CELL TUMOUR [B] unilateral	NOBS		0	1	0	0
	I		0	1	0	0
	F		0	0	0	0
	P-VALUE	#	0.68	0.42	1.00	1.00
ADRENAL GLAND	NEX		50	50	50	50
CORTICAL ADENOMA [B] unilateral	NOBS		3	1	0	2
	I		3	1	0	2
	F		0	0	0	0
	P-VALUE	#	0.51	0.32	1.00	0.77
ADRENAL GLAND	NEX		50	50	50	50
PHAEOCHROMOCYTOMA [B]	NOBS		0	1	2	1
	I		0	1	2	1
	F		0	0	0	0
	P-VALUE	#	0.32	0.47	0.19	0.51

NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ/Finding		0 mg/kg/day (N=50)	0.075 mg/kg/day (N=50)	0.25 mg/kg/day (N=50)	0.75 mg/kg/day (N=50)
PITUITARY GLAND	NEX	50	49	49	50
CARCINOMA ANTERIOR LOBE [M] locally invasive	NOBS	1	0	1	5
	I	0	0	1	3
	F	1	0	0	2
	P-VALUE #	0.008	1.00	0.69	0.039
PITUITARY GLAND	NEX	50	49	49	50
ADENOMA ANTERIOR LOBE [B]	NOBS	34	27	35	28
	I	22	15	21	22
	F	12	12	14	6
	P-VALUE	0.85	0.76	0.20	0.89
PITUITARY GLAND	NEX	50	49	49	50
ADENOMA INTERMEDIATE LOBE [B]	NOBS	0	0	1	1
	I	0	0	1	1
	F	0	0	0	0
	P-VALUE #	0.19	1.00	0.43	0.51
PANCREAS (ENDOCRINE)	NEX	50	50	50	49
ISLET CELL ADENOMA [B]	NOBS	0	0	0	1
	I	0	0	0	1
	F	0	0	0	0
	P-VALUE #	0.28	1.00	1.00	0.51
OVARY	NEX	50	50	50	50
GRANULOSA/THECAL CELL TUMOUR [B]	NOBS	0	1	0	0
	I	0	1	0	0
	F	0	0	0	0
	P-VALUE #	0.73	0.47	1.00	1.00
OVARY	NEX	50	50	50	50
FIBROMA [B] unilateral	NOBS	0	1	0	1
	I	0	1	0	1
	F	0	0	0	0
	P-VALUE #	0.33	0.47	1.00	0.51
UTERUS	NEX	50	50	50	50
STROMAL POLYP [B]	NOBS	5	5	7	10
	I	5	5	7	10
	F	0	0	0	0
	P-VALUE	0.058	0.46	0.18	0.060
UTERUS	NEX	50	50	50	50
ADENOCARCINOMA [M]	NOBS	1	0	0	0
	I	1	0	0	0
	F	0	0	0	0
	P-VALUE #	1.00	1.00	1.00	1.00
UTERUS	NEX	50	50	50	50
STROMAL SARCOMA [M]	NOBS	3	1	2	0
	I	1	1	2	0
	F	2	0	0	0
	P-VALUE #	0.94	0.95	0.86	1.00
UTERUS	NEX	50	50	50	50
FIBROMA [B]	NOBS	1	0	0	0
	I	1	0	0	0
	F	0	0	0	0
	P-VALUE #	1.00	1.00	1.00	1.00
UTERUS	NEX	50	50	50	50
ADENOMA [B]	NOBS	0	0	0	1
	I	0	0	0	1
	F	0	0	0	0
	P-VALUE #	0.18	1.00	1.00	0.35
TONGUE	NEX	50	49	50	50
SQUAMOUS-CELL CARCINOMA [M]	NOBS	1	0	0	0
	I	0	0	0	0
	F	1	0	0	0
	P-VALUE #	1.00	1.00	1.00	1.00
DUODENUM	NEX	49	50	49	49
LEIOMYOSARCOMA [M]	NOBS	0	0	1	0
	I	0	0	1	0
	F	0	0	0	0
	P-VALUE #	0.49	1.00	0.43	1.00
DUODENUM	NEX	49	50	49	49
LEIOMYOMA [B]	NOBS	0	1	0	0
	I	0	1	0	0
	F	0	0	0	0
	P-VALUE #	0.73	0.47	1.00	1.00

NEX = Number of animals examined, NOBS = Number of animals with finding
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F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

Organ;Finding			0 mg/kg/day (N=50)	0.075 mg/kg/day (N=50)	0.25 mg/kg/day (N=50)	0.75 mg/kg/day (N=50)
LIVER	NEX		50	50	50	50
HEPATOCELLULAR ADENOMA [B]	NOBS		1	1	1	1
	I		1	1	1	1
	F		0	0	0	0
	P-VALUE	#	0.52	0.74	0.74	0.74
LIVER	NEX		50	50	50	50
CHOLANGIOMA [B]	NOBS		0	0	1	0
	I		0	0	1	0
	F		0	0	0	0
	P-VALUE	#	0.40	1.00	0.48	1.00
BRAIN	NEX		50	50	50	50
GRANULAR CELL TUMOUR [B]	NOBS		0	0	0	1
	I		0	0	0	1
	F		0	0	0	0
	P-VALUE	#	0.18	1.00	1.00	0.36
BRAIN	NEX		50	50	50	50
OLIGODENDROGLIOMA [M]	NOBS		1	0	0	0
	I		0	0	0	0
	F		1	0	0	0
	P-VALUE	#	1.00	1.00	1.00	1.00
SPINAL CORD	NEX		50	50	50	50
MENINGEAL SARCOMA [M] locally invasive	NOBS		0	0	1	0
	I		0	0	0	0
	F		0	0	1	0
	P-VALUE	#	0.49	1.00	0.50	1.00
SKIN AND SUBCUTIS	NEX		50	50	50	50
FIBROSARCOMA [M]	NOBS		0	2	0	0
	P-VALUE	#	0.81	0.23	1.00	1.00
SKIN AND SUBCUTIS	NEX		50	50	50	50
DERMAL FIBROMA [B]	NOBS		0	1	1	0
	P-VALUE	#	0.68	0.48	0.48	1.00
SKIN AND SUBCUTIS	NEX		50	50	50	50
BASAL CELL ADENOMA [B]	NOBS		1	1	0	0
	P-VALUE	#	0.93	0.72	1.00	1.00
SKIN AND SUBCUTIS	NEX		50	50	50	50
SQUAMOUS-CELL PAPILLOMA [B]	NOBS		0	1	0	0
	P-VALUE	#	0.74	0.48	1.00	1.00
SKIN AND SUBCUTIS	NEX		50	50	50	50
SQUAMOUS-CELL CARCINOMA [M]	NOBS		0	1	0	0
	P-VALUE	#	0.35	0.48	1.00	1.00
SKIN AND SUBCUTIS	NEX		50	50	50	50
SARCOMA (NOT OTHERWISE SPECIFIED) [M]	NOBS		0	0	1	0
	P-VALUE	#	0.50	1.00	0.50	1.00
SKIN AND SUBCUTIS	NEX		50	50	50	50
FIBROMA [B]	NOBS		4	1	1	1
	P-VALUE	#	0.05	0.95	0.95	0.97
SKIN AND SUBCUTIS	NEX		50	50	50	50
LIPOMA [B]	NOBS		1	1	0	1
	P-VALUE	#	0.53	0.72	1.00	0.74
SKIN AND SUBCUTIS	NEX		50	50	50	50
MYXOMA [B]	NOBS		0	0	0	1
	P-VALUE	#	0.27	1.00	1.00	0.51
SKIN AND SUBCUTIS	NEX		50	50	50	50
KERATOACANTHOMA [B]	NOBS		0	1	0	1
	P-VALUE	#	0.30	0.47	1.00	0.47
MAMMARY GLAND	NEX		50	50	50	50
FIBROCARCINOMA [M]	NOBS		0	0	1	0
	P-VALUE	#	0.49	1.00	0.49	1.00
MAMMARY GLAND	NEX		50	50	50	50
FIBROADENOMA [B]	NOBS		28	25	26	20
	P-VALUE		0.93	0.65	0.42	0.92
MAMMARY GLAND	NEX		50	50	50	50
CARCINOMA [B]	NOBS		10	13	8	6
	P-VALUE		0.06	0.16	0.62	0.90
MAMMARY GLAND	NEX		50	50	50	50
ADENOMA [B]	NOBS		4	5	5	2
	P-VALUE		0.45	0.51	0.53	0.37

NEX = Number of animals examined, NOBS = Number of animals with finding
I = Number of animals with incidental finding (defined as incidental or probably incidental)
F = Number of animals with fatal finding (defined as fatal or probably fatal)
# = Exact permutation test
P-VALUE = p-values under the control group are trend test, under dosed groups are pairwise comparisons (one-sided)

[N000 4.2.3.4.1.1 compiled from P252-273]

APPENDIX 2 FOR RAT CARCINOGENICITY STUDY REVIEW: HISTORICAL CONTROL GROUP DATA OF TUMOR INCIDENCE IN SPRAGUE DAWLEY RATS FROM 2 YEAR CARCINOGENICITY STUDIES

_____ from 2002 to 2004. b(4)

TABLE 2
INCIDENCES OF NEOPLASTIC AND FOCAL HYPERPLASTIC LESIONS IN UNTREATED Crl: CD SPRAGUE-DAWLEY RATS IN 104 WEEK STUDIES

ADRENAL GLAND (MEDULLA)

	Male				Female			
Study No.	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	49	6	8 phaeochromocytoma	1 phaeochromocytoma	48	5	6 phaeochromocytoma	0
009	59	9	10 phaeochromocytoma	1 phaeochromocytoma	60	4	1 phaeochromocytoma	0
059	48	9	7 phaeochromocytoma	0	50	3	1 phaeochromocytoma	0
441	50	8	8 phaeochromocytoma	5 phaeochromocytoma	50	3	3 phaeochromocytoma	0
499	49	13	4 phaeochromocytoma	1 phaeochromocytoma	49	5	2 phaeochromocytoma	0
530	50	11	4 phaeochromocytoma	1 phaeochromocytoma	49	5	1 phaeochromocytoma	0
588	50	5	6 phaeochromocytoma	0	50	1	0	0
939	83	12	7 phaeochromocytoma	3 phaeochromocytoma	78	13	1 phaeochromocytoma	0
970	75	2	12 phaeochromocytoma	0	77	0	3 phaeochromocytoma	1 phaeochromocytoma
089	50	14	8 phaeochromocytoma	0	50	3	2 phaeochromocytoma	0
911	50	8	7 phaeochromocytoma	0	50	3	1 phaeochromocytoma	0
332	100	50	17 phaeochromocytoma	1 phaeochromocytoma	100	15	2 phaeochromocytoma	0
348	100	21	15 phaeochromocytoma	1 phaeochromocytoma	100	6	3 phaeochromocytoma	0
678	120	23	7 phaeochromocytoma	0	120	6	2 phaeochromocytoma	1 phaeochromocytoma
277	100	0	1 phaeochromocytoma	1 phaeochromocytoma	100	0	0	0
430	120	13	14 phaeochromocytoma	4 phaeochromocytoma	117	15	6 phaeochromocytoma	2 phaeochromocytoma
687	65	21	9 phaeochromocytoma	2	65	15	1 phaeochromocytoma	0
TOTAL	1218	225	144	21	1213	102	35	4
RATE		18.5%	11.8 %	1.7%		8.4%	2.9%	0.3%
RANGE		0-50%	1-16.9%	0-10%		0-23.1%	0-12.5%	0-1.7%

ADRENAL GLAND (SUBCAPSULAR CELL)

	Male				Female			
Study No.	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	49	0	0	0	48	0	0	0
009	59	0	0	0	60	0	0	0
059	48	0	0	0	50	0	0	0
441	50	0	0	0	50	0	0	0
499	49	0	0	0	49	0	0	0
530	50	0	0	0	50	0	0	0
588	50	0	0	0	50	0	0	0
939	83	0	0	0	78	0	0	0
970	75	0	0	0	77	0	0	0
089	50	0	0	0	50	0	0	0
911	50	0	0	0	50	0	0	0
332	100	0	0	0	100	0	0	0
348	100	0	0	0	100	0	0	0
678	120	1	0	0	120	0	0	0
277	100	1	0	0	100	1	0	0
430	120	3	0	0	117	3	0	0
687	65	0	0	0	65	0	0	0
TOTAL	1218	5	0	0	1214	4	0	0
RATE		0.4%	0.0%	0.0%		0.3%	0.0%	0.0%
RANGE		0-2.5%	0.0%	0.0%		0-2.6%	0.0%	0.0%

ADRENAL GLAND (CORTEX)

	Male				Female			
Study No.	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	49	1	2 adenoma	0	48	1	0	0
009	59	6	0	0	60	3	0	1 carcinoma
059	48	1	0	0	50	1	1 adenoma	0
441	50	11	1 adenoma	0	50	3	2 adenoma	0
499	49	14	0	2 carcinoma	49	12	0	0
530	50	8	0	0	50	5	2 adenoma	0
588	50	6	0	0	50	6	1 adenoma	0
939	83	18	1 adenoma	0	78	20	3 adenoma	0
970	75	4	0	0	77	3	1 adenoma	1
089	50	21	1 adenoma	1 carcinoma	50	28	1 adenoma	0
911	50	8	0	1 carcinoma	50	10	1 adenoma	0
332	100	30	3 adenoma	1 carcinoma	100	62	0	0
348	100	16	1 adenoma	0	100	8	2 adenoma	1 carcinoma
678	120	26	2 adenoma	2 carcinoma	120	7	2 adenoma	1 carcinoma
277	100	48	2 adenoma	1 carcinoma	100	14	0	0
430	120	37	1 adenoma	0	117	17	2 adenoma	1 carcinoma
687	65	16	2 adenoma	0	65	21	0	2 carcinoma
TOTAL	1218	271	16	8	1214	221	18	7
RATE		22.2%	1.3%	0.7%		18.2%	1.5%	0.6%
RANGE		2-48%	0-4.1%	0-4.1%		2-62%	0-4%	0-3.1%

BRAIN

	Male				Female			
Study No.	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	0	2 glioma	49	0	0	0
009	60	0	0	0	60	0	0	0
059	50	0	1 glioma	0	50	0	1 glioma	0
441	50	0	1 granular cell tumour	2 glioma	50	0	0	2 reticulosis
499	49	0	0	1 astrocytoma	50	0	1 meningioma	1 granular cell tumour
530	50	0	1 granular cell tumour	1 reticulosis	50	0	0	0
588	49	0	1 granular cell tumour	2 glioma	50	0	0	0
939	82	1	2 granular cell tumour	2 astrocytoma	78	0	0	0
970	74	0	0	1 astrocytoma	77	0	0	1 astrocytoma
089	50	0	1 granular cell tumour	0	50	0	0	0
911	50	0	1 granular cell tumour	0	50	0	0	0
332	100	0	1 meningioma	0	100	0	1 granular cell tumour	0
348	100	0	1 meningioma	2 astrocytoma	100	0	1 ependymoma	0
678	120	0	0	4 astrocytoma 1 sarcoma	120	0	0	0
277	100	0	2 granular cell tumour	0	100	0	1 granular cell tumour	0
430	120	0	1 granular cell tumour	0	119	0	0	1 astrocytoma
687	65	0	1 granular cell tumour	0	65	0	0	2 astrocytoma
TOTAL	1219	1	14	16	1218	0	5	7
RATE		0.0%	1.1%	1.5%		0.0%	0.4%	0.6%
RANGE		0.0%	0-2.3%	0-4.2%		0.0%	0-2%	0-4%

DUODENUM

	Male				Female			
Study No.	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	47	0	0	0	47	0	0	0
009	59	0	0	0	60	0	0	0
059	45	0	0	0	47	0	0	0
441	46	0	0	0	50	0	0	0
499	49	0	0	0	50	0	0	0
530	48	0	0	0	50	0	0	0
588	50	0	0	0	50	0	0	0
939	81	0	0	0	77	0	0	0
970	76	0	0	0	77	0	0	0
089	50	0	0	0	50	0	0	0
911	49	0	0	0	47	0	0	0
332	95	0	0	0	94	0	0	1 leiomyosarcoma
348	100	0	0	0	100	0	1 leiomyoma	0
678	117	0	0	0	115	0	0	1 leiomyosarcoma
277	100	0	0	0	99	0	0	0
430	110	0	0	0	114	1	0	0
687	59	0	0	0	65	0	0	0
TOTAL	1181	0	0	0	1192	1	1	2
RATE		0.0%	0.0%	0.0%		0.1%	0.1%	0.2%
RANGE		0.0%	0.0%	0.0%		0-0.9%	0-1%	0-1.1%

HEART

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	0	0	49	0	0	0
009	60	0	0	0	60	0	0	0
059	50	0	0	0	50	0	0	0
441	50	0	0	0	50	0	0	0
499	50	0	0	0	50	0	0	0
530	50	0	0	0	50	0	0	0
588	50	0	0	1 mesothelioma	50	0	0	0
939	84	0	0	1 mesothelioma	78	0	0	0
970	76	0	0	0	77	0	0	0
089	50	0	0	0	50	0	0	0
911	50	0	0	0	50	0	0	0
332	100	0	0	1 mesothelioma	100	0	0	0
348	100	0	0	1 mesothelioma	100	0	0	0
678	120	0	2 schwannoma	0	118	0	1 schwannoma	0
277	100	0	0	0	100	0	0	0
430	119	1	0	0	120	2	0	0
687	65	0	0	0	65	0	0	0
TOTAL	1224	1	2	4	1217	2	1	0
RATE		0.1%	0.2%	0.3%		0.2%	0.1%	0.0%
RANGE		0-0.8%	0-1.7%	0-2%		0-1.7%	0-0.8%	0.0%

JEJUNUM

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	43	0	0	0	45	0	0	0
009	57	0	0	0	59	0	0	1 leiomyosarcoma
059	45	0	0	0	44	0	0	0
441	43	0	0	0	46	0	0	0
499	0	0	0	0	0	0	0	0
530	45	0	0	0	48	0	0	0
588	46	0	0	0	50	0	0	0
939	81	0	0	0	77	0	0	0
970	76	0	0	0	76	0	0	0
089	50	0	0	0	50	0	0	0
911	49	0	0	0	47	0	0	0
332	85	0	0	0	94	0	0	0
348	100	0	0	0	100	0	0	0
678	113	0	0	0	112	0	0	0
277	99	0	0	0	98	0	0	0
430	106	0	0	0	112	0	0	1 sarcoma
687	59	0	0	0	64	0	0	0
TOTAL	1097	0	0	0	1122	0	0	2
RATE		0.0%	0.0%	0.0%		0.0%	0.0%	0.2%
RANGE		0.0%	0.0%	0.0%		0.0%	0.0%	0-1.7%

KIDNEY

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	1 adenoma	0	50	0	0	1 mesenchymal tumour
009	60	0	0	0	60	0	0	1 liposarcoma
059	49	0	1 adenoma	0	50	0	0	0
441	50	0	0	0	50	0	0	0
499	50	0	0	0	50	0	0	0
530	50	0	0	0	50	0	1 lipoma	1 liposarcoma
588	50	0	1 polyp	0	50	0	0	0
939	99	3	0	0	100	2	1 adenoma	1 liposarcoma
970	100	0	0	1 carcinoma	100	0	0	1 carcinoma
089	50	0	0	0	50	0	0	0
911	50	0	0	0	50	0	0	0
332	99	0	0	0	100	0	1 adenoma 1 papilloma	0
348	100	0	0	0	100	0	0	0
678	120	0	0	0	120	0	0	0
277	100	2	1 adenoma	1 carcinoma	100	0	0	0
430	120	2	0	3 carcinoma	120	0	0	1 carcinoma 1 liposarcoma
687	65	0	0	0	65	0	0	1 liposarcoma
TOTAL	1262	7	4	5	1265	2	4	8
RATE		0.6%	0.3%	0.4%		0.2%	0.3%	0.6%
RANGE		0-3%	0-2%	0-2.5%		0-2%	0-2%	0-2%

LIVER (HEPATOCELLULAR)

	Male						Female					
Study No.	Number Examined	Eosinophilic / Clear cell foci	Basophilic foci	Amphophilic foci	Benign Neoplasia	Malignant Neoplasia	Number Examined	Eosinophilic / Clear cell foci	Basophilic foci	Amphophilic foci	Benign Neoplasia	Malignant Neoplasia
623	50	22	10	0	2 adenoma	0	50	12	22	0	0	0
009	60	25	21	0	6 adenoma	2 carcinoma	60	23	31	0	3 adenoma	0
059	50	19	13	0	1 adenoma	1 carcinoma	50	12	33	0	3 adenoma	0
441	49	13	7	0	2 adenoma	2 carcinoma	50	9	16	0	0	0
499	50	30	11	0	5 adenoma	2 carcinoma	50	24	35	0	0	0
530	50	11	13	0	2 adenoma	4 carcinoma	50	7	27	0	0	0
588	50	15	17	0	1 adenoma	4 carcinoma	50	10	27	0	0	0
939	84	36	17	0	0	1 carcinoma	76	30	36	0	0	1 carcinoma
970	76	23	14	0	2 adenoma	0	77	18	25	0	1 adenoma	0
089	50	27	12	0	2 adenoma	0	50	12	22	0	1 adenoma	1 unknown origin
911	50	31	2	0	0	1 carcinoma	50	9	13	0	0	0
332	100	52	40	0	3 adenoma	4 carcinoma	100	26	75	0	0	0
348	100	72	11	0	4 adenoma	1 carcinoma	100	20	50	0	0	0
678	120	60	24	9	2 adenoma	2 carcinoma	120	26	84	7	1 adenoma	0
277	100	11	7	0	1 adenoma	0	100	7	11	0	0	0
430	119	74	39	0	1 adenoma	5 carcinoma	118	52	77	3	0	2 carcinoma
687	65	45	22	0	0	1 carcinoma	65	17	53	0	0	0
TOTAL	1123	616	280	9	34	30	1218	312	637	10	9	4
RATE		54.9%	24.9%	0.8%	3.0%	2.7%		25.6%	52.2%	0.8%	0.7%	0.3%
RANGE		11-72%	4-40%	0-7.5%	0-10%	0-8%		7-48%	11-81.5%	0-5.8%	0-6%	0-2%

Lymphoreticular / Haemopoietic System

	Male				Female			
Study No.	Number Examined	Lymphoma	Leukaemia	Histiocytic sarcoma	Number Examined	Lymphoma	Leukaemia	Histiocytic sarcoma
623	50	0	0	1	50	1	0	3
009	60	2	0	2	60	2	0	0
059	50	3	0	1	50	1	0	2
441	50	1	0	0	50	2	0	0
499	50	0	2	3	50	1	0	2
530	50	0	0	1	50	0	0	2
588	50	4	0	2	50	0	0	2
939	82	5	4	3	77	0	0	3
970	76	4	0	1	77	1	0	1
089	50	0	3	2	50	0	0	3
911	50	1	1	4	50	1	0	3
332	100	0	1	0	99	0	0	2
348	100	0	2	3	100	3	2	0
678	120	3	1	4	120	2	0	3
277	100	0	6	0	100	0	0	0
430	119	1	1	0	119	3	0	2
687	65	0	3	0	65	0	0	4
TOTAL	1222	24	24	27	1217	17	2	32
RATE		2.0%	2.0%	2.2%		1.4%	0.2%	2.6%
RANGE		0-8%	0-6%	0-6%		0-4%	0-2%	0-6.2%

MAMMARY GLAND

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	45	0	0	0	50	0	29 fibroadenoma 2 adenoma	7 carcinoma
009	54	1	0	0	60	0	22 fibroadenoma	10 carcinoma
059	49	0	2 fibroadenoma	1 carcinoma	50	2	34 fibroadenoma 1 adenoma	10 carcinoma
441	49	0	3 fibroadenoma	0	50	6	27 fibroadenoma	4 carcinoma
499	46	0	4 fibroadenoma	0	50	4	28 fibroadenoma	5 carcinoma
530	44	0	1 fibroadenoma 1 adenoma	0	50	1	29 fibroadenoma 1 adenoma	3 carcinoma
588	46	0	0	0	50	0	36 fibroadenoma 1 adenoma	6 carcinoma
939	74	0	1 fibroadenoma	0	79	11	44 fibroadenoma	12 adenocarcinoma
970	75	6	1 adenoma	1 carcinoma	77	4	40 adenoma	3 carcinoma
089	50	0	1 fibroadenoma	0	50	0	26 fibroadenoma 2 adenoma	10 carcinoma
911	46	0	2 fibroadenoma	0	50	0	24 fibroadenoma	8 carcinoma
332	96	4	1 fibroadenoma	0	100	37	42 fibroadenoma 10 adenoma 1 fibroma 1 lipoma	17 carcinoma
348	81	0	1 fibroadenoma	1 carcinoma	100	4	44 fibroadenoma 2 adenoma 1 cystadenoma	23 carcinoma
678	120	0	1 fibroadenoma	0	120	11	54 fibroadenoma 7 adenoma 2 cystadenoma	12 carcinoma 1 carcinosarcoma
277	98	16	2 fibroadenoma	0	100	59	41 fibroadenoma 5 adenoma	5 carcinoma
430	107	1	2 fibroadenoma	1 adenocarcinoma	120	34	70 fibroadenoma 3 adenoma 2 fibroma	24 adenocarcinoma
687	53	0	2 fibroadenoma	0	65	15	37 fibroadenoma 2 adenoma 1 fibroma	12 adenocarcinoma 1 carcinosarcoma
TOTAL	1133	30	25	4	1220	260	671	173
RATE		2.6%	2.2%	0.4%		21.3%	55%	14.2%
RANGE		0-16.3%	0-8.7%	0-2%		0-59%	36.7-74%	3.4-23%

EXOCRINE PANCREAS

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	4	3 adenoma	0	49	0	0	1 carcinoma
009	59	0	0	0	59	0	1 adenoma	0
059	50	0	3 adenoma	0	50	0	0	0
441	48	1	0	0	50	0	0	0
499	49	0	0	0	50	2	1 adenoma	0
530	49	0	0	0	49	0	0	0
588	50	0	0	0	50	0	0	0
939	81	4	0	0	76	0	0	0
970	76	2	0	0	77	0	0	0
089	50	0	0	0	50	0	0	0
911	49	1	1 adenoma	0	48	1	1 adenoma	0
332	98	6	0	0	100	0	0	0
348	100	1	0	0	100	0	0	0
678	120	0	0	0	120	0	0	0
277	99	0	0	0	100	0	0	0
430	116	0	1 adenoma	0	117	0	0	1 carcinoma
687	62	1	0	0	65	0	0	0
TOTAL	1206	20	8	0	1210	3	3	2
RATE		1.7%	0.7%	0.0%		0.2%	0.2%	0.2%
RANGE		0-8%	0-6%	0.0%		0-4%	0-2.1%	0-2%

ENDOCRINE PANCREAS

	Male				Female			
Study No.	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	2	7 adenoma	0	49	0	1 adenoma	0
009	59	0	12 adenoma	1 carcinoma	59	0	1 adenoma	0
059	50	1	6 adenoma	0	50	1	1 adenoma	0
441	48	2	6 adenoma	3 carcinoma	50	1	1 adenoma	0
499	49	4	7 adenoma	1 carcinoma	50	0	0	0
530	49	0	2 adenoma	1 carcinoma	49	0	3 adenoma	0
588	50	0	2 adenoma	1 carcinoma	50	0	1 adenoma	0
939	81	6	2 adenoma	3 carcinoma	76	1	0	0
970	76	0	5 adenoma	0	77	0	1 adenoma	0
089	50	26	3 adenoma	0	50	10	0	0
911	49	2	2 adenoma	0	48	1	0	0
332	98	2	8 adenoma	0	99	0	1 adenoma	0
348	100	9	5 adenoma	0	99	2	0	0
678	120	4	3 adenoma	1 carcinoma	120	2	3 adenoma	0
277	99	0	6 adenoma	0	100	0	1 adenoma	0
430	116	8	2 adenoma	2 carcinoma	119	3	3 adenoma	0
687	62	7	2 adenoma	1 carcinoma	62	2	4 adenoma	0
TOTAL	1206	73	80	14	1207	23	21	0
RATE		6.1%	6.6%	1.2%		1.9%	1.7%	0.0%
RANGE		0-52%	1.7-20.3%	0-6.3%		0-20%	0-6.5%	0.0%

PITUITARY GLAND (ANTERIOR)

	Male				Female			
Study No.	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	4	28 adenoma	1 carcinoma	49	0	35 adenoma	7 carcinoma
009	60	0	30 adenoma	1 carcinoma	60	0	44 adenoma	6 carcinoma
059	50	5	33 adenoma	0	50	4	38 adenoma	1 carcinoma
441	50	3	28 adenoma	0	49	1	34 adenoma	1 carcinoma
499	49	12	31 adenoma	0	50	10	36 adenoma	1 carcinoma
530	50	0	23 adenoma	2 carcinoma	98	0	26 adenoma	5 carcinoma
588	50	0	22 adenoma	0	50	0	27 adenoma	6 carcinoma
939	82	25	32 adenoma	1 carcinoma	77	10	58 adenoma	3 carcinoma
970	76	8	35 adenoma	0	75	2	61 adenoma	0
089	50	8	21 adenoma	0	49	10	36 adenoma	3 carcinoma
911	49	12	19 adenoma	0	49	8	34 adenoma	2 carcinoma
332	100	27	43 adenoma	0	99	26	60 adenoma	3 carcinoma
348	98	10	29 adenoma	0	97	10	62 adenoma	6 carcinoma
678	120	23	44 adenoma	1 carcinoma	117	19	61 adenoma	2 carcinoma
277	99	19	36 adenoma	0	98	12	35 adenoma	0
430	119	29	29 adenoma	0	120	20	70 adenoma	5 adenocarcinoma
687	64	20	12 adenoma	1 carcinoma	64	14	39 adenoma	1 carcinoma
TOTAL	1216	205	495	7	1251	146	754	52
RATE		16.9%	40.7%	0.6%		11.7%	60.3%	4.2%
RANGE		0-31.3%	18.8-66%	0-4%		0-26.7%	26.5-81.3%	0-14.3%

SUBMANDIBULAR (SALIVARY GLAND)

Study Number	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	1	1 fibroma	0	50	1	0	0
009	59	0	0	0	60	0	0	0
059	50	0	0	0	49	0	0	0
441	48	0	0	0	48	0	0	0
499	49	1	0	0	50	0	0	0
530	49	0	0	0	49	0	0	0
588	50	0	0	0	50	0	0	0
939	73	0	1 adenoma	0	78	0	0	0
970	74	0	0	0	76	0	0	0
089	49	0	0	0	50	0	0	0
911	50	0	0	0	48	0	0	0
332	96	0	0	0	97	0	0	0
348	99	0	0	0	100	0	0	0
678	119	0	0	0	116	0	0	0
277	100	0	0	0	100	0	0	0
430	118	0	0	0	117	0	0	0
687	65	0	0	0	65	0	0	0
TOTAL	1195	2	2	0	1203	1	0	0
RATE		0.2%	0.2%	0.0%		0.1%	0.0%	0.0%
RANGE		0-2%	0-2%	0.0%		0-2%	0.0%	0.0%

SEMINAL VESICLE

Study No.	Male			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	2	0	0	0
009	60	0	0	0
059	49	0	0	0
441	50	1	0	0
499	5	0	0	0
530	2	0	0	0
588	49	0	0	0
939	82	0	0	0
970	76	0	0	0
089	50	0	0	0
911	49	0	1 adenoma	0
332	100	0	0	0
348	100	0	0	0
678	120	0	0	0
277	100	0	0	0
430	115	2	0	0
687	65	1	0	0
TOTAL	1074	4	1	0
RATE		0.4%	0.1%	0.0%
RANGE		0-2%	0-2%	0.0%

SKIN (EPITHELIUM)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	1	1 intracutaneous cornifying epithelioma	1sebaceous carcinoma	50	0	1intracutaneous cornifying epithelioma	1 sebaceous carcinoma
009	60	0	3 intracutaneous cornifying epithelioma 1 papilloma 1 basal cell adenoma	0	60	0	0	0
059	50	0	2 intracutaneous cornifying epithelioma 1 papilloma	0	50	0	0	0
441	50	2	8 intracutaneous cornifying epithelioma 1 basal cell adenoma	1 basal cell carcinoma	50	0	2 intracutaneous cornifying epithelioma	1 basal cell carcinoma 1 hibernosarcoma
499	49	1	3 keratoacanthoma 2 basal cell adenoma 2 papilloma	1 squamous cell carcinoma	49	0	0	0
530	50	5	2 intracutaneous cornifying epithelioma 1 papilloma	1 basal cell carcinoma	50	0	0	1 squamous cell carcinoma
558	50	4	3 intracutaneous cornifying epithelioma 1 papilloma 1 sebaceous adenoma	1 squamous cell carcinoma	50	3	1 papilloma	0
939	83	0	2 papilloma 2 keratoacanthoma	0	78	0	1 papilloma	0
970	75	0	10 basal cell adenoma 4 papilloma	0	77	0	1 basal cell adenoma	1 carcinoma
089	50	1	2 keratoacanthoma	1 squamous cell carcinoma	50	4	0	0
911	50	0	0	1 squamous cell carcinoma	50	0	0	0
332	100	2	5 keratoacanthoma 2 sebaceous adenoma 2 papilloma 1 basal cell adenoma	0	100	3	2 keratoacanthoma	1 basal cell carcinoma
348	100	2	3 keratoacanthoma 2 papilloma 1 sebaceous adenoma	1 squamous cell carcinoma 1 basal cell carcinoma	100	2	1 keratoacanthoma	0
678	120	0	6 keratoacanthoma 1 basal cell adenoma 1 sebaceous adenoma	1 basal cell carcinoma	120	0	1 keratoacanthoma	0
277	100	0	3 keratoacanthoma	0	100	0	2 keratoacanthoma	0
430	120	0	8 keratoacanthoma 2 papilloma 1 sebaceous adenoma	0 basal cell carcinoma 1 squamous cell carcinoma 1 sebaceous carcinoma	120	0	1 keratoacanthoma	1 basal cell carcinoma
687	65	3	7 keratoacanthoma 1 basal cell adenoma 1 sebaceous adenoma 1 papilloma	0	65	2	0	1 squamous cell carcinoma
TOTAL	1222	21	101	18	1219	14	13	8
RATE		1.7%	8.3%	1.5%		1.1%	1.1%	0.7%
RANGE		0-10%	0-19.7%	0-6.7%		0-8%	0-4%	0-2%

SKIN/SUBCUTIS (MESODERM)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	8 fibroma 2 dermal fibroma 1 lipoma	1 fibrosarcoma	50	0	1 fibroma	1 sarcoma
009	60	0	3 fibroma 1 lipoma	3 fibrosarcoma 1 fibrous histiocytoma	60	0	2 fibroma	0
059	50	0	10 fibroma 1 lipoma	2 fibrosarcoma	50	0	1 fibroma	0
441	50	0	14 fibroma 2 lipoma	2 fibrosarcoma 1 sarcoma	50	0	1 fibroma	1 fibrosarcoma
499	49	0	7 fibroma	1 fibrosarcoma 1 sarcoma	49	0	1 fibroma	0
530	50	0	10 fibroma 4 lipoma	5 fibrosarcoma	50	0	3 fibroma	1 sarcoma
558	50	0	7 fibroma	2 fibrosarcoma	50	0	0	3 fibrosarcoma
939	83	0	12 fibroma 2 lipoma	0	78	0	4 fibroma	1 fibrosarcoma
970	75	0	9 fibroma	2 sarcoma	77	0	3 fibroma	2 sarcoma
089	50	0	9 fibroma 2 lipoma	1 sarcoma	50	0	2 fibroma 1 lipoma	0
911	50	0	11 fibroma 1 fibrous histiocytoma 1 lipoma	3 fibrosarcoma 2 sarcoma	50	0	1 fibroma	0
332	100	0	9 fibroma 7 dermal fibroma 3 lipoma 1 fibrolipoma	4 fibrosarcoma	100	0	1 fibroma 1 lipoma 1 fibrolipoma	1 liposarcoma
348	100	0	9 fibroma 3 lipoma	4 sarcoma 2 fibrosarcoma	100	0	7 lipoma 4 fibroma	3 fibrosarcoma
678	120	0	9 fibroma 6 lipoma 6 dermal fibroma	5 fibrosarcoma	120	0	7 fibroma 1 dermal fibroma	3 fibrosarcoma 2 sarcoma
277	100	0	9 fibroma 4 dermal fibroma	1 fibrosarcoma 1 sarcoma	100	0	5 fibroma 1 lipoma	1 sarcoma
430	120	0	13 fibroma 5 lipoma 3 dermal fibroma 1 fibrolipoma	4 fibrosarcoma 3 liposarcoma 2 fibrous histiocytoma 2 sarcoma	120	0	5 fibroma 2 lipoma	2 fibrosarcoma
687	65	0	5 fibroma 4 dermal fibroma 1 myxoma 1 lipoma	3 fibrosarcoma	65	0	3 fibroma	0
TOTAL	1222	0	216	58	1219	0	58	21
RATE		0.0%	17.8%	4.7%		0.0%	4.8%	1.7%
RANGE		0.0%	6.7-32%	0-10%		0.0%	0-11%	0-6%

SPINAL CORD

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	0	0	50	0	0	0
009	60	0	0	0	60	0	0	0
059	50	0	0	0	50	0	0	0
441	50	0	0	0	50	0	0	0
499	50	0	0	0	50	0	0	0
530	0	0	0	0	0	0	0	0
588	49	0	0	0	50	0	0	0
939	82	0	0	0	78	0	0	0
970	75	0	0	0	77	0	0	0
089	50	0	0	1 astrocytoma	50	0	0	0
911	50	0	0	0	50	0	0	0
332	100	0	0	0	100	0	0	1 astrocytoma
348	100	0	0	0	100	0	0	0
678	120	0	0	1 astrocytoma	119	0	0	0
277	100	0	0	0	100	0	0	0
430	120	0	0	0	119	0	0	0
687	65	0	0	0	65	0	0	0
TOTAL	1171	0	0	2	1168	0	0	1
RATE		0.0%	0.0%	0.2%		0.0%	0.0%	0.1%
RANGE		0.0%	0.0%	0-2%		0.0%	0.0%	0-1%

STOMACH (NON-GLANDULAR)

Study No.	Male Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Female Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	1	0	0	48	0	0	0
009	60	3	0	0	60	4	0	0
059	49	0	0	0	50	2	0	0
441	49	9	0	0	50	6	0	0
499	49	3	0	0	50	2	0	0
530	50	6	0	0	50	8	0	0
588	48	5	0	1 squamous cell carcinoma	50	6	1 squamous cell papilloma	0
939	82	1	0	0	77	0	0	0
970	74	0	0	0	77	0	0	0
089	50	0	0	0	50	0	0	0
911	50	0	0	0	50	0	0	0
332	99	1	0	0	99	0	0	0
348	100	1	0	0	99	1	0	0
678	117	1	0	0	119	2	0	0
277	100	0	1 squamous cell papilloma	0	100	0	0	0
430	115	0	0	0	119	1	0	0
687	63	0	0	1 squamous cell carcinoma	65	0	0	0
TOTAL	1205	31	1	2	1213	32	1	0
RATE		2.6%	0.1%	0.2%		2.6%	0.1%	0.0%
RANGE		0-18.4%	0-1%	0-2.1%		0-16%	0-2%	0.0%

TESTIS / EPIDIDYMUS

Study No.	Interstitial Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Rete testis Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	3 adenoma	0	50	0	0	0
009	60	1	3 adenoma	0	60	0	0	0
059	50	2	4 adenoma	0	50	0	0	0
441	50	2	5 adenoma	0	50	0	0	0
499	50	2	3 adenoma	0	50	0	0	0
530	50	4	4 adenoma	0	50	0	0	0
588	49	2	3 adenoma	0	49	0	0	0
939	84	13	10 adenoma	0	84	0	0	0
970	75	0	4 adenoma 1 mesothelioma	0	75	0	0	0
089	50	6	5 adenoma	0	50	0	0	0
911	50	3	2 adenoma	0	50	0	0	0
332	100	12	5 adenoma 1 sertoli cell tumour	0	100	0	0	0
348	100	2	2 adenoma	0	100	0	0	0
678	120	1	2 adenoma	0	120	0	0	0
277	100	8	6 adenoma	1 carcinoma	100	0	0	0
430	119	6	6 adenoma	0	119	0	0	0
687	65	5	3 adenoma	0	65	0	0	0
TOTAL	1222	69	72	1	1222	0	0	0
RATE		5.6%	5.9%	0.1%		0.0%	0.0%	0.0%
RANGE		0-15.5%	1.7-11.9%	0-1%		0.0%	0.0%	0.0%

THYMUS

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	48	2	1 thymoma	0	48	3	0	0
009	56	4	0	0	59	14	0	0
059	49	0	0	0	50	5	0	0
441	44	1	0	0	50	6	0	0
499	48	0	0	0	50	0	1 hibernoma	1 carcinoma
530	48	0	0	0	46	0	0	0
588	47	0	0	0	49	6	0	0
939	77	0	2 thymoma	1 thymoma	74	0	0	0
970	70	0	0	1 carcinoma	74	0	2 thymoma	1 carcinoma
089	45	8	0	0	49	11	1 thymoma	1 thymoma
911	49	2	2 thymoma	0	48	10	1 thymoma	0
332	97	0	0	0	98	1	0	0
348	74	3	0	0	89	19	0	0
678	117	0	0	0	116	0	0	2 thymoma
277	94	10	1 thymoma	0	95	35	2 thymoma	0
430	107	0	0	2 thymoma	114	0	0	0
687	60	4	0	0	60	38	0	2 thymoma
TOTAL	1130	34	6	4	1169	148	7	7
RATE		3.0%	0.5%	0.4%		12.7%	0.6%	0.6%
RANGE		0-17.8%	0-4.1%	0-1.9%		0-63.3%	0-2.7%	3.3%

THYROID GLAND (FOLLICULAR CELL)

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	0	0	50	0	0	0
009	60	0	1 adenoma	0	60	0	0	0
059	47	0	0	0	48	1	1 adenoma	0
441	49	2	5 adenoma	0	50	0	1 adenoma	0
499	49	0	2 adenoma	0	50	0	0	0
530	50	0	1 adenoma	0	49	0	2 adenoma	0
588	49	0	3 adenoma	0	50	0	0	0
939	75	3	1 adenoma	1 carcinoma	77	0	2 adenoma	0
970	75	0	0	0	76	5	2 adenoma	0
089	50	1	0	0	49	0	1 adenoma	0
911	50	0	1 adenoma	0	50	1	0	0
332	94	4	1 adenoma	1 carcinoma	97	1	0	0
348	97	0	0	0	99	2	1 adenoma	0
678	117	1	1 adenoma	1 carcinoma	114	0	0	1 carcinoma
277	100	2	0	0	100	1	3 adenoma	0
430	118	3	1 adenoma	0	118	1	1 adenoma	2 carcinoma
687	61	2	0	0	65	0	0	0
TOTAL	1191	18	17	3	1202	12	14	3
RATE		1.5%	1.4%	0.3%		1.0%	1.2%	0.2%
RANGE		0-4.3%	0-10.2%	0-1.3%		0-6.6%	0-4.1%	0-1.7%

THYROID GLAND (C-CELL)

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	3	9 adenoma	0	50	10	8 adenoma	0
009	60	6	9 adenoma	1 carcinoma	60	1	6 adenoma	1 carcinoma
059	47	5	5 adenoma	1 carcinoma	48	9	3 adenoma	0
441	49	6	7 adenoma	0	50	4	5 adenoma	2 carcinoma
499	49	7	8 adenoma	1 carcinoma	50	6	5 adenoma	0
530	50	2	2 adenoma	1 carcinoma	49	1	3 adenoma	1 carcinoma
588	49	1	3 adenoma	1 carcinoma	50	3	3 adenoma	1 carcinoma
939	75	9	4 adenoma	1 carcinoma	77	14	6 adenoma	2 carcinoma
970	75	0	3 adenoma	0	76	0	1 adenoma	0
089	50	2	5 adenoma	0	49	4	6 adenoma	1 carcinoma
911	50	1	3 adenoma	0	50	4	4 adenoma	0
332	94	6	12 adenoma	0	97	11	11 adenoma	0
348	97	4	10 adenoma	1 carcinoma	99	3	4 adenoma	0
678	117	11	13 adenoma	0	114	8	7 adenoma	0
277	100	10	11 adenoma	0	100	9	9 adenoma	0
430	118	13	25 adenoma	1 carcinoma	118	14	10 adenoma	1 carcinoma
687	61	9	10 adenoma	0	65	18	7 adenoma	0
TOTAL	1191	95	129	8	1202	119	98	9
RATE		7.8%	10.8%	0.7%		9.9%	8.2%	0.7%
RANGE		0-14.3%	4-21.1%	0-2.1%		0-20%	1.3-16%	0-4%

TONGUE

Study Number.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	0	0	0	0	0	0	0	0
009	60	0	0	0	60	0	0	0
059	50	0	0	0	49	0	0	0
441	49	0	0	0	50	0	0	0
499	0	0	0	0	0	0	0	0
530	49	0	0	0	49	0	0	0
588	49	0	0	0	50	0	0	0
939	75	0	0	0	77	0	0	0
970	74	0	0	0	76	0	0	0
089	50	0	0	0	50	0	0	0
911	50	0	0	0	50	0	0	0
332	98	0	0	0	98	0	0	0
348	97	0	0	0	100	0	0	0
678	119	0	0	0	115	0	0	0
277	100	0	0	0	100	0	0	0
430	118	0	0	0	118	0	0	0
687	65	0	0	0	65	0	0	0
TOTAL	1103	0	0	0	1107	0	0	0
RATE		0.0%	0.0%	0.0%		0.0%	0.0%	0.0%
RANGE		0.0%	0.0%	0.0%		0.0%	0.0%	0.0%

UTERUS/CERVIX

Study No.	Endometrium				Smooth muscle				Stroma			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	49	0	3 polyp 1 adenoma	0	49	0	0	0	49	0	0	0
009	60	2	2 polyp	0	60	0	0	0	60	0	0	0
059	50	4	4 polyp	0	50	0	0	0	50	0	1 stromal tumour	1 sarcoma
441	50	0	2 polyp	0	50	0	0	0	50	0	0	0
499	50	0	3 polyp	0	50	0	0	0	50	0	0	0
530	50	0	1 polyp	0	50	0	0	0	50	0	0	0
588	50	0	3 polyp 1 papilloma	0	50	0	0	0	50	1	0	0
939	78	0	0	1adenocarcinoma	78	0	0	0	78	0	4 polyp	0
970	77	0	0	0	77	0	3 leiomyoma	0	77	0	0	0
089	50	0	1 polyp	0	50	0	0	0	50	0	0	0
911	50	0	0	0	50	0	0	0	50	0	2 polyp	0
332	100	0	0	0	100	0	1 leiomyoma	0	100	0	8 polyp	0
348	100	0	7 polyp	0	100	0	1 leiomyoma	0	100	0	0	0
678	120	2	1 adenoma	1 carcinoma	120	0	0	0	120	0	7 polyp	3 sarcoma
277	100	0	0	0	100	0	0	0	100	0	7 polyp	0
430	119	5	0	1adenocarcinoma	119	0	0	0	119	0	8 polyp 1 fibroma	1 schwannoma
687	65	17	0	0	65	0	0	0	65	0	4 polyp 1 fibroma	0
TOTAL	1218	30	29	3	1218	0	5	0	1218	1	43	5
RATE		2.5%	2.4%	0.2%		0.0%	0.4%	0.0%		0.1%	3.5%	0.4%
RANGE		0-26.2%	0-8.2%	0-1.3%		0.0%	0-3.9%	0.0%		0-2%	0-7.6%	0-2.5%

VASCULAR SYSTEM

Study No.	Male				Female			
	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia	Number Examined	Focal Hyperplasia	Benign Neoplasia	Malignant Neoplasia
623	50	0	1 haemangioma	0	50	0	0	0
009	60	0	0	1 haemangiosarcoma	60	0	0	0
059	50	0	2 haemangioma	0	50	0	0	0
441	50	0	0	2 haemangiosarcoma	50	0	0	0
499	50	0	2 haemangioma	3 haemangiosarcoma	50	0	0	0
530	50	0	0	1 haemangiosarcoma	50	0	3 haemangioma	0
588	50	0	0	0	50	0	0	0
939	99	0	0	4 haemangiosarcoma	100	0	0	0
970	76	0	1 haemangioma	0	77	0	0	0
089	50	0	0	0	50	0	0	0
911	50	0	0	1 haemangiosarcoma	50	0	0	0
332	100	0	1 haemangioma 1 lymphangioma (mesenteric node)	1 haemangiosarcoma	100	0	0	0
348	100	0	1 haemangioma	0	100	0	0	1 haemangiosarcoma
678	120	0	1 haemangioma	0	118	0	0	0
277	100	0	1 haemangioma	2 haemangiosarcoma	100	0	0	0
430	120	0	2 haemangioma	4 haemangiosarcoma 1 lymphangiosarcoma (mesenteric node)	120	0	0	0
687	65	0	1 haemangioma	1 haemangiosarcoma	65	0	0	0
TOTAL	1240	0	14	21	1240	0	3	1
RATE		0.0%	1.1%	1.7%		0.0%	0.2%	0.1%
RANGE		0.0%	0-4%	0-6%		0.0%	0-6%	0-1%

Appendix C: Mechanistic Studies of Liraglutide-Induced Rodent C-cell Tumors

Mechanistic Studies (Rodent Thyroid C-cell tumors)

- c-cell / Rodent C-cell findings: Assessment of human relevance
- 204370 / An immunohistochemical investigation of the GLP-1R in tissue from mice, rats, cynomolgus monkeys, and humans
- 20040515PR4 / Investigation of GLP-1 receptor mRNA expression in mouse, rat cynomolgus monkey, and human thyroid C-cells and in pancreatic islets studied by in situ hybridization
- 14725-006 / GLP-1 receptor expression in rat and human cell lines
- 205088 / Quantitative analysis of GLP-1 receptor levels on 2 rat (rMTC 6-23 and CA-77) and one human (TT) C-cell line
- 205218 / Western blot analysis of GLP-1R expression in rats and human C-cell lines
- 204415 / Real-time (TaqMan) RT-PCR quantification of glucagon-like peptide 1 receptor in C-cell lines
- 13737-025 / Thyroid C-cell line GLP-1 receptor functional data: cAMP accumulation and calcitonin release
- 14725-062 / Further human C-cell lines
- 205295 / Investigation of the mitogenic potential of liraglutide in rats and human C-cell lines
- 14718-007 / Calcitonin receptor binding studies
- 13736-092 / Liraglutide binding to rat gastrin (CCK2R) and bombesin (BB2R) receptors in AR42J cells b(4)
- 040301 / Assessment of beta and non-beta cell mass in pancreatic islets of cynomolgus monkeys treated with liraglutide for 52 weeks: ——— study 577863, NN study 200241
- 205106 / NNC 90-1170 single dose study in mice with subcutaneous administration
- 204268 / A 9 week exploratory study with reversibility in mice – Combined evaluation of the in life phase, hormone analysis, molecular analysis, pathology, and statistical analysis
- 204289 / 13 week toxicity study in mice with subcutaneous administration. Calcitonin determinations in mouse plasma
- 203281 / Effects on calcium homeostasis after a single subcutaneous administration to male rats in a fasted condition – Combined evaluation of the in life phase, hormone analysis, statistical analysis, and molecular analysis
- 203258 / The effects on calcium homeostasis after a single subcutaneous administration to male rats in a nonfasted condition – Combined evaluation of the in life phase, hormone analysis, and statistical analysis
- 203282 / Study on acute effects on calcium homeostasis related hormones after single dose subcutaneous administration in fasted and calcium treated rats
- 203317 / Effects on calcium homeostasis related parameters and thyroid volume fractions after up to six weeks daily subcutaneous administration followed by a 2 week reversibility period in male rats – Combined evaluation of in life phase, hormone analysis, and statistical analysis
- 204163 / Effects on plasma calcitonin, thyroid C-cell mass, and formation of antibodies after up to 483 days daily subcutaneous administration in young aged male rats – Combined evaluation of the in life phase including antibody analysis, calcitonin
- 204021 / Quantification of thyroid C-cells by digital image analysis on histological sections prepared from specimens from ——— studies 577863 (cynomolgus monkeys) and 455476 (Crl: CD rats) b(4)
- 203262 / Effects on calcium homeostasis related parameters after up to 87 weeks daily subcutaneous administration in male and female cynomolgus monkeys – combined evaluation of in life phase including thyroid histopathological evaluation

- 204402 / Study on the acute effects on calcitonin and toxicokinetics after single dose subcutaneous administration in fasted mice
- 205074 / In vivo study with administration of NNC 0113-0000-0000 by subcutaneous administration as bolus injections (once, twice, three times daily) or continuous infusion in female mice
- 205050 / NNC 0113-0000-0000 and liraglutide. Study on calcitonin and toxicokinetics after 3–days of subcutaneous administration in fasted male mice
- 205025 / Preliminary investigative study by subcutaneous administration (3 times a day) to CD-1 mice for 2 or 13 weeks – Combined evaluation of the in life phase including hormone analysis and C-cell pathology of the thyroid gland and molecular analysis
- 205205 / Investigatory toxicity study by osmotic minipump subcutaneous administration to CD-1 mice for 12 or 16 weeks
- 2005 001 / Modeling of exendin-4 concentration and effect on plasma calcitonin in mice
- 2005 005 / Modeling of pharmacokinetics and effect on plasma calcitonin after once daily dose administration of liraglutide
- 205121 / Characterization of the distribution of C-cells in thyroids from cynomolgus monkeys

BACKGROUND

Liraglutide, a palmitoylated recombinant human GLP-1 analog with a prolonged elimination half-life due to increased peptidase resistance of the highly protein bound drug, is being developed to treat type 2 diabetes under IND 61,040. In 2 year repeat subcutaneous dose carcinogen bioassays in rats and mice, liraglutide was a non-genotoxic carcinogen increasing the incidence of thyroid c-cell tumors in rats and mice and fibrosarcomas on the dorsal surface, the body surface for drug administration, in male mice. The sponsor performed mechanistic studies to evaluate the human relevance of thyroid c-cell tumor findings. Mechanistic studies were reviewed and presented to the Executive Carcinogenicity Committee at a meeting on 9 December 2008 for concurrence with the Division's recommendation that the lack of clinical relevance of liraglutide-induced thyroid c-cell tumors in rodents was not supported by mechanistic studies.

EFFECT OF LIRAGLUTIDE ON THYROID PARAMETERS IN HUMANS

Because liraglutide caused proliferative lesions of thyroid c-cells in rats and mice, the sponsor monitored thyroid parameters in clinical studies. Plasma calcitonin was measured in several clinical trials and a calcium-stimulated calcitonin test was performed on a subgroup of subjects in 2 long term studies (1573 and 1574). Thyroid biochemistry parameters were monitored in 8 clinical studies and thyroid structure, determined by ultrasonography, was monitored in 4 studies. A search of adverse events related to calcitonin or thyroid was performed across 38 completed and 4 ongoing clinical studies. The sponsor evaluated GLP-1R radioligand binding, second messenger coupling, and calcitonin secretion in a human thyroid C-cell line, TT cells.

Thyroid

In completed clinical trials, the rate of total, serious, and non-serious thyroid adverse events (Table 2-16, number of events divided by subject years of exposure multiplied by 1000) was higher in liraglutide treated subjects (event rates of 35.7, 4.5, and 31.2 for total, serious, and non-serious AEs, respectively) compared to non-liraglutide-treated subjects (placebo or comparator, event rates of 22.0, 0.9, and 21.1 for total, serious, and non-serious AEs, respectively).

Table 2–16 Summary of Treatment Emergent Thyroid AEs - All Completed Trials - Safety Analysis Set

	Liraglutide	Non-liraglutide
Safety Analysis Set	4211	2272
Total Exposure (yrs)	2241.4	1138.6
Number of Subjects with Serious Thyroid Adverse Events (events)	7 (10)	1 (1)
Number of Subjects with Non-serious Thyroid Adverse Events (events)	57 (70)	(24) 24
Total Number of Subjects with Thyroid Adverse Events (events)	61 (80)	24 (25)
Rate of Thyroid Serious Adverse Events (R)	4.5	0.9
Rate of Thyroid Non-serious Adverse Events (R)	31.2	21.1
Rate of All Thyroid Adverse Events (R)	35.7	22.0

R: Number of events divided by subject years of exposure multiplied by 1000

[N000 Module 2.5 P107]

Table 2-20 shows thyroid adverse events that occurred after at least 1 dose in clinical studies. Thyroid adverse events that increased in liraglutide-treated clinical trial subjects were goiter, hyperthyroidism, thyroid cyst, and thyroid disorder. The incidence of treatment-emergent benign and malignant thyroid neoplasms was higher in liraglutide-treated compared to non-liraglutide treated subjects. The incidence of papillary thyroid tumors were notably higher in liraglutide-treated subjects with the earliest onset occurring within 50 days of initiating treatment with 1.8 mg liraglutide + metformin + rosiglitazone (Table 2-23). Despite dose-related elevated plasma calcitonin levels in liraglutide-treated subjects, thyroid c-cell tumors were not noted in liraglutide-treated subjects.

Table 2–20 Treatment Emergent AEs - Thyroid (AE Onset Date after First Drug Date) - by SOC and Preferred Term - All Completed Trials - Safety Analysis Set

	Liraglutide				Non-Liraglutide			
	N	(%)	E	R	N	(%)	E	R
Safety Analysis Set	4211				2272			
Total Exposure (yrs)	2241.4				1138.6			
All Adverse Events related to Thyroid	46	1.1	62	27.7	19	0.8	19	16.7
Endocrine Disorders	19	0.5	24	10.7	7	0.3	7	6.1
Goitre	13	0.3	14	6.2	1	0.0	1	0.9
Hypothyroidism	3	0.1	3	1.3	4	0.2	4	3.5
Hyperthyroidism	2	0.0	2	0.9	0	0.0	0	0.0
Thyroid Cyst	2	0.0	2	0.9	0	0.0	0	0.0
Thyroid Disorder	2	0.0	2	0.9	0	0.0	0	0.0
Autoimmune Thyroiditis	1	0.0	1	0.4	2	0.1	2	1.8
Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)	19	0.5	22	9.8	4	0.2	4	3.5
Thyroid Neoplasm	15	0.4	16	7.1	4	0.2	4	3.5
Papillary Thyroid Cancer	4	0.1	4	1.8	0	0.0	0	0.0
Benign Neoplasm Of Thyroid Gland	1	0.0	1	0.4	0	0.0	0	0.0
Parathyroid Tumour Benign	1	0.0	1	0.4	0	0.0	0	0.0
Investigations	14	0.3	16	7.1	8	0.4	8	7.0
Blood Calcitonin Increased	10	0.2	11	4.9	6	0.3	6	5.3
Blood Thyroid Stimulating Hormone Incr	2	0.0	3	1.3	1	0.0	1	0.9
Blood Calcitonin Abnormal	1	0.0	1	0.4	0	0.0	0	0.0
Thyroxine Decreased	1	0.0	1	0.4	0	0.0	0	0.0
Blood Thyroid Stimulating Hormone Decr	0	0.0	0	0.0	1	0.0	1	0.9

N: Number of Subjects with adverse events
%: Proportion of subjects in analysis set having adverse events
E: Number of adverse events
R: Number of events divided by subject years of exposure multiplied by 1000

[N000 Module 2.5 P108]

Table 2–23 Treatment Emergent Adverse Events of Papillary Thyroid Cancer – All Completed Trials and Ongoing Trials

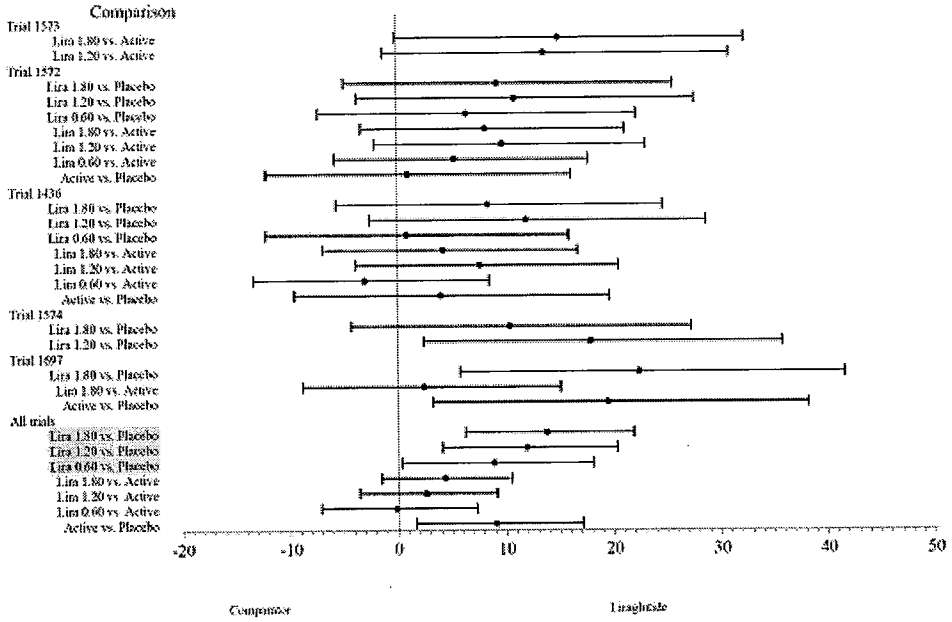
Trial	Subject ID	Age Yrs/Gender	Treatment	Preferred Term [MedDRA] (Relationship) (Outcome) (severity)	Duration of Therapy at Onset	Duration of Event
1334	16004	70/F	Liraglutide 0.6 mg	Papillary thyroid cancer (U) (R*) (mild)	99 days	NA
1573	261006	62/F	Liraglutide 1.2 mg	Thyroid disorder (P) (R) (moderate) Papillary thyroid cancer (P) (R) (moderate) Benign neoplasm of the thyroid gland (P) (R)	356 days	113 days
1436	506001	59/M	Liraglutide 1.8 mg+glimepiride	Papillary thyroid cancer Possible (liraglutide) Unlikely (glimepiride) (R) (moderate)	175 days	149 days
1574	326016	53/F	Liraglutide 1.8 mg+metformin+ rosiglitazone	Goitre (U) (R) (mild) Papillary thyroid cancer (U) (R) (moderate)	22 days 50 days	30 days 63 days
	326008	59/M	Metformin+rosiglitazone	Papillary thyroid cancer (U) (R) (moderate)	1 day	91 days

*Updated based on information received after end of trial (Appendix 7.4, Listing 5)
Gender: M=male and F=female
Relationship: P=evaluated as possibly or probably related by investigator, U=unlikely related
Outcome: R=recovered, NR=not recovered

[N000 Module 2.5 P115]

Calcitonin

A Forest plot of individual clinical trials and pooled results from week 26/28 of long term clinical trials showed significant, dose-dependent increased plasma calcitonin compared to placebo at all liraglutide doses, but no significant difference between liraglutide and active comparator at any dose.



Cross-reference: Appendix 7.3, Figure 84

Figure 3–9 Forest plot of Calcitonin Continous Analysis – Week 26/28 – All Long-term Trials - Safety analysis set

[N000 Module 2.5 P192]

At week 52, calcitonin was significantly higher than placebo at both 1.2 or 1.8 mg/day liraglutide, and calcitonin was significantly higher than active comparator at 1.8 mg/day (Figure 85 in Appendix 7.3 not found, but the report containing the appendix wasn't hyperlinked. Data from clinical trial 1573.)

A calcium stimulated calcitonin test was performed in a subset of subjects from long-term clinical studies 1573 (90 subjects) and 1574 (54 subjects). There were no significant differences in calcium-stimulated calcitonin secretion between comparator or liraglutide (1.2 or 1.8 mg/day) groups prior to initiating treatment or after 52 weeks of treatment.

GLP-1 Receptors in Human Thyroid C-cells

Autoradiography using ^{125}I-GLP-1(7–36)amide in thyroid tissue slices showed GLP-1Rs occurred in thyroid of 1 of 18 humans, compared to 60% of thyroids from mice and 100% of thyroids from rats (Table 3) (Körner M et al., . J Nucl Med(2007) 48: 736–743). Although specific thyroid cell types binding ^{125}I-GLP-1(7–36)amide were not identified, the receptor density in human thyroid was 60% of the receptor density in mouse thyroid, 52% of the receptor density in rat thyroid, and similar to the receptor density in human pancreas islets.

TABLE 3
GLP-1 Receptor (GLP-1 R) Expression in Lung and Thyroid Gland of Rat, Mouse, and Human; Comparison of Receptor Incidence and Density

Organ	GLP-1 R	Rat*	Mouse*	Human*
Lung	Incidence	3/3 (100)	6/6 (100)	11/28 (39)
	Density†	3,477 ± 1,539	1,677 ± 439	636 ± 164
Thyroid gland	Incidence	12/12 (100)	3/5 (60)	1/18 (6)
	Density†	2,289 ± 282	1,982 ± 470	1,193

*Values in parentheses are percentages.
†Mean ± SEM of receptor-positive cases (dpm/mg tissue).

TABLE 2
GLP-1 Receptor Density in Receptor-Positive Human Normal Tissues

Organ	Tissue compartment	GLP-1 receptor density*	
Central nervous system	Neurohypophysis	5,207 ± 472	(n = 6)
	Leptomeninges	1,453 ± 276	(n = 6)
Normal pancreas	Islets	1,322 ± 143	(n = 24)
	Acini	693 ± 49	(n = 9)
Chronic pancreatitis	Islets	960 ± 251	(n = 6)
	Acini	567 ± 112	(n = 6)
Duodenum	Brunner's glands	2,752 ± 522	(n = 5)
Ileum	Myenteric plexus	887 ± 285	(n = 6)
Colon	Myenteric plexus	788 ± 84	(n = 9)
Breast	Ducts and lobuli	519 ± 136	(n = 3)
Lung	Small blood vessels	636 ± 164	(n = 11)
Kidney	Large- and medium-sized arteries	674 ± 127	(n = 2)

*Mean ± SEM of receptor-positive cases (dpm/mg tissue).

[Körner M et al., . J Nucl Med 48: 736–743, 2007]

Specific GLP-1(1-7) binding was more frequently found in human medullary thyroid carcinomas (MTCs, 27.7% of samples) than in normal thyroid tissue (5.5% of samples). GLP-1Rs were identified in 5/18 of human thyroid medullary carcinoma tumors with an average receptor density comparable to that observed in the human thyroid sample positive for ^{125}I-GLP-1(7–36)amide binding.

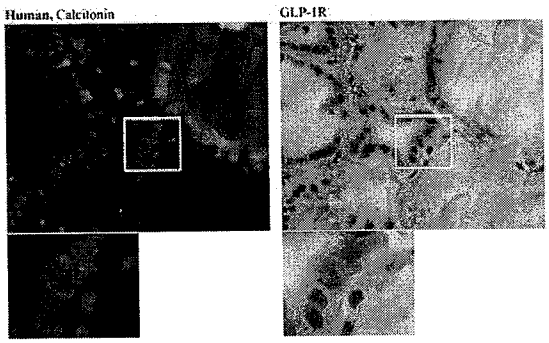
TABLE 1
GLP-1 Receptor Incidence and Density in Human Tumors

Tumor type	GLP-1 receptor incidence*	GLP-1 receptor density†
Endocrine tumors		
Pheochromocytomas	12/20 (60)	3,970 ± 1,002
Paragangliomas	5/18 (28)	1,353 ± 601
Medullary thyroid carcinomas	5/18 (28)	1,326 ± 264
Adrenal cortical adenomas	0/7 (0)	
Parathyroid carcinomas	0/4 (0)	
Pituitary adenomas	0/36 (0)	
Tumors of nervous system		
Meningiomas	7/20 (35)	989 ± 271
Astrocytomas	4/16 (25)	1,069 ± 398
Glioblastomas	2/21 (9)	790 ± 120
Ependymomas	1/6 (16)	1,075
Schwannomas	0/9 (0)	
Embryonic tumors		
Medulloblastomas	3/12 (25)	1,246 ± 726
Nephroblastomas	2/9 (22)	421 ± 21
Neuroblastomas	3/16 (18)	932 ± 518
Carcinomas		
Ovarian adenocarcinomas	2/12 (16)	686 ± 384
Prostate carcinomas	1/20 (5)	1,283
Breast carcinomas	0/22 (0)	
Colorectal adenocarcinomas	0/21 (0)	
Gastric adenocarcinomas	0/20 (0)	
Pancreatic adenocarcinomas	0/21 (0)	
Cholangiocellular carcinomas	0/17 (0)	
Hepatocellular carcinomas	0/16 (0)	
Non–small cell lung carcinomas	0/20 (0)	
Small cell lung carcinomas	0/6 (0)	
Renal cell carcinomas	0/20 (0)	
Non–Hodgkin's lymphomas	0/10 (0)	

*Values in parentheses are percentages.
†Mean ± SEM of receptor-positive cases (dpm/mg tissue).

[Körner M et al., . J Nucl Med 48: 736–743, 2007]

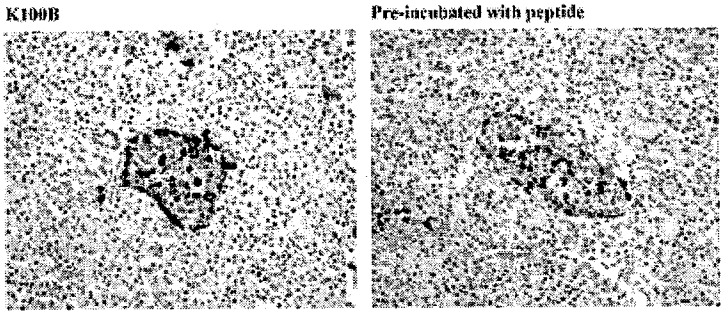
In study report 204370, colocalization of GLP-1Rs and calcitonin immunoreactivity in human thyroid tissue were equivocal for GLP-1R because the specificity of the rabbit anti-human GLP-1R antibody, K100B, was not adequately demonstrated (staining only partially blocked by preabsorption with the antigenic peptide, antibody stains pancreatic islets in GLP-1R knockout mice) and staining was weak and not always colocalized with calcitonin (see Figure below).



[N000 4.2.3.7.3 P20]

K100B strongly stained islets from pancreatic tissue, but preabsorption of K100B with the antigenic peptide only partially blocked staining (Figure 12, below).

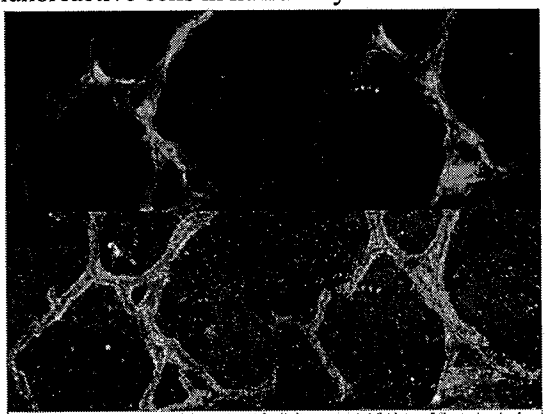
Figure 12 Pancreas serial sectioned, human



[N000 4.2.3.7.3 P30]

In situ hybridization of species specific ^{35}S-labeled riboprobes to GLP-1R mRNA was evaluated in paraffin-embedded thyroid tissue sections from humans (study 20040515PR4). Thyroid c-cells were identified by indirect fluorescent microscopy after staining with an Alexa488-coupled anti-calcitonin antibody. In situ hybridization to pancreatic islets served as a positive control for GLP-1R probes and hybridization of a ^{35}S-labeled riboprobes to calcitonin served as a control for mRNA quality in thyroid tissue. A ^{35}S-labeled probe to cyclophilin, a low to medium abundance transcript, served as a addition control for mRNA quality in samples of thyroid and pancreas.

GLP-1R mRNA was not detectable in thyroid c-cells identified by anti-calcitonin antibody staining and Figure 6 shows colocalization of GLP-1R mRNA and calcitonin was not compelling (Figure 6). Anti-sense human GLP-1R probes labeled human pancreatic islet cells and an antisense calcitonin probe labeled calcitonin immunoreactive cells in human thyroid tissue sections (data not shown)



Top row: calcitonin immunoreactivity in human thyroid C-cells; bottom row: dark field view of silver grains after 5 weeks exposure of emulsion. Left column: in-situ hybridisation with antisense riboprobe to hGLP-1R mRNA; right column: in-situ hybridisation with sense riboprobe to hGLP-1R mRNA.

Figure 6 Human thyroid GLP-1R mRNA co-localisation with C-cell calcitonin

[N000 4.2.3.7.3 P20]

GLP-1 Receptors in Human Thyroid C-cell Lines

The human thyroid C-cell line, TT, was devoid of functional GLP-1Rs coupled to calcitonin secretion, but cAMP-coupled receptor pathways may be perturbed in this cell line. In TT cells, forskolin, a direct activator of cAMP, increased calcitonin secretion, but pentagastrin, a potent human and rat calcitonin secretagogue, did not. Furthermore, known TT cell mitogens pentagastrin and epidermal growth factor did not stimulate TT cell mitosis. More detailed reviews of studies characterizing GLP-1Rs

in human TT cells are included in reviews of studies characterizing GLP-1R signaling in rat c-cell lines in Section 2.6.6.8 because rat and human cell lines experiments were included in the same reports.

Western blotting of TT cell proteins after separation by SDS-PAGE using the anti-GLP-1R polyclonal antibody K102B did not identify the GLP-1R protein, but the results are not valid because the specificity of the K102B antibody was not adequately demonstrated (study report 205218). TT cell GLP-1R transcript levels were very low to undetectable by real-time PCR with estimated transcript levels of 1 GLP-1R transcript / 1000 beta actin transcripts (report 204415).

Although the sponsor claims specific binding of radiolabeled, but not fluorescent GLP-1(7-37) was demonstrated in human TT cells, specific ligand binding wasn't demonstrated in either study (study report 1425-006 and 205088 using [^{125}I]GLP-1(7-37) or GLP-1(7-36)-Lys(6-FAM), respectively).

GLP-1R agonists liraglutide or exenatide didn't stimulate intracellular cAMP accumulation or calcitonin secretion from TT cells, but the positive control forskolin did. In vivo in humans, pentagastrin stimulates CCK_2 receptor mediated calcitonin release, but pentagastrin had no effect on cAMP accumulation or calcitonin secretion from TT cells (study report 13737-025). Micromolar concentrations of forskolin did not elicit calcitonin secretion from 3 other human thyroid c-cell lines: SINJ, SHER-1 or MTC-SK cells (report 14725-062).

Liraglutide, GLP-1(7-37) or exenatide were not mitogenic in a [^{3}H]thymidine DNA incorporation assay using human TT cells, but these cells didn't respond to the positive control mitogens gastrin or epidermal growth factor (study report 205295).

Liraglutide doesn't bind to human calcitonin receptors. In a scintillation proximity format assay, up to 5 μM liraglutide or GLP-1(7-37) did not inhibit binding of 53 pM [^{125}I]calcitonin (salmon) to BHK cell expressing a recombinant human calcitonin receptor (report 14718-007).

In conclusion, specific GLP-1(1-37)amide binding occur in normal thyroid in at least a subgroup of people, but GLP-1Rs have not been localize to specific cells in thyroid. When it occurs, the density of receptor binding sites in thyroid was similar to pancreas. GLP-1Rs are more commonly found in human MTCs than in normal thyroid. In clinical studies, liraglutide dose-dependently increased plasma calcitonin compared to placebo at 0.6, 1.2, and 1.8 mg/day at 26 or 28 months of treatment. In clinical studies, the incidence of treatment-emergent benign and malignant thyroid neoplasms was higher in liraglutide-treated compared to non-liraglutide treated subjects.

MECHANISTIC STUDIES: OVERALL SUMMARY

MECHANISTIC STUDIES OF LIRAGLUTIDE-INDUCED PROLIFERATIVE THYROID C-CELL LESIONS IN RATS AND MICE

To evaluate the human relevance of liraglutide-induced thyroid c-cell tumors, the sponsor performed mechanistic studies to support their proposed mode of action that:

6. Circulating liraglutide binds to and activates GLP-1Rs on thyroid C-cells.
7. GLP-1R activation on C-cells induces calcitonin release.
8. Continued calcitonin release leads to increased calcitonin synthesis.
9. Persistent stimulation of calcitonin secretion and synthesis in C-cells leads to C-cell hyperplasia in rodents.
10. Long-term C-cell hyperplasia may lead to C-cell neoplasia in rodents.

A schematic of the sponsor's hypothetical mode of action is shown below.

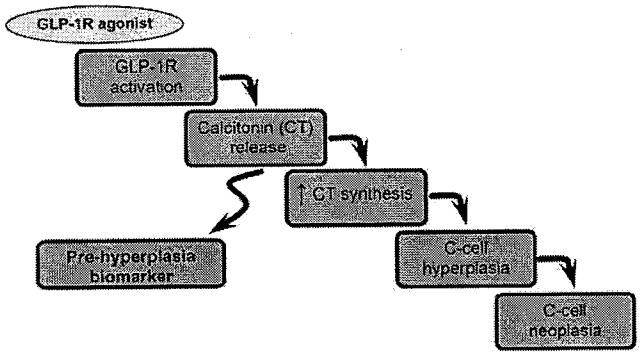


Figure 2 Key events in the process leading to rodent C-cell proliferation after long-term treatment with GLP-1 receptor (GLP-1R) agonists

[N000 4.2.3.7.3 Assessment Document P15]

The key events are 1) persistent liraglutide-induced GLP-1R-mediated calcitonin release from thyroid C-cells results in c-cell hyperplasia and 2) persistent hyperplasia progresses to adenomas, then carcinomas. The sponsor proposed that GLP-1R agonist-induced calcitonin secretion from c-cells is more robust in rodents compared to primates, therefore this mode of action is relevant to liraglutide induced C-cell tumors in rats and mice, but not humans.

Rats and mice have different susceptibilities to naturally occurring and xenobiotic-induced thyroid c-cell tumors. In rats, plasma calcitonin, diffuse c-cell hyperplasia (considered a physiologic response), focal c-cell hyperplasia (considered a preneoplastic lesion), and c-cell adenomas increase with age. In Sprague Dawley rats, thyroid c-cell adenomas are common in control groups of 2 year studies (incidence > 1%), but c-cell carcinomas are not (incidence < 1%). In mice, focal c-cell hyperplasia, adenomas, and carcinomas are rare in control groups of 2 year studies (incidence < 1%). In rats, proliferative c-cell lesions progress from diffuse hyperplasia to focal hyperplasia to adenomas, but in mice, when adenomas occur, they are rarely preceded by focal c-cell hyperplasia. Seven marketed drugs with rat thyroid c-cell tumor findings in their label were identified (including exenatide), but none of them caused c-cell tumors in mice and a mechanism for drug-induced c-cell tumors wasn't established for any of them (see Overall Conclusions and Recommendations section).

The sponsor used rat and human C-cell lines to characterize species differences in GLP-1R agonist binding, signal transduction, coupling to calcitonin secretion, GLP-1R agonist-induced regulation of calcitonin and GLP-1R transcription, or ligand-induced mitogenesis. However, the behavior of the human TT cells, a thyroid C-cell line, did not agree with previously published studies with respect to known mitogens or known calcitonin secretagogues, therefore any differences in GLP-1R agonist effects in rat and human cell lines are not proof of species differences occurring in vivo.

Since rats and mice differ with respect to their susceptibility to drug-induced thyroid c-cell tumors and the incidence and course of development of spontaneous c-cell tumors, mechanistic studies addressing the mode of action of liraglutide induced proliferative C-cell lesions were considered separately.

Rats

Thyroid c-cell GLP-1 receptor in rats

There is no direct evidence of rat thyroid c-cell GLP-1Rs coupled to calcitonin secretion. Rat c-cell GLP-1Rs are inferred from autoradiography of rat thyroid tissue using radiolabeled GLP-1, in vitro pharmacology studies of GLP-1R agonist binding and adenylyl cyclase activation in rat c-cell lines, GLP-1R mRNA in rat c-cell lines, and GLP-1 induced calcitonin secretion from perfused rat thyroid and rat c-cell lines.

Published studies suggest GLP-1Rs occur on rat thyroid c-cells, and studies with perfused rat thyroid and rat c-cell lines suggest the receptor mediates calcium-dependent calcitonin secretion.

Autoradiography of thyroid tissue slices labeled with ^{125}I-GLP-1(7–36)amide showed detectable GLP-1 binding sites, but binding wasn't attributed to a specific cell type (Korner et al. J Nucl Med 48: 736–743, 2007). GLP-1Rs were demonstrated in rat c-cell lines CA77 (Lamari et al, FEBS Lett. 393(2-3): 248 – 52, Crespel et al, Endocrin 137: 3674 – 80) and MTC 6-23 (Vertongen et al, Endocrin 135: 1537 – 42). In CA77 cells, GLP-1R mRNA was detected by RT-PCR amplification using transcript specific primers and by Northern blot. GLP-1Rs in CA77 cells were coupled to adenylyl cyclase activation via Gs, calcitonin secretion (up to 52% increase over baseline), and increased calcitonin mRNA (2.9 fold). GLP-1R expression in MTC 6-23 cells were demonstrated by radioligand binding, the presence of the receptor transcript by PCR amplification using receptor specific probes, and GLP-1 (7-36)amide activation of adenylyl cyclase.

An immunohistochemical study of GLP-1R in rat thyroid tissue sections stained with anti-calcitonin antibodies to identify c-cells did not confirm because the specificity of the rabbit polyclonal anti-human GLP-1R antibody, K102B, wasn't demonstrated (study 204370). GLP-1R specificity of K102B was not adequately demonstrated because; 1) K102B staining wasn't blocked in the presence of the peptide antigen used to generate the antibody and 2) Western blot analysis of protein from c-cell lines did not demonstrate GLP-1R specific staining (study 205218). Furthermore, results from Western blots of SDS-PAGE electrophoresed proteins from rat c-cell lines CA77 and MTC 6-23 and the human TT c-cell using K102B were equivocal because stained proteins were unlikely to be GLP-1Rs (study 205218).

An in situ hybridization study of GLP-1R mRNA in tissue sections from rats showed GLP-1R transcript levels were low to undetectable in thyroid, but much higher in pancreas, a positive control (study 20040515PR4).

GLP-1Rs were demonstrated in rat thyroid c-cell lines CA77 and MTC 6-23 by ^{125}I-GLP1(7-37) radioligand binding (study 14725-006), GLP-1(7-36)-Lys(6-FAM) fluorescent ligand binding (study205088), PCR amplification of the receptor transcript, and GLP-1R agonist induced cAMP accumulation (study 13737-025). GLP-1(7-37) was 48 fold more potent than liraglutide at stimulating cAMP accumulation in MTC 6-23 cells. The presence of GLP-1Rs in rat c-cell lines doesn't confirm the presence of the receptor in thyroid c-cells in vivo.

C-cell GLP-1 receptor activation linked to calcitonin release

There is no direct evidence that rat thyroid calcitonin secretion is mediated by a c-cell GLP-1R. In subchronic and chronic repeat dose studies of liraglutide in male Sprague Dawley rats, the magnitude of any effect was small, typically < 2 fold, and transient because it didn't persist after a few months of treatment. Although GLP-1R agonist appear to increase it, plasma calcitonin levels probably remain within a normal physiologic range and elicit a counter-regulatory hypocalcemic response.

The best evidence for GLP-1 mediated calcitonin release in rats comes from a published study by Crespel (Endocrinol 137(9): 3674 – 3680). Perfusion of rat thyroid glands with 1 or 10 nM GLP-1 in the presence of low calcium (1 mM) or high calcium (3 mM) showed GLP-1 induced calcitonin secretion was calcium dependent (Figure 5). However, it should be noted that 1 mM calcium is a subphysiologic concentration (4 mg/dL) whereas 3 mM is within a normal physiologic range (12 mg/dL). Persistent calcitonin secretion in the presence of GLP-1 probably doesn't reflect normal physiology because the major counter-regulatory response, decreased serum calcium (due to inhibition of osteoclast-mediated resorption), can't occur.

[Crespel et al. Endocrinol 137(9): 3674 – 3680)]

This study also demonstrated GLP-1 elicited calcitonin release from CA-77 cells, a rat c-cell line, but calcitonin secretion from MTC 6-23 cells was calcium independent (Scherub et al Horm Met Res [Suppl] 21: 18 – 21). Differences in calcium dependence of GLP-1 elicited calcitonin release from perfused thyroid and MTC 6-23 cells indicates cell lines may not accurately reflect regulation of calcitonin secretion from c-cells in vivo.

GLP-1R agonists induce calcitonin secretion from cultured MTC 6-23 cells with the rank order potency expected for GLP-1R mediation: exenatide (EC_{50} 55 pM) > GLP-1 (1-37) (EC_{50} 80 pM)>> liraglutide (EC_{50} 5,300 pM). Calcium dose-dependently stimulated calcitonin release from MTC 6-23 cells and liraglutide enhanced calcium-stimulated calcitonin secretion. Pentagastrin, a potent calcitonin secretagogue in humans and rats, had no effect on calcitonin secretion from rat MTC 6-23 cells suggesting that receptor-coupled calcitonin secretion in the cell line was different from thyroid c-cells in vivo.

In young rats (~ 2 months old at the start of treatment), single and repeat dosing with liraglutide for up to 6 weeks increased plasma calcitonin, but the effects didn't persist in chronically treated rats. Liraglutide-induced increased calcitonin provokes a counter-regulatory response of decreased plasma calcium and subsequently, increased PTH. The effect of subcutaneously administered liraglutide on plasma calcitonin in rats was determined after single doses and repeat dosing up to 69 weeks.

A single dose study of subcutaneously injected 0 (vehicle) or 0.75 mg/kg liraglutide in male Sprague Dawley rats with monitoring plasma calcium parameters for up to 6 hours after dosing showed plasma calcitonin was modestly, transiently, but significantly increased compared to concurrent controls 0.5 and 1 hour after dosing and PTH levels increased 6 hours post-dose (study 203281). The transient increase in calcitonin was countered by decreased blood calcium, increased PTH, and increased excretion of calcium in urine (not monitored in this study). In calcium loaded rats (intraperitoneal injection of 1 mM/kg calcium) treated with 0 or 0.75 mg/kg liraglutide, calcitonin levels peaked within 15 minutes of dosing with higher levels in the liraglutide group (study 203282). The magnitude of increased plasma calcitonin in response to calcium loading was > 10 fold greater than any increase due to liraglutide. Plasma calcitonin levels were similar or below control group levels from 0.5 – 6 hours after dosing. Within 24 hours of a single s.c. injection of 0 or 0.75 mg/kg liraglutide to male Sprague Dawley rats, liraglutide increased urine volume and calcium excretion, but without significantly increasing plasma calcitonin. Decreased plasma calcium was considered an effect of increased calcium excretion, and increased PTH was a counter-regulatory response to decreased plasma calcium.

A 6 week study of 0 or 0.75 mg/kg liraglutide injected s.c. once a day to male Sprague Dawley rats included a 4 week interim sacrifice group, a 2 week recovery group. On day 45, fasting treated rats were calcium loaded to determine its effect on any liraglutide-induced changes in plasma PTH and calcitonin. Calcium loading vehicle or liraglutide treated rats on day 45 markedly reduced plasma PTH and increased calcitonin with return to baseline levels within 24 hours after calcium loading. Calcitonin levels in the liraglutide-treated group trended higher than concurrent controls in non-fasted rats sampled in week 4 and in fasted rats sampled in week 5. After a 2 week recovery period, plasma calcitonin levels in rats treated with liraglutide for 6 weeks trended lower than concurrent controls.

In a chronic repeat dose study of liraglutide in young and old male Sprague Dawley rats, any liraglutide-related increase in plasma calcitonin was transient and occurred early in treatment. In a 69 week repeat dose study of 0 (vehicle), 0.025, 0.25, or 0.75 mg/kg/day liraglutide in young male Sprague Dawley rats (2 months old) treated for 7, 10, 13, or 16 months or aged rats (8 months old) treated for 1, 4, 7, or 10 months, calcitonin levels were > 1.3 fold higher than concurrent controls prior to and after dosing at 0.25 and 0.75 mg/kg/day on day 28 in young rats, and at 0.75 mg/kg/day in aged rats. Calcitonin levels > 1.3 fold higher than concurrent controls occurred sporadically at all doses in aged rats, but the increase was small (at or near 1.3) and these increases were considered incidental because they weren't related to dose, duration of therapy, or time of drug administration. Calcium levels were unaffected by liraglutide treatment.

GLP-1 receptor agonist-induced calcitonin release increases calcitonin synthesis

In normal rats, a single dose of liraglutide decreased thyroid calcitonin peptide and mRNA levels, but in calcium loaded rats, it increased both. Repeat dosing up to 4 weeks did not significantly increase thyroid calcitonin transcript levels.

A single subcutaneous injection of 0.75 mg/kg liraglutide decreased thyroid calcitonin and calcitonin transcript levels in fasted rats, but in calcium loaded rats, liraglutide increased thyroid calcitonin and calcitonin transcript levels. Thyroid calcitonin and calcitonin transcript levels were determined 6 hours after a single subcutaneous injection of 0 (vehicle) or 0.75 mg/kg liraglutide to male Sprague Dawley rats (study 203281) or calcium loaded rats (single intraperitoneal injection of 1 mM/kg calcium). Calcium loading reduced thyroid calcitonin up to 2.2 fold up to 6 hours in vehicle treated controls, but in the liraglutide group, calcitonin levels were up to 4.7 fold higher than concurrent controls 6 hours after calcium loading. In fasted rats (without calcium loading), liraglutide decreased thyroid calcitonin up to 2.7 fold up to 6 hours compared to controls. Calcium loading liraglutide treated rats resulted in increased thyroid calcitonin up to 3.8 fold up to 6 hours after dosing indicating concurrent liraglutide treatment and calcium loading increases calcitonin synthesis, whereas liraglutide treatment alone or calcium loading alone do not. In fasted rats, liraglutide decreased thyroid calcitonin mRNA up to 3.5 fold compared to controls, for up to 6 hours after dosing whereas calcium loading increased calcitonin mRNA levels in liraglutide treated rats.

After 4 weeks of dosing male Sprague Dawley rats with 0 or 0.75 mg/kg/day liraglutide (s.c. injections once a day), there were no treatment-related differences in relative thyroid calcitonin mRNA levels (study 203317).

Persistent c-cell stimulation (persistent elevated plasma calcitonin) leads to c-cell hyperplasia

There was no compelling evidence of liraglutide-induced diffuse thyroid c-cell hyperplasia, an expected physiologic response to increased calcitonin demand, but liraglutide increased the incidence of age-dependent focal c-cell hyperplasia, a preneoplastic lesion. There was no evidence of diffuse c-cell hyperplasia preceding focal hyperplasia in liraglutide-treated rats. Liraglutide appears to be a tumor promoter in rats because liraglutide-induced focal c-cell hyperplasia was age-dependent while liraglutide-induced c-cell tumors were treatment-duration dependent.

Treatment with up to 1 mg/kg/day liraglutide subcutaneously injected once a day in male and female Sprague Dawley rats for up to 26 weeks, did not increase the incidence of focal thyroid c-cell hyperplasia or c-cell adenoma. In a 2 year repeat dose carcinogenicity study of 0, 0.025, 0.25, or 0.75 mg/kg/day liraglutide, an increased incidence and severity of focal thyroid c-cell hyperplasia was dose-related at $\geq$ 0.25 mg/kg/day in males and females. Retrospective quantitative analysis of thyroid c-cells in rats from the 26 week chronic rat toxicity and the 104 week carcinogenicity study did not find any evidence of liraglutide-induced diffuse c-cell proliferation or any effect on the ratio of thyroid c-cells to follicular cells in the high dose groups (1 mg/kg/day liraglutide in the 26 week study and 0.75 mg/kg/day in the 104 week study).

In a repeat dose study of 0 or 0.75 mg/kg/day liraglutide administered to male Sprague Dawley rats for up to 6 weeks with BrdU administered within 48 hours of the terminal sacrifice to label proliferating cells, group mean absolute and relative thyroid weight in the liraglutide treated group was significantly lower than concurrent controls. However, quantitative analysis of c-cells (immunoreactive with anti-calcitonin antibody) and BrdU labeled c-cells showed despite differences in thyroid weight, there were no treatment-related differences in follicular cell volume, c-cell volume, or volume of proliferating c-cells. Elevated plasma calcium increases calcitonin secretion from thyroid c-cells in rats and mice, but elevated calcium doesn't necessarily result in c-cell hyperplasia. Hypercalcemia induced by hypervitaminosis D3 in rats (25,000 IU/day D3 concurrently administered with or without $CaCl_2$) did not cause c-cell hyperplasia (Fernández-Santos et al, Histol Histopathol. (2001) 16(2):407-14).

To determine the time course and characteristics of liraglutide-induced c-cell hyperplasia, the sponsor carried out single and repeat subcutaneous dose studies of up to 69 weeks with monitoring of calcium parameters including plasma calcium, calcitonin, and PTH, and quantitative and qualitative thyroid microscopic pathology. In a repeat dose study of 0, 0.075, 0.25, or 0.75 mg/kg/day liraglutide injected once a day in young (2 months old) or old (8 months old) male Sprague Dawley rats for up to 69 weeks with sacrifices occurring after 30, 43, 56, and 69 weeks for young rats and 4, 17, 30, or 43 week for aged rats (study 204163), focal thyroid c-cell hyperplasia first occurred in the 0.75 mg/kg/day group after 30 weeks of dosing in young rats and after just 4 weeks of dosing in aged rats. The age of onset, 9 months, was the same in both young and aged rats. C-cell adenomas first occurred in the 0.75 mg/kg/day group after 30 weeks of dosing in young or aged rats (9 months at age of onset in young rats, 15 month age of onset in aged rats), so the duration of treatment was the same, 30 weeks. In young rats, both focal c-cell hyperplasia and adenoma occurred after 30 weeks of treatment. Therefore, liraglutide-induced focal c-cell hyperplasia appears to age-dependent, but liraglutide-induced c-cell adenomas are treatment-duration dependent. Although c-cell carcinomas occurred in a 104 week rat carcinogen bioassay, c-cell carcinomas didn't occur in "young rats" treated for up to 69 weeks or "aged rats" treated for up to 43 weeks in repeat dose mechanistic studies using the same doses.

Persistent liraglutide-induced c-cell hyperplasia progresses to c-cell neoplasms

Progression of liraglutide-induced focal thyroid c-cell hyperplasia to adenoma was treatment-duration dependent, but it occurred in the absence of any evidence of persistent elevated plasma calcitonin over and above the age-related increase that normally occurs in rats. Repeat dose mechanistic studies of

subcutaneously administered liraglutide up to 69 weeks in young male Sprague Dawley rats and up to 43 weeks in aged rats showed liraglutide-induced focal thyroid c-cell hyperplasia was age dependent. In a 2 year repeat subcutaneous dose carcinogenicity study of 0.03, 0.2, 1, or 3 mg/kg/day liraglutide in Sprague Dawley rats, a strain not susceptible to thyroid c-cell tumors, the NOAEL for focal c-cell hyperplasia was < 0.075 mg/kg/day liraglutide in males and 0.075 mg/kg/day in females with minimal to marked c-cell hyperplasia occurring at the lowest observed effect level (LOEL) in both sexes (0.075 mg/kg/day in males and 0.25 mg/kg/day in females). C-cell adenomas occurred at ≥ 0.25 mg/kg/day in males and at ≥ 0.075 mg/kg/day in females. The LOEL for c-cell adenomas in females was lower than the LOEL for hyperplasia. The incidence of c-cell carcinomas exceeded the concurrent and historical control range occurred at ≥ 0.075 mg/kg/day in males and at ≥ 0.25 mg/kg/day in females. In the 2 year carcinogenicity study, the incidence of combined c-cell tumors (adenoma / carcinoma) exceeded the incidence of focal hyperplasia at ≥ 0.25 mg/kg/day in males and at 0.75 mg/kg/day in females. Although a prolonged period of diffuse and nodular c-cell hyperplasia and elevated serum calcitonin typically precedes the development of c-cell tumors in both humans and rats, that didn't occur in the mechanistic studies of liraglutide induced c-cell tumors in rats.

Mice

Thyroid c-cell GLP-1 receptor in mice

Immunohistochemical and in situ hybridization studies of GLP-1Rs in mouse thyroid did not provide sufficient evidence of GLP-1Rs on c-cells.

A published autoradiographic ligand binding study of [^{125}I]GLP-1(7-36) in thyroid tissue sections from mice showed mice are heterogeneous with specific tissue binding occurring in thyroid from 3/6 mice (Korner M et al, J Nucl Med(2007) 48: 736-743). Mouse thyroid cell type(s) labeled by [^{125}I]GLP-1(7-36) were not identified.

An immunohistochemical colocalization study using mouse thyroid tissue slices was equivocal for colocalization of GLP-1R and calcitonin immunoreactivities on the same cells because GLP-1R immunoreactivity was weak and the specificity of the anti-GLP-1R antibody was not demonstrated. The specificity of K100B, a polyclonal rabbit anti-human GLP-1R antibody, was not adequately demonstrated because; 1) the antibody stained pancreas from GLP-1R knockout mice and 2) immunohistochemical staining in the presence of the peptide antigen used to generate the antibody did not block staining.

An in situ hybridization study of GLP-1R mRNA in tissue sections from mice was equivocal with low to undetectable levels of GLP-1R transcript in thyroid, but much higher levels in pancreas, a positive control.

C-cell GLP-1 receptor activation linked to calcitonin release

There were no in vitro studies in mouse thyroid c-cells or mouse c-cell lines linking GLP-1R activation to calcitonin release. There is no direct evidence of liraglutide induced, thyroid c-cell GLP-1R mediated calcitonin release in mice, but GLP-1R agonists liraglutide and exenatide increase plasma calcitonin and thyroid calcitonin mRNA in mice prior to inducing focal c-cell hyperplasia. The magnitude of any GLP-1R agonist elicited increase in plasma calcitonin was substantially smaller than that of intraperitoneally injected calcium. There was a trend of increased plasma calcitonin after the first liraglutide dose, and increased plasma calcitonin was sustained for up to 2 years of continuous treatment in a mouse carcinogenicity study. Focal c-cell hyperplasia develop after 4 - 9 weeks of liraglutide treatment and neoplasms develop after 64 weeks. Proliferative c-cell lesions account for increased basal and GLP-1R agonist stimulated calcitonin release in liraglutide-treated mice.

A methodological issue confounded results from studies of GLP-1R agonist effects on plasma calcitonin in mice. Mouse plasma calcitonin was quantified using a rat calcitonin immunoradiometric assay (IRMA), but reports of the sensitivity, specificity, and validity of the assay for mouse calcitonin weren't submitted to the NDA (reports 205089 & 205189). Although peptide sequences of rat and mouse calcitonin differ by only a single amino acid, cross-reactivity of the rat IRMA with human calcitonin is only 12%, despite human and rat sequences differing by only 2 amino acids.

A recently published study characterizing bone and mineral homeostasis in GLP-1R deficient mice supports the sponsor's hypothesis that the GLP-1R showed receptor signaling is linked to bone resorption by a calcitonin dependent pathway (C. Yamada et al., Endocrinology (2008) 149(2):574–579). GLP-1R knockout mice had increased osteoclasts, increased bone resorption, and decreased thyroid calcitonin mRNA, but plasma levels of ionized calcium and intact PTH were unaffected. Administering 10 IU/kg eel calcitonin suppressed elevated urinary excretion of deoxypyridinoline, a biomarker of increased bone resorption, in GLP-1R knockout mice. Furthermore, GLP-1R agonists don't directly affect osteoclast or osteoblast activity.

The effect of subcutaneously administered liraglutide and exenatide on plasma calcitonin in mice was determined after single and repeat dosing. Plasma calcitonin levels were measured for up to 16 weeks of exenatide treatment and up to 2 years of liraglutide treatment.

Single bolus subcutaneous doses of 0.2, 1, or 3 mg/kg liraglutide increased calcitonin in CD-1 mice (male and females combined)1.8, 2.4, or 2.4 fold compared to concurrent controls, respectively, within 1.5 to 36 hours after dosing. In nearly all dose groups at all time points, some mice were considered liraglutide non-responsive because plasma calcitonin levels were within the range of values for the control group.

In a 3 day repeat subcutaneous dose study of 0.06 or 25 mg/kg/day liraglutide in male CD-1 mice, day 3 pre-dose group mean plasma calcitonin dose-dependently increased in both liraglutide groups. However, there was evidence that some high dose group mice didn't respond to liraglutide treatment (plasma calcitonin < 50 pg/mL).

Liraglutide increased plasma calcitonin within 2 weeks of daily subcutaneous dosing in CD-1 mice, the increase was sustained with continued treatment for up to 9 weeks, and it was reversed within 6 weeks after treatment was stopped. In a 9 week study of 0.2 or 5 mg/kg/day liraglutide in CD-1 mice, the time course of liraglutide effects on plasma calcitonin were determined prior to dosing and 0.5 and 3 hours after on day 14 and at a single time point after dosing on days 14 and 63 (at the end of 2 and 9 weeks of treatment). Liraglutide increased plasma calcitonin in males 0.5 and 3 hours after dosing on day 14 and in females, calcitonin was above concurrent control levels 3 hours after dosing at 0.2 mg/kg/day and at all time points in females treated with 5 mg/kg/day. In males, calcitonin was increased only at 5 mg/kg/day and only at the end of the of the 9 week treatment period, but in 5 mg/kg/day liraglutide females, calcitonin was elevated after 2 and 9 weeks. Calcitonin levels in both males and females in the 5 mg/kg/day group returned to control group levels by the end of a 6 week recovery period.

In a 13 week repeat subcutaneous dose toxicity study of 0.2, 1, or 5 mg/kg/day liraglutide in CD-1 mice, plasma calcitonin levels increased at all liraglutide doses within 24 hours post-dose after the first dose and in week 13. In a 2 year carcinogenicity study of 0.03, 0.2, 1, or 3 mg/kg/day liraglutide subcutaneously injected once a day, plasma calcitonin was measured in weeks 26, 52, and 104. Group mean calcitonin in males was significantly higher the concurrent control group at $\geq$ 0.2 mg/kg/day in week 26 (male and female), then at all doses in weeks 52 and 104. In females, calcitonin increased at $\geq$ 0.2 mg/kg/day liraglutide in weeks 26 and 52, and at all doses in week 104. Between weeks 26 and 104, group mean plasma calcitonin increased more than 2 fold at 3 mg/kg/day in males and females, but not at lower doses. Proliferative C-cell lesions in liraglutide treated mice accounts for increased calcitonin at 3 mg/kg/day at the end of the carcinogenicity study.

To support their hypothesis that liraglutide-associated increased plasma calcitonin is GLP-1R mediated, the sponsor evaluated the effects of a second agonist, exenatide, on plasma calcitonin and proliferative c-cell lesions in mice. Pharmacokinetic / pharmacodynamic modeling of liraglutide effects on plasma calcitonin in mice indicated that more frequent or continuous dosing with exenatide would be necessary to achieve comparable effects on plasma calcitonin due the shorter elimination half life of exenatide compared to liraglutide.

In a single subcutaneous bolus dose study of 0.25, 1, or 5 mg/kg exenatide in CD-1 mice, exenatide had little or no discernable affect on plasma calcitonin for up to 24 hours after dosing, particularly compared to the robust response elicited by intraperitoneal infusion of calcium.

Because of its short half life, exenatide was administered more frequently, up to 3 times daily, by subcutaneous bolus dosing or by continuous subcutaneous infusion using implanted ALZet osmotic minipumps.

Subcutaneous bolus injections of 0.25 mg/kg/day exenatide once a day or in divided doses 2 or 3 times a day (0.125 or 0.083 mg/kg/injection, respectively) in female CD-1 mice didn't affect plasma calcitonin levels after 2 days of treatment, but continuous subcutaneous infusion of 0.25 mg/kg/day liraglutide (ALZet osmotic minipump) significantly increased plasma calcitonin above control group levels on study day 2.

In a 3 day repeat subcutaneous bolus injection study of 0.06 or 0.25 mg/kg/day exenatide administered once a day or 0.03 or 0.125 mg/kg/injection administered twice a day (0.06 or 0.25 mg/kg/day) to male CD-1 mice, day 3 predose plasma calcitonin levels were higher in mice dosed twice a day, but there was no significant difference between 0.03 and 0.125 mg/kg/injection doses. Within 6 hours after dosing, plasma calcitonin levels were similar to controls. The effect of once a day exenatide dosing on plasma calcitonin was minimal.

In a repeat subcutaneous dose study of 0.083, 0.33, or 1.67 mg/kg/injection exenatide administered 3 times daily, (0.25, 1, or 5 mg/kg/day total dose) for 2 weeks, group mean calcitonin was significantly higher in all exenatide treated groups; up to 6.2 fold higher than concurrent controls in males and up to 8.1 fold higher in females. In a 13 week study of 0.33 mg/kg/injection exenatide administered 3 times daily for 8 days (1 mg/kg/day total dose) followed by 1 mg/kg/injection administered 3 times daily for 12 additional weeks (3 mg/kg/day total dose), calcitonin levels were significantly increased in exenatide treated males, but not in females, at the end of the 13 week period. At the end of the 13 week treatment with multiple daily subcutaneous injections of exenatide, increased plasma calcitonin and increased thyroid calcitonin mRNA lacked correlative focal c-cell hyperplasia in males.

Plasma calcitonin levels were determined in a 16 week repeat dose study of 0.25 or 1 mg/kg/day exenatide administered by continuous subcutaneous infusion or 0.25 mg/kg injected once a day in CD-1 mice. Compared to concurrent controls, daily subcutaneous injections of 0.25 mg/kg/day did not significantly increase plasma calcitonin levels after 12 or 16 weeks of treatment. In mice treated by continuous infusion, calcitonin levels were higher than concurrent controls in weeks 4, 8, 12 and 16. In weeks 12 and 16, calcitonin levels in exenatide groups treated by continuous infusion were at least 4 fold lower than in weeks 4 and 8, probably because treatment was stopped 24 hours prior to sampling in weeks 12 and 16, but in weeks 4 and 8, samples were taken while treatment was ongoing.

Pharmacokinetic / pharmacodynamic modeling of exenatide effects on plasma calcitonin in mice, using an EC_{90} of 270 pM exenatide to increase plasma calcitonin, estimated continuous infusion of 0.25 mg/kg/day would be sufficient to cause sustained elevated blood levels of calcitonin while subcutaneous bolus injections of $\leq$ 1.67 mg/kg/injection administered 3 times daily would not. This modeling result is consistent with the absence of thyroid c-cell proliferative lesions in a carcinogenicity study of 0, 0.018, 0.08, or 0.25 mg/kg/day exenatide subcutaneously injected once a day in CD-1 mice for up to 98 weeks in males and up to 96 weeks in females and the presence of c-cell hyperplasia in mice after 12 or 16 weeks of treatment with a constant subcutaneous infusion of 0.25 or 1 mg/kg/day exenatide for 12 or 16 weeks.

GLP-1 receptor agonist-induced calcitonin release increases calcitonin synthesis

There is no direct evidence of liraglutide induced, thyroid c-cell GLP-1R mediated calcitonin release in mice, but GLP-1R agonists liraglutide and exenatide increase plasma calcitonin and thyroid calcitonin mRNA prior to inducing focal c-cell hyperplasia. A recently published study showed GLP-1R knockout mice ($Glp\text{-}1r^{-/-}$) mice had cortical osteopenia, bone fragility, increased numbers of osteoclasts, increased bone resorption, higher levels of urinary deoxypyridinoline (a marker of bone resorption), and reduced levels of thyroid calcitonin mRNA (Yamada et al. Endocrin (2008), 149(2):574–579). GLP-1 had no direct effect on osteoclasts and osteoblasts, so in mice, GLP-1Rs control bone resorption through a calcitonin-dependent pathway. Subcutaneous injection of 24 nmol/kg exenatide (0.09 mg/kg) increased calcitonin transcript levels in thyroid of wild-type mice, and calcitonin transcript levels were significantly

reduced in GLP-1R knockout mice (see Figure 4 below from Yamada et al., Endocrinology (2008) 149(2):574–579) without affecting blood levels of ionized calcium or iPTH (data not shown) .

[Excerpted from Yamada et al. Endocrin (2008), 149(2):574–579]

In a 9 week study of 0, 0.2, or 5 mg/kg/day liraglutide in CD-1 mice, thyroid calcitonin mRNA levels in the 5 mg/kg/day group significantly increased 3.9 fold over concurrent controls.

After 2 weeks of repeat subcutaneous dosing with 0, 0.083, 0.33, or 1.67 mg/kg/injection exenatide administered 3 times daily, (0.25, 1, or 5 mg/kg/day total dose), calcitonin mRNA levels in thyroid were significantly, dose-dependently increased 2.3 - 4.8 fold at 0.25, 1, and 5 mg/kg/day exenatide, and GLP-1R mRNA was unaffected.

Persistent c-cell stimulation (persistent elevated plasma calcitonin) leads to c-cell hyperplasia

Evaluation of GLP-1R agonist-induced c-cell hyperplasia in mice was confounded by inconsistent definitions of c-cell hyperplasia across studies. A Pathology Peer Review and Pathology Working Group Review to peer review thyroid c-cell histopathology findings in 4, 9, and 13 week studies in mice, chaired by Peter C. Mann, DVM, reached a consensus diagnosis for c-cell findings in these studies.

To determine the time course of liraglutide-induced thyroid c-cell hyperplasia in CD-1 mice, c-cells in thyroid tissues sections were identified by calcitonin immunoreactivity and examined microscopically from mice treated with subcutaneously injected liraglutide for 2 weeks (study 204338), 4 weeks (study 203261), 9 weeks (study 204338), or 13 weeks (study 203261) and for 2 years. In the 9 week study, the time course of reversal of c-cell hyperplasia was determined after 6 and 15 week recovery periods.

In a 9 week study of 0, 0.2 or 5 mg/kg/day liraglutide with an interim sacrifice in week 2 and recovery periods lasting 6 or 15 weeks, there were no qualitative or quantitative microscopic changes in thyroid c-cells in week 2. After 9 weeks of treatment, a low incidence of minimal c-cell hyperplasia occurred in males at $\geq$ 0.2 mg/kg/day (1/16 at 0.2 or 5 mg/kg/day), and a dose-related increased incidence and severity of up to mild c-cell hyperplasia occurred in females at $\geq$ 0.2 mg/kg/day (1/16 at 0.2 mg/kg/day, 6/16 at 5 mg/kg/day). C-cell hyperplasia was fully reversed in males and partially reversed in females at the end of a 6 week recovery period, and after a 15 week recovery period, minimal hyperplasia only occurred in 1/16 females at 5 mg/kg/day. In a 4 week repeat subcutaneous dose toxicity study of 0, 0.1, 0.5, 1, or 5 mg/kg/day liraglutide in CD-1 mice, minimal to moderated c-cell hyperplasia occurred in 2/10 females in the 5 mg/kg/day group, but review of the finding by the Pathology Working Group

dismissed the finding as "developmental disturbances associated with incomplete fusion of the ultimobranchial duct with the thyroid lobe resulting in only partial delivery of c-cells in the thyroid, and were not considered related to treatment."

In a 13 week repeat dose study of 0, 0.2, 1, or 5 mg/kg/day liraglutide in CD-1 mice, dose-related increased incidence and severity of minimal to mild c-cell hyperplasia occurred at $\geq$ 0.2 mg/kg/day in males and females. The Pathology Working Group agreed with the study pathologists diagnosis of c-cell hyperplasia, but disagreed with the characterization as focal.

Persistent calcitonin release resulting in C-cell hyperplasia would expected for treatments that induce hypercalcemia in mice and rats. However, hypercalcemia induced by implanting canine CAC8 adenocarcinomas in nude mice (Okada et al., Vet Path (1994) 341: 339-347) or hypervitaminosis D3 in rats (25,000 IU/day D3 concurrently administered with or without $CaCl_2$) (Fernández-Santos et al, Histol Histopathol. (2001) 16(2):407-14) did not cause c-cell hyperplasia. These results suggest that hypercalcemia itself may not be sufficient to induce c-cell hyperplasia in rats or mice.

Persistent liraglutide-induced c-cell hyperplasia progresses to c-cell neoplasms

Repeat dose studies of subcutaneously administered liraglutide up to 13 weeks in CD-1 mice showed focal thyroid c-cell hyperplasia occurred after $\geq$ 9 weeks of treatment, and liraglutide-induced hyperplasia was largely reversible in males and females. Diffuse hyperplasia, an expected physiologic response to increased calcitonin demand, was not liraglutide treatment related. In a 2 year repeat subcutaneous dose carcinogenicity study of 0.03, 0.2, 1, or 3 mg/kg/day liraglutide, the NOAEL for proliferative c-cell lesions was 0.03 mg/kg/day with minimal to marked focal c-cell hyperplasia occurring at $\geq$ 0.2 mg/kg/day in males and females, c-cell adenomas occurring at $\geq$ 1 mg/kg/day in males and females, and c-cell carcinomas occurring at 3 mg/kg/day in females. In the 2 year study, focal c-cell hyperplasia was considered a preneoplastic lesion because:

5. the incidence and severity of focal hyperplasia increased with dose in both males and females.
6. focal hyperplasia occurs at lower doses than c-cell tumors
7. the incidence of focal c-cell hyperplasia in mice with adenomas in the 3 mg/kg/day group was 56% in males and 33% in females.
8. in decedents, a finding of c-cell hyperplasia preceded c-cell tumors by 17 weeks in both males and females.

Cynomolgus Monkeys

Subcutaneously administered liraglutide had no effect on plasma calcitonin, thyroid c-cell proliferation, or calcium homeostasis parameters including plasma calcium and iPTH in studies up to 87 weeks long. Four mechanistic studies were performed: 1) immunohistochemical colocalization of calcitonin and GLP-1R immunoreactivity in thyroid and pancreas tissue sections, 2) in situ hybridization determining GLP-1R transcript levels in c-cells of thyroid tissue sections and pancreatic tissue, 3) quantifying c-cells in thyroid tissue sections from control and high dose monkeys from a pivotal 52 week repeat dose toxicity study, and 4) determining calcium homeostasis parameters (plasma calcium, iPTH, and calcitonin) and thyroid histopathology in monkeys treated with 0, 0.25, or 5 mg/kg/day liraglutide for up to 87 weeks.

In a dedicated study characterizing thyroid c-cells in male and female cynomolgus monkeys (study 205121), the sponsor determined calcitonin immunoreactive c-cells were primarily located in the middle third of each thyroid lobe in clusters of 2 to 10 cells attached to thyroid follicular epithelium or in parafollicular positions (cell clusters between follicles).
type(s) labeled by [^{125}I]GLP-1(7-36) were not identified.

GLP-1Rs were not localized on thyroid c-cells in monkeys. An immunohistochemical colocalization study using monkey thyroid tissue slices was equivocal for colocalization of GLP-1R and calcitonin immunoreactivities on the same cells because GLP-1R immunoreactivity was weak and the specificity of the anti-GLP-1R antibody, K100B, was not demonstrated (study 204370). The specificity of

K100B, a polyclonal rabbit anti-human GLP-1R antibody, was not adequately demonstrated because; 1) the antibody stained pancreas from GLP-1R knockout mice and 2) immunohistochemical staining in the presence of the peptide antigen used to generate the antibody did not block staining. An in situ hybridization study of GLP-1R mRNA in tissue sections from monkeys was equivocal with undetectable levels of GLP-1R transcript in thyroid, but much higher levels in pancreas, a positive control (study 20040515PR4).

Repeat subcutaneous dosing of up to 5 mg/kg/day liraglutide for up to 87 weeks in cynomolgus monkeys had no effect on plasma calcitonin or thyroid c-cells. In a definitive 52 week chronic toxicity study in monkeys, there were no thyroid c-cell proliferative lesions or plasma calcium changes. PCNA immunohistochemical staining thyroid tissue from control group and 5 mg/kg/day high dose monkeys in the 52 week study showed liraglutide had no effect on c-cell proliferation. In an 87 week mechanistic study identifying calcitonin immunoreactive c-cells in thyroid tissue sections of monkeys treated with 0, 0.25, or 5 mg/kg/day liraglutide, high plasma liraglutide levels interfered with the anti-liraglutide antibody screening and neutralization assays, and in the absence of any pharmacodynamic effect, the inability to characterize the anti-liraglutide antibody response confounds interpretation of the study. In the 87 week study, single or repeat doses of 0.25 or 5 mg/kg liraglutide had no effect on plasma calcium, plasma calcitonin, plasma iPTH, or calcium-induced secretion of calcitonin or iPTH. At the end of 87 weeks, liraglutide had no effect on macroscopic or microscopic pathology of calcitonin immunoreactive thyroid c-cells.

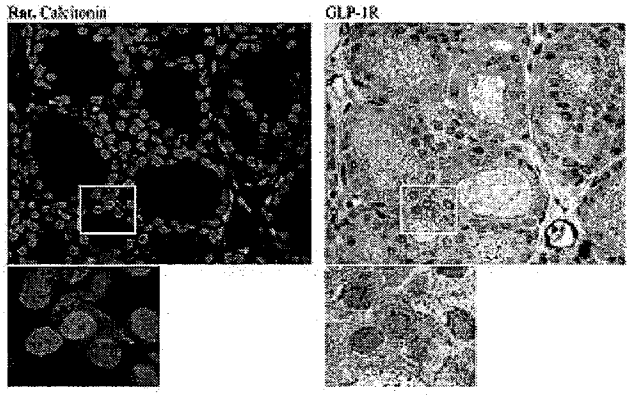
REVIEW OF MECHANISTIC STUDIES OF LIRAGLUTIDE-INDUCED THYROID C-CELL PROLIFERATIVE LESIONS

GLP-1 RECEPTOR LOCALIZATION AND SIGNALING

- 204370 / An immunohistochemical investigation of the GLP-1R in tissue from mice, rats, cynomolgus monkeys, and humans

Immunohistochemical studies of thyroid tissue sections from mice, rats, cynomolgus monkeys and humans using anti-calcitonin antibody to identify c-cells and an anti-GLP-1R antibody to determine if c-cells express GLP-1Rs were inconclusive. A fluorescent Alexa488-labeled anti-human calcitonin polyclonal antibody and a primary rabbit anti-human GLP-1R polyclonal antibody (recognizing the receptor's amino terminus) labeled with chromogenic secondary goat anti-rabbit secondary antibody coupled to HRP (biotinylated antibody, avidin coupled HRP) were used for colocalization. Three different rabbit anti-human GLP-1R antibodies were produced. Due to species differences in GLP-1R immunoreactivity, antibody K100B was used to stain human, mouse, and monkey tissues and K102B was used for rat tissues. Pancreas tissue slices were used for GLP-1R positive controls in all 4 species. Results using human tissues were already presented. Specificity of both anti-GLP-1R polyclonal antibodies (K100B and K102B) was not demonstrated because in both cases, staining was weak and staining wasn't blocked or it was only partially blocked by preincubation with the antigenic peptide. Furthermore, K100B stained cells in thyroid and pancreas from GLP-1R knockout mice, and Western blot of GLP-1R containing cell lines using K102B didn't demonstrate receptor specific labeling.

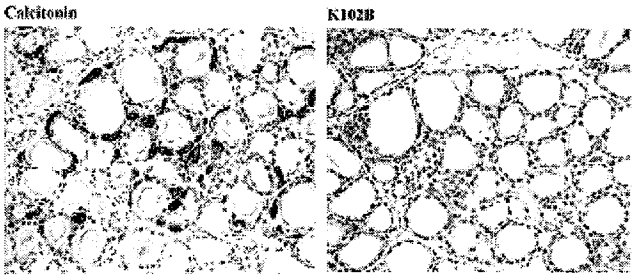
The figures below show specific calcitonin staining (left) and only weak GLP-1R staining in rat thyroid.



[N000 4.2.3.7.3 P19]

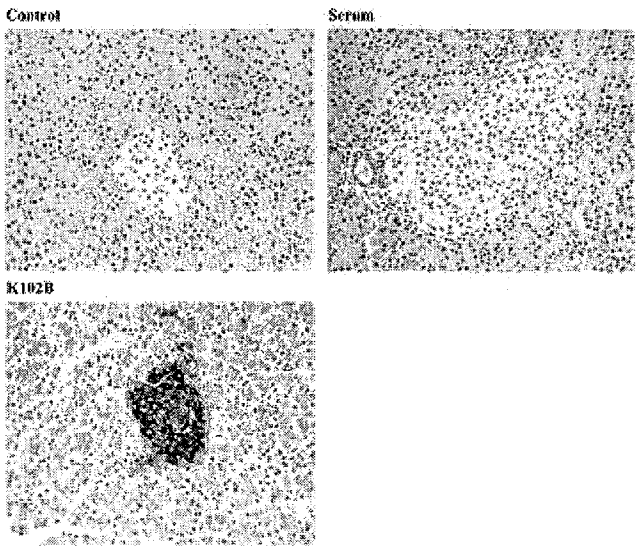
Figure 7 shows calcitonin immunoreactivity in rat thyroid was strongly labeled, but GLP-1R labeling with K102B was weak with questionable specificity because staining wasn't blocked by preabsorption of the antibody with the GLP-1R peptide antigen (data not shown). Figure 8 shows K102B stained specific cells in rat pancreas, a GLP-1R positive control, but stained cell types were not determined.

Figure 7 Thyroid gland serial sectioned, rat



[N000 4.2.3.7.3 P25]

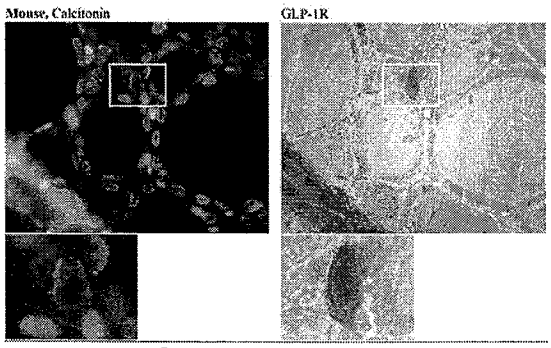
Figure 8 Pancreas serial sectioned, rat



[N000 4.2.3.7.3 P26]

In mouse thyroid tissue sections stained with Alexa488-labeled anti-human calcitonin polyclonal antibody or K100B, a polyclonal anti-GLP-1R antibody, calcitonin staining had high background levels(Figure 1, left). GLP-1R staining was evident in one calcitonin positive cell (Figure 1, right, enclosed in a square), but other cells not labeled by anti-calcitonin antibody were also stained by K100B.

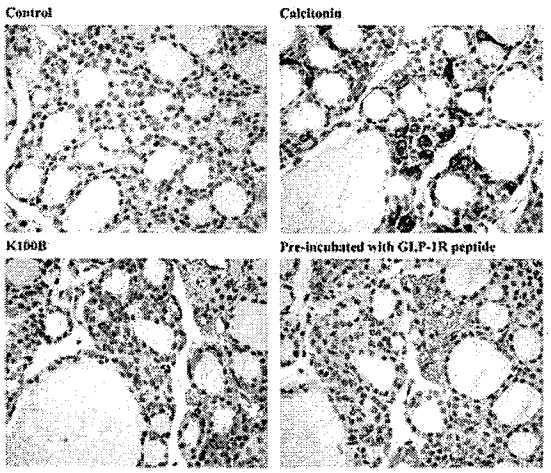
Figure 1 Mouse and rat thyroid gland, double staining



[N000 4.2.3.7.3 P19]

Figure 3 shows calcitonin immunoreactive cells in mouse thyroid, but GLP-1R immunoreactivity with K100B was weak and specificity wasn't clearly demonstrated because preabsorption of the antibody with the GLP-1R peptide antigen had little effect on staining.

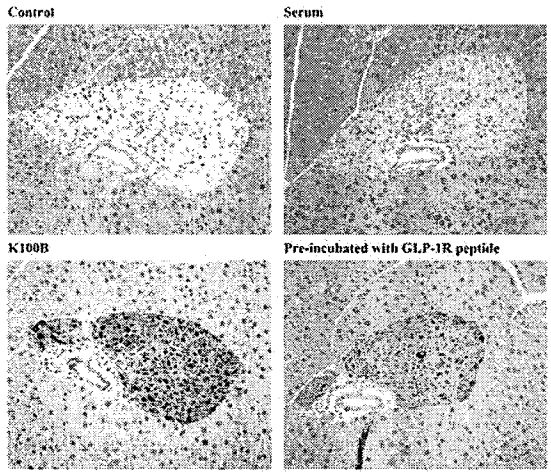
Figure 3 Thyroid gland serial sectioned, mouse



[N000 4.2.3.7.3 P21]

Figure 4 shows specific cell staining by K100B in mouse pancreas, a GLP-1R positive control. However, the specificity of K102B was not clearly demonstrated because staining was only partially blocked by preincubating the antibody with the antigenic peptide.

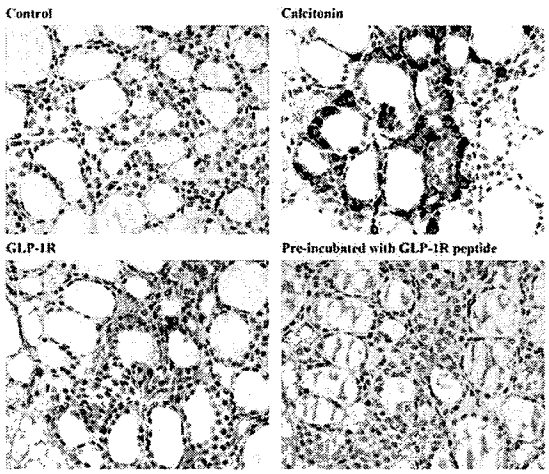
Figure 4 Pancreas serial sectioned, mouse



[N000 4.2.3.7.3 P22]

K100B cell staining in thyroid and pancreas tissue sections from GLP-1R knockout mice, GLP-1R negative control tissues, was similar to wild type mice. GLP-1R staining in GLP-1R knockout mice was attributed to expression of a non-functional GLP-1R amino terminus. Figure 5 shows calcitonin immunoreactive cells in thyroid tissue sections from GLP-1R knockout mice, but GLP-1R labeling with K100B was weak, although preabsorption of the antibody with the GLP-1R peptide antigen did inhibit staining to a greater extent than in thyroid from wild-type mice.

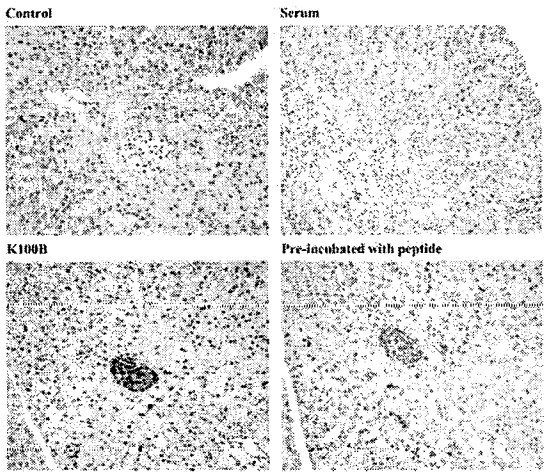
Figure 5 Thyroid gland serial sectioned, KO-mouse



[N000 4.2.3.7.3 P23]

K100B labeled cells in pancreas from GLP-1R knockout mice, but labeled cell types were not identified. Preabsorption of K100B with the peptide antigen partially decreased islet staining.

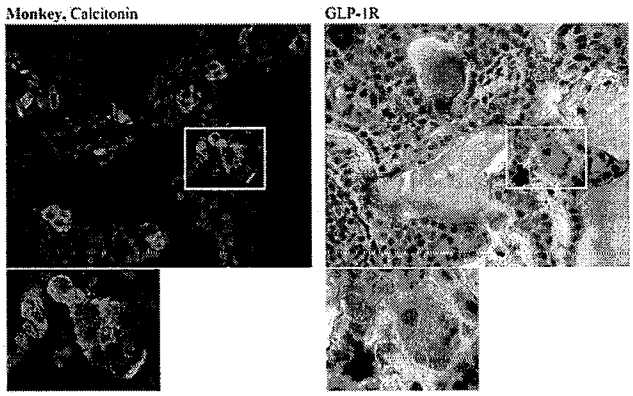
Figure 6 Pancreas serial sectioned, KO-mouse



[N000 4.2.3.7.3 P24]

Results of immunohistochemical studies colocalizing calcitonin and GLP-1R immunoreactivity in thyroid tissue sections from cynomolgus monkeys to identify GLP-1Rs on c-cells was equivocal. GLP-1R immunoreactivity may not be confined to calcitonin immunoreactive cells and not all identified c-cells reacted with the anti-GLP-1R antibody. A fluorescent Alexa488-labeled anti-human calcitonin polyclonal antibody and a primary rabbit anti-human GLP-1R polyclonal antibody (antibody K100B directed against the receptor's amino terminus) labeled with chromogenic secondary goat anti-rabbit secondary antibody coupled to HRP (biotinylated antibody, avidin coupled HRP) were used for colocalization. Figure 2 shows specific calcitonin staining (left) and weak GLP-1R staining (right). In some instances, calcitonin immunoreactive cells were not GLP-1R immunoreactive and visa versa.

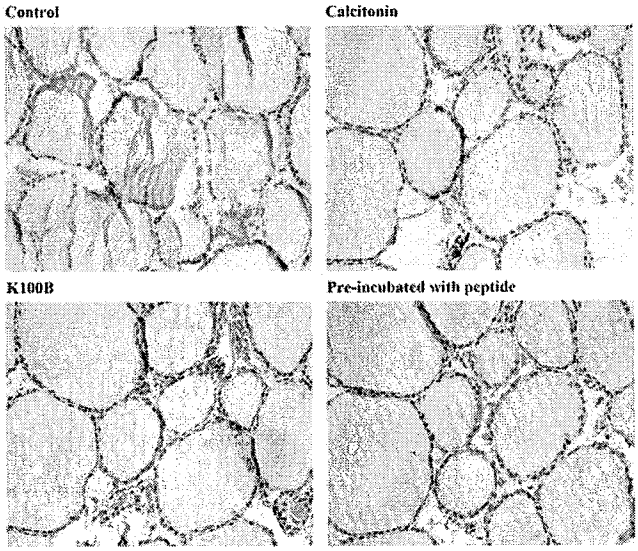
Figure 2 Cynomolgus Monkey and human thyroid gland, double staining



[N000 4.2.3.7.3 P20]

Figure 9 shows K100B labeled specific cells in monkey thyroid, but GLP-1R labeling with the K100B polyclonal antibody was weak and specificity wasn't demonstrated because preabsorption of the antibody with the GLP-1R peptide antigen had little effect on staining.

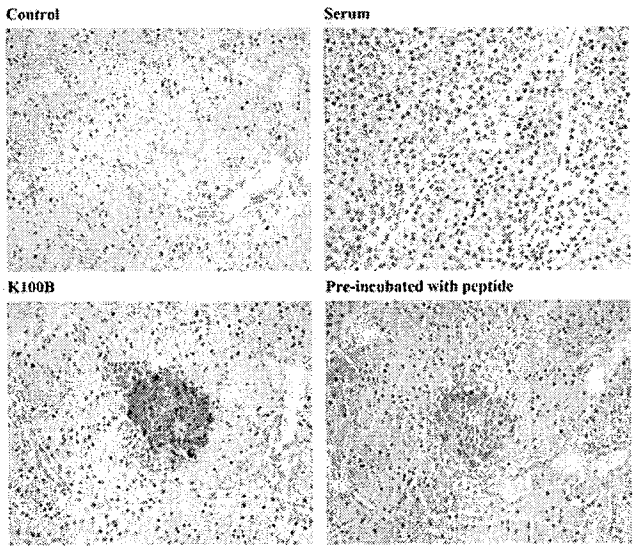
Figure 9 Thyroid gland serial sectioned, Cynomolgus monkey



[N000 4.2.3.7.3 P27]

Figure 10 shows K100B labeled specific cells in monkey pancreas, a GLP-1R positive control. Pancreas cell types labeled by K100B were not identified. Specificity of K100B immunoreactivity was not clearly demonstrated because staining was only partially blocked by preincubating the antibody with the antigenic peptide.

Figure 10 Pancreas serial sectioned, Cynomolgus monkey



[N000 4.2.3.7.3 P28]

- 20040515PR4 / Investigation of GLP-1 receptor mRNA expression in mouse, rat cynomolgus monkey, and human thyroid C-cells and in pancreatic islets studied by in situ hybridization

In situ hybridization of species specific ^{35}S-labeled riboprobes to GLP-1R mRNA was evaluated in paraffin-embedded thyroid tissue sections from mice, rats, cynomolgus monkeys, and humans. Thyroid c-cells were identified by indirect fluorescent microscopy after staining with an Alexa488-coupled anti-

calcitonin antibody. In situ hybridization to pancreatic islets served as a positive control for GLP-1R probes and hybridization of an ^{35}S-labeled riboprobes to calcitonin served as a control for mRNA quality in thyroid tissue. An ^{35}S-labeled probe to cyclophilin, a low to medium abundance transcript, served as a addition control for mRNA quality in samples of thyroid and pancreas.

Thyroid c-cells from mice, rat, cynomolgus monkeys, or humans have very low to undetectable levels of GLP-1R transcript. Evidence of GLP-1R mRNA in thyroid tissue was equivocal in all species tested. Results are summarized in Table 1 (below). GLP-1R mRNA levels in calcitonin-positive thyroid cells (identified by immunofluorescence after staining with anti-calcitonin antibody) determined by autoradiography was weak in mice and rats and undetectable in monkeys and humans. In situ hybridization using a calcitonin mRNA probe showed thyroid cells stained with anti-calcitonin antibody contained calcitonin mRNA demonstrating thyroid tissue was suitable for in situ hybridization.

Table 1 Summary of results with GLP in situ hybridisation and control experiments

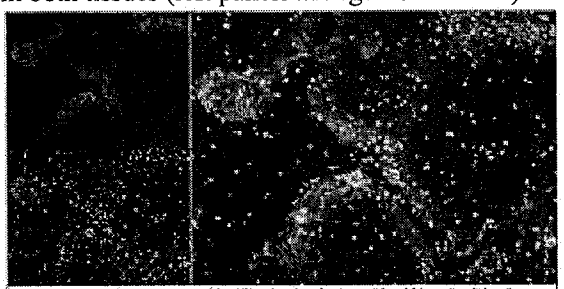
Species	In-situ hybridisation with ^{35}S labelled riboprobe to mRNA in tissue, with or without double labelling by immunofluorescent antibody to calcitonin in thyroids.									
	Tissue	GLP-1R mRNA (5-6 weeks exposure)			Calcitonin mRNA (3-6 days exposure)			Cyclophilin mRNA(2-3 weeks exposure)		
		as	s[§]	calc IFL	as	s	calc IFL	as	s	calc IFL
Mouse	pancreas*	very strong	OK					strong	OK	
	thyroid	weak	OK	strong	very strong	OK	strong	very strong	OK	strong
Rat	pancreas*	medium	OK					strong	OK	
	thyroid	weak	OK	strong	very strong	OK	strong	very strong	OK	strong
Cynomolgus monkey	pancreas	medium	OK					strong	OK	
	thyroid	below limit	-	strong	strong	OK	strong	strong	OK	strong
Human	pancreas	weak	OK					strong	OK	
	thyroid	below limit	-	strong	strong	OK	strong	strong	OK	strong

*) Pancreas sections from mice and rats were only exposed for 20-25 days with the autoradiographic emulsion before development

§) OK for hybridisations of sense riboprobes means that the signal over islets in pancreas or C-cells in thyroids was similar to surrounding tissue elements.

[N000 4.2.3.7.3 P35]

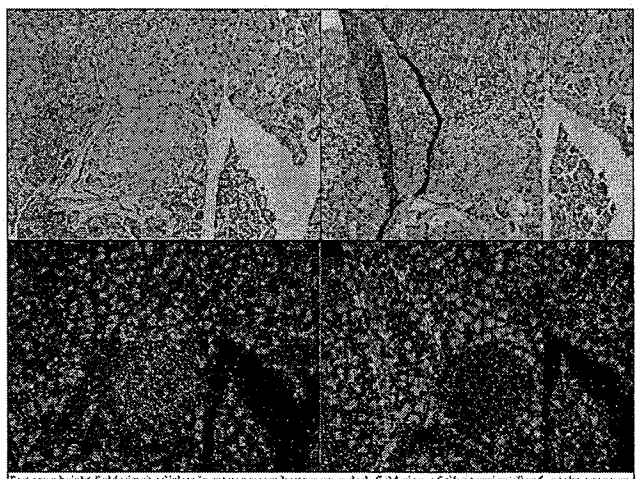
Although the sponsor claims GLP-1R mRNA was detectable in thyroid c-cells identified by anti-calcitonin antibody staining, Figure 3 shows colocalization of GLP-1R mRNA and calcitonin was equivocal due to high background staining by the anti-calcitonin antibody and weak to undetectable autoradiographic signals in cells. As a positive control, anti-sense rat GLP-1R radiolabeled probes labeled cells in rat pancreas (Figure 16, left) and an antisense calcitonin radiolabeled probe labeled anti-calcitonin immunoreactive cells (Figure 8, left) in rat thyroid tissue sections. Sense probes for GLP-1R and calcitonin were inactive in both tissues (left panels in Figures 16 and 8).



Top left: Calcitonin demonstrated by IFL after incubation with rabbit anti-calcitonin-Alexa488 complex; lower left: silver grains in autoradiographs after in-situ hybridisation with ^{35}S labelled antisense riboprobe to ratGLP-1R; right: composite of the IFL and in-situ hybridisation frames. Cryostat sections.

Figure 3 Rat thyroid GPL-1R mRNA co-localisation with C-cell calcitonin IFL

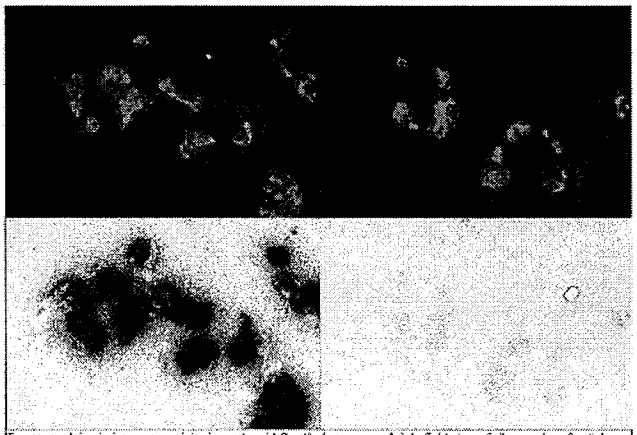
[N000 4.2.3.7.3 P17]



Top row: bright field views of islets in rat pancreas; bottom row: dark field view of silver grains after 5 weeks exposure of emulsion. Left column: in-situ hybridisation with antisense riboprobe to rGLP-1R mRNA; right column: in-situ hybridisation with sense riboprobe to rGLP-1R mRNA. Paraffin sections.

Figure 16 GLP-1R mRNA expression in rat pancreatic islets

[N000 4.2.3.7.3 P30]

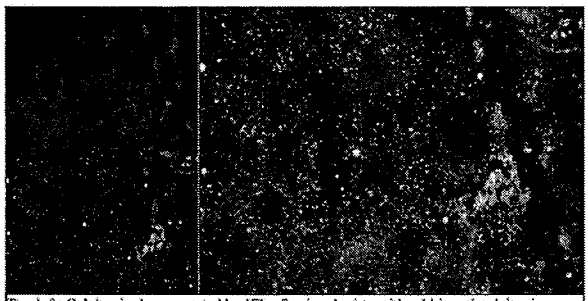


Top row: calcitonin immunoreactivity in rat thyroid C-cells; bottom row: bright field view of silver grains after 6 days exposure of emulsion. Left column: in-situ hybridisation with antisense riboprobe to calcitonin mRNA; right column: in-situ hybridisation with sense riboprobe to calcitonin mRNA. Pictures taken from beneath the silver grains via an inverted fluorescence microscope.

Figure 8 Rat thyroid co-localisation of calcitonin mRNA and immunoreactivity

[N000 4.2.3.7.3 P22]

In mouse thyroid tissue sections, high background anti-calcitonin antibody staining precludes the identification of c-cells (Figures 1 and 2). Since GLP-1R mRNA was at or below the level of visual detection using the anti-sense probe and high background calcitonin immunoreactivity, colocalization of calcitonin immunoreactivity and GLP-1R mRNA was equivocal. Non-specific mRNA detection using a sense probe confirms that GLP-1R mRNA was at or below the level of detection (Figure 2).

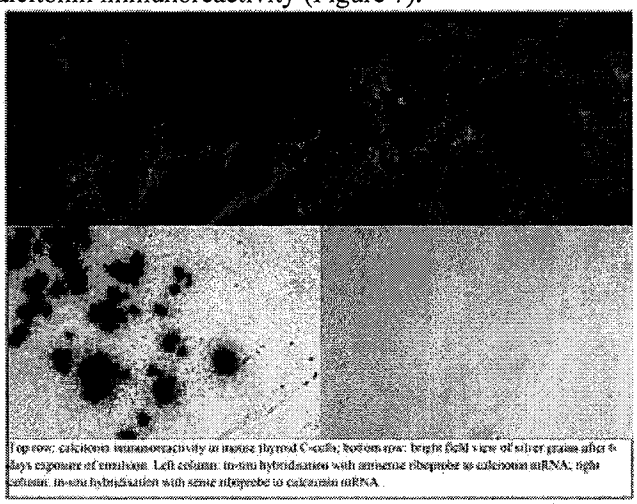


Top left: Calcitonin demonstrated by IFL after incubation with rabbit anti-calcitonin-Alexa488 complex; lower left: silver grains in autoradiographs after in situ hybridisation with ^{35}S labelled sense riboprobe to ratGLP-1R; right: composite of the IFL and in-situ hybridisation frames. Cryostat sections.

Figure 2 Mouse thyroid GLP-1R mRNA in-situ hybridisation sense control with C-cell calcitonin IFL

[N000 4.2.3.7.3 P16]

As a positive control, anti-sense human GLP-1R RNA probes labeled cells in rat pancreas and an antisense calcitonin probe labeled anti-calcitonin immunoreactive cells in human thyroid tissue sections. ^{35}S-labeled calcitonin riboprobes labeled specific cells in mouse thyroid tissue sections, but due to high background staining with the anti-calcitonin antibody, it was not possible to discern if mRNA labeled cells were positive for calcitonin immunoreactivity (Figure 7).



Top row: calcitonin immunoreactivity in mouse thyroid C-cells; bottom row: bright field view of silver grains after 6 days exposure of emulsion. Left column: in-situ hybridisation with antisense riboprobe to calcitonin mRNA; right column: in-situ hybridisation with sense riboprobe to calcitonin mRNA.

Figure 7 Mouse thyroid co-localisation of calcitonin mRNA and immunoreactivity

[N000 4.2.3.7.3 P21]

Figure 5 shows colocalization of GLP-1R mRNA and calcitonin immunoreactivity was negative in monkeys. As a positive control, anti-sense monkey GLP-1R RNA probes labeled specific cells in monkey pancreas and an antisense calcitonin probe labeled calcitonin immunoreactive cells in monkey thyroid tissue sections (not shown).

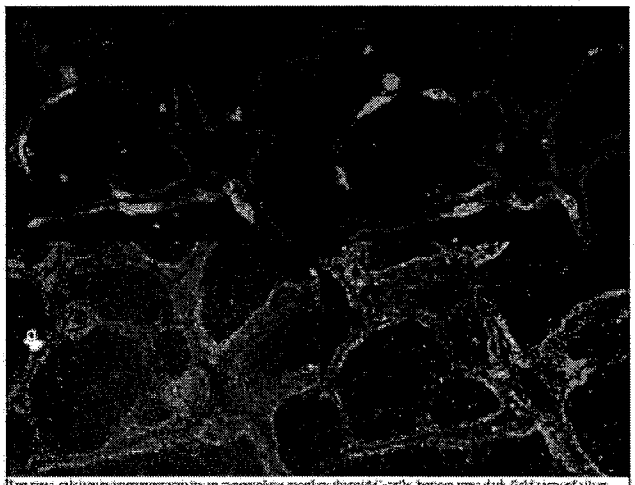


Figure 5 Cynomolgus monkey thyroid GLP-1R mRNA co-localization with C-cell calcitonin

[N000 4.2.3.7.3 P20]

- 14725-006 / GLP-1 receptor expression in rat and human cell lines

Functional GLP-1Rs in rat thyroid c-cell lines (MTC 6-23 and CA-77), a human c-cell line (TT), and a rat pancreatic beta cell line (INS-1E) were characterized by saturation radioligand binding of [^{125}I]GLP-1 (7-37) to estimate the number of receptors/cell and radioligand affinity. Non-specific binding was determined in the presence of unlabeled GLP-1.

Rat MTC 6-23 cells had 16,000 GLP-1Rs/cell with an affinity of K_d 47.0 pM (Figure 1). The number and receptors / cell may be underestimated because MTC 6-23 cells were detached from culture plates by protease treatment 24 hours prior to performing radioligand binding experiments.

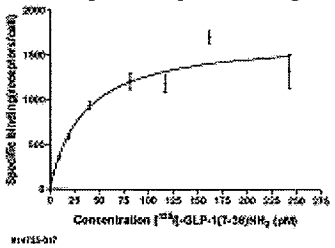


Figure 1 GLP-1 receptor saturation plots for MTC 6-23 cell line.

[N000 4.2.3.7.3 P10]

Rat CA-77 cells expressed 13,000 GLP-1 binding sites/cell with and affinity of K_d 31 pM.

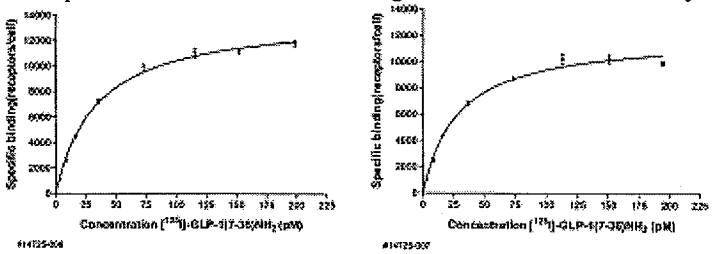


Figure 2 GLP-1 receptor saturation plots for the CA-77 cell line.

[N000 4.2.3.7.3 P11]

Rat INS-1E cells, derived from pancreatic beta cells, expressed 8,780 GLP-1 binding sites/cell with and affinity of K_d 82 pM.

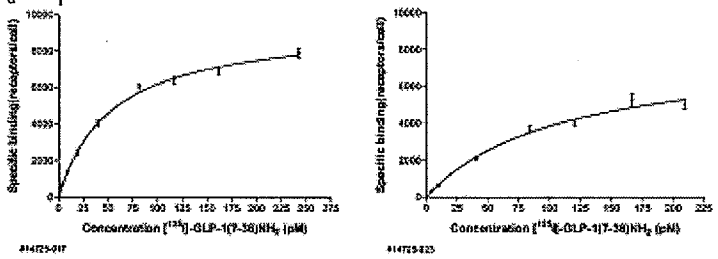


Figure 4 GLP-1 receptor saturation plots for the INS-1E cell line.

[N000 4.2.3.7.3 P13]

The finding that rat thyroid c-cell lines and a pancreatic beta cell line express similar levels of GLP-1Rs, despite a large difference in GLP-1R antibody reactivity and mRNA levels in thyroid and pancreas tissue sections suggests the density of GLP-1Rs in rat lines may not reflect the receptor density in thyroid c-cells or rat pancreas in vivo.

Figure 3 shows specific saturable binding of [^{125}I]GLP-1 (7-37) was not demonstrated in human TT cells.

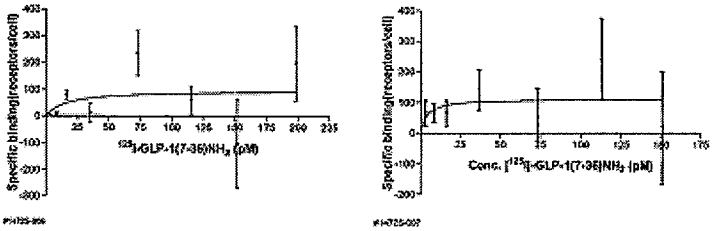


Figure 3 GLP-1 receptor saturation plots for the TT cell line

[N000 4.2.3.7.3 P12]

- 205088 / Quantitative analysis of GLP-1 receptor levels on 2 rat (rMTC 6-23 and CA-77) and one human (TT) C-cell line

Functional GLP-1Rs in thyroid c-cell lines were quantified in a flow cytometric fluorescent ligand-binding assay. Specifically bound fluorescence of rat MTC 6-23 and CA-77 cells or human TT cells labeled with varying concentrations of fluorescently labeled GLP-1 [GLP-1 (7-36)-Lys(6-FAM)] in the absence or presence of 2 µM exendin(9-39) was quantified by flow cytometry to estimate the number of GLP-1R sites and ligand affinity.

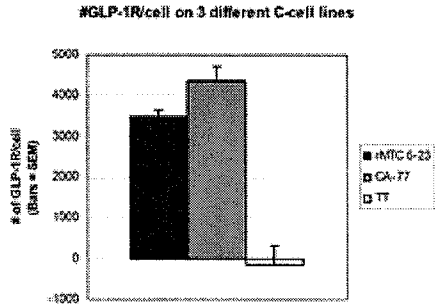


Figure 4 Number of surface-expressed GLP-1R on the rat C-cell lines rMTC 6-23 and CA-77, and on the human C-cell line TT.

Results are means of 3 independent experiments. The expression of GLP-1R on the rat C-cell lines was significantly higher that GLP-1R expression on the human C-cell line (see Table 1).

[N000 4.2.3.7.3 P17]

GLP-1R sties on thyroid c-cell lines were demonstrated on rat MTC 6-23 and CA-77 cells, but not on human TT cells (Figure 4). Estimated GLP-1R density on rat MTC 6-23 cells and CA-77 cells was similar (3,499 and 4,369 GLP-1Rs / cell, respectively). Compared to radioligand binding saturation studies, the number of GLP-1R per cell estimated by flow cytometry of fluorescent GLP-1 labeled cells was 4.6 fold lower for MTC 6-23 cells and 2 fold lower for CA-77 cells.

- 205218 / Western blot analysis of GLP-1R expression in rats and human C-cell lines

The sponsor claims GLP-1R was identified in Western blots of rat thyroid and rat thyroid c-cell lines MTC 6-23 and CA77, but not human c-cell line TT using anti-human GLP-1R antibody K102B. However, these results are equivocal because the specificity of the antibody for GLP-1Rs was not demonstrated and the protein size and SDS-PAGE pattern were not consistent with published results for GLP-1Rs (Widman et al, Biochem. J. (1995) 310, 203-214).

GLP-1R protein in rat thyroid c-cell lines MTC 6-23 and CA-77 and human c-cell line TT was quantified by western blotting using rabbit anti-human GLP-1R antibody targeted to the peptide sequence TVSLWETVQKWREYRRQC corresponding to human GLP-1R amino acids 29 – 46 in the receptor's amino terminus (rabbit antibody 102B, 2 non-identical amino acids in the corresponding sequence of the rat GLP-1R), an HRP-conjugated horse anti-rabbit IgG secondary antibody, and a chemiluminescent substrate (ECL, GE Healthcare).

Antibody 102B identified a 51 kDa protein in rat thyroid and rat c-cell lines and immunoreactivity with the 51 kDa protein was reduced by preabsorbing it with the peptide antigen (Figure 1).

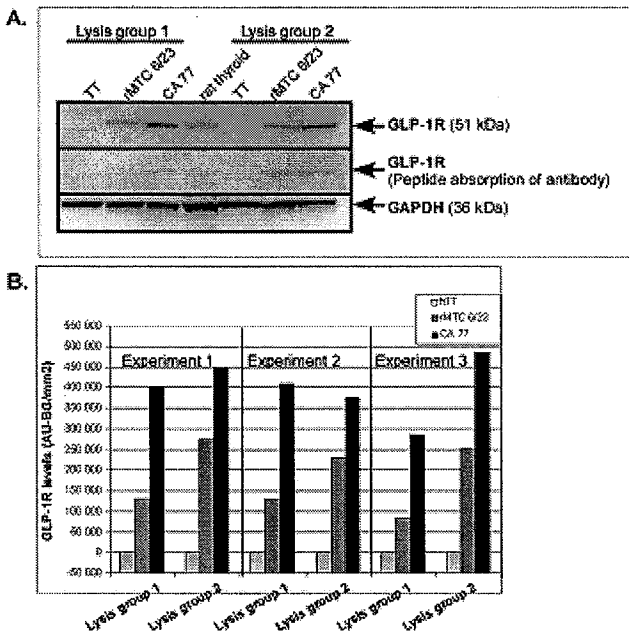
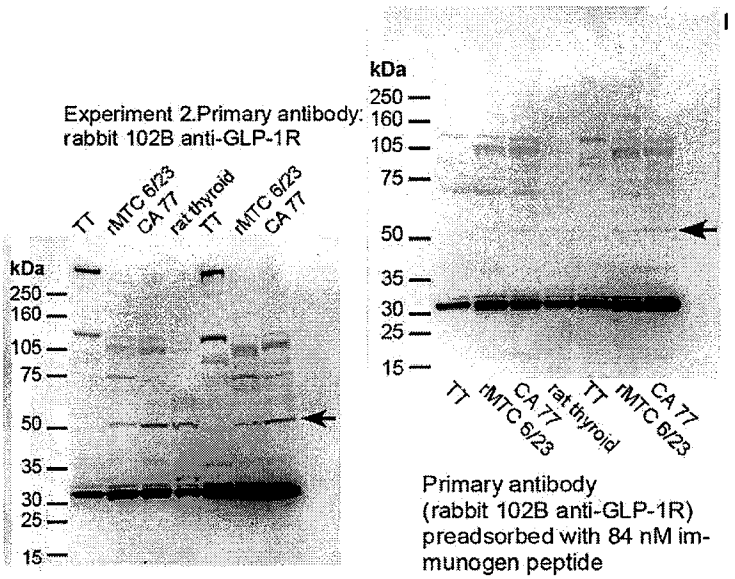


Figure 1 GLP-1R cannot be detected in a human C-cell line.
Two sets of C-cell line lysates: lysis group 1 and lysis group 2, with high and low passage numbers, respectively (see table 2 for passage numbers), were analysed by Western immunoblotting for GLP-1R expression. Lysate of rat thyroid was included as a positive control. A, representative result from three independent Western blot experiments (see appendix B for whole filter image). Top panel, the GLP-1R band location is indicated by an arrow. Middle panel, absorption with 84 nM human GLP-1R peptide lead to reduction of the 51 kDa GLP-1R band, demonstrating the specificity of the reaction. Bottom panel, a parallel membrane was probed with anti-GAPDH antibody, to verify equal protein loading. B, Quantitative analysis of GLP-1R expression levels. Data from both C-cell line lysis groups for three independent Western blotting experiments are shown in the bar diagram.

[N000 4.2.3.7.3 P14]

Reviewer note: Because of the heterogeneity of glycosylated receptors, immunoreactive GLP-1Rs would be expected to be diffuse bands at a molecular weight > 55 with nonglycosylated receptor appearing as more discrete bands at a molecular weight < 55 (Widmann et al, Biochem. J. (1995) 310, 203-214). The staining pattern in Figure 1 not consistent with a glycosylated G-protein coupled receptor.

Images of the entire western blot from a representative experiment showed intense, specific immunoreactivity in TT cells at > 250 KDa, probably consisting of protein complexes that didn't enter or barely entered the gel, and between 105 and 160 kDa. This data doesn't support the sponsor's conclusions about the absence of specific staining in TT cells or the specificity of antibody 102B.



[N000 4.2.3.7.3 P19]

- 204415 / Real-time (TaqMan) RT-PCR quantification of glucagon-like peptide 1 receptor in C-cell lines

Consistent with low to undetectable functional GLP-1Rs in TT cells, a human thyroid c-cell line, GLP-1R transcript levels in TT cells were much lower compared to transcript levels in rat thyroid c-cell lines MTC 6-23 or CA77. Relative GLP-1R transcript levels in rat and human c-cell lines were measured by real-time quantitative RT-PCR incorporating fluorescent primers into amplified cDNA and normalizing GLP-1R transcript levels to transcript levels encoding for beta-actin. Summary results in Figure 3 show relative levels of GLP-1R mRNA were significantly higher in rat c-cell lines MTC 6-23 and CA77 (18 – 27 GLP-1R transcripts / 1000 beta actin transcripts) compared to human TT cells (1 GLP-1R transcript / 1000 beta actin transcripts).

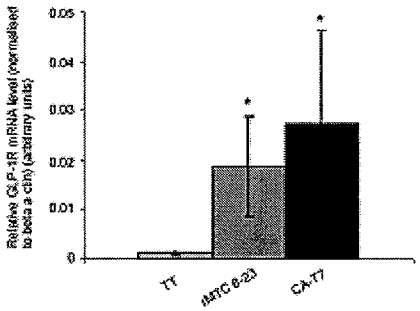


Figure 3 Comparison of GLP-1R mRNA expression levels between rat and human C-cell lines
Relative GLP-1R mRNA levels were determined by normalization to beta actin, and expressed as $2^{-\Delta Ct}$ (arbitrary units, Table 3). The columns represent mean $2^{-\Delta Ct}$ values from table 3; Bars, one standard deviation; *Both rat C-cell lines had significantly higher relative GLP-1R mRNA content that the human TT C-cell line ($p<0.01$, student's t-test).

[N000 4.2.3.7.3 P17]

- 13737-025 / Thyroid C-cell line GLP-1 receptor functional data: cAMP accumulation and calcitonin release

Functional GLP-1R coupled to adenylyl cyclase activation and calcitonin secretion was demonstrated in rat MTC 6-23 and CA77 thyroid c-cell lines, but not in human TT cells. Pentagastrin, a calcitonin secretagogue used as a positive control, did not stimulated calcitonin release from any of the 3 c-cell lines suggesting these cell lines are not representative of receptor coupled calcitonin secretion in vivo.

The rank order potency of the GLP-1R agonists human GLP-1 (7 – 37), exenatide, or liraglutide to stimulate cAMP accumulation and calcitonin secretion in rat and human c-cell lines was determined. In these in vitro experiments, the lower potency of liraglutide compared to GLP-1 (7 – 37) and exenatide was attributed to liraglutide binding to protein in culture media supplemented with 15% horse serum.

In rat MTC 6-23 cells, GLP-1 (7 – 37), exenatide, and liraglutide dose-dependently increased cAMP accumulation (EC_{50}s 120, 90, and 5,800 pM, respectively, Figure 3) with a maximum response < 50% of the 100 µM forskolin control, a phorbol ester that directly activates adenylyl cyclase (Figure 4A).

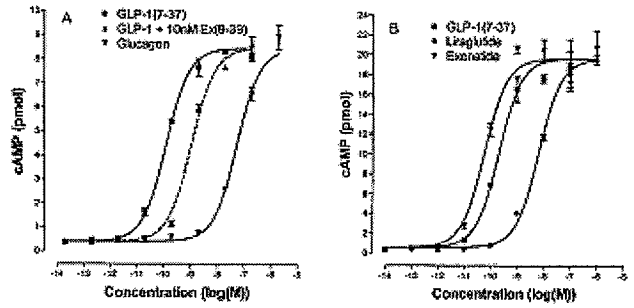


Figure 3 GLP-1 receptor signalling and pharmacology in the rat thyroid C-cell line MTC 6-23.

[N000 4.2.3.7.3 P14]

To demonstrate GLP-1R specificity of liraglutide's effect in MTC 6-23 cells, the liraglutide dose-response curve shifted right-ward in the presence of 10 nM exendin (9 – 39), a GLP-1R antagonist (Figure 4B).

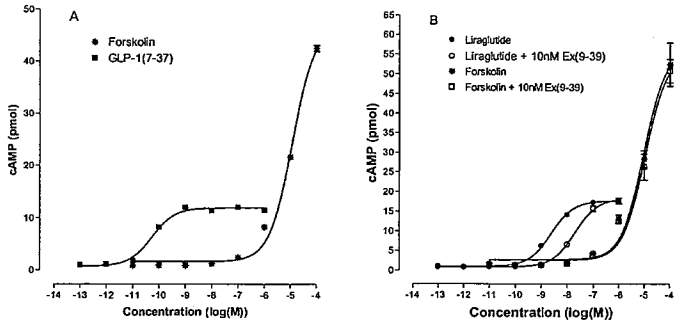


Figure 4 Comparison of GLP-1 and forskolin induced cAMP activation in the rat thyroid cell line, MTC 6-23.

[N000 4.2.3.7.3 P15]

GLP-1 (7-37), exenatide, and liraglutide had a similar rank order potency and EC_{50}s for calcitonin secretion (Figure 5A, 80, 55, and 5,300 pM, respectively) and the maximal effect of liraglutide was up to 67% of the maximum response to 10 μM forskolin.

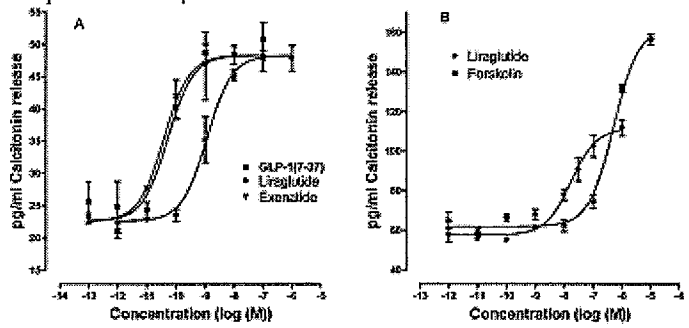


Figure 5 Calcitonin release from the rat C-cell line MTC 6-23.

Data are from experiment 14725-001 and 13737-060. In A, the serum concentration was 1% and in B 15%.

[N000 4.2.3.7.3 P16]

Exendin(9-39), a GLP-1R specific agonist, inhibits liraglutide stimulated calcitonin secretion from MTC 6-23 cells (Figure 6).

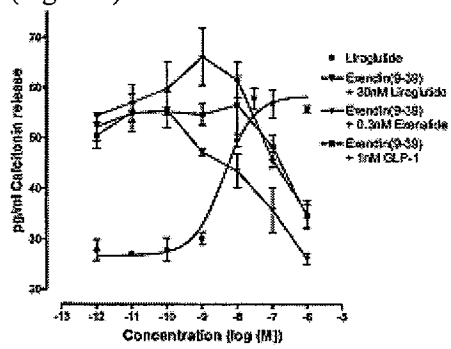


Figure 6 Exendin(9-39) antagonised GLP-1, exenatide and liraglutide induced calcitonin release.

[N000 4.2.3.7.3 P17]

Pentagastrin stimulates calcitonin release by activating thyroid CCK_2 receptors in humans and rats. Pentagastrin didn't stimulate calcitonin release from rat MTC 6-23 cells (Figure 7), rat CA-77 cells, or human TT c-cell lines (figures not shown).

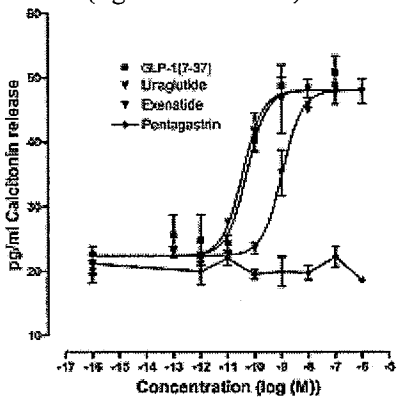


Figure 7 Pentagastrin did not stimulate calcitonin release from MTC 6-23 cells.

Data are from experiment 14725-001.

[N000 4.2.3.7.3 P18]

Calcium dose-dependently stimulates calcitonin release from MTC 6-23 cells (Figure 8A). Liraglutide enhances calcium-stimulated calcitonin release, and the effect is greater at higher calcium concentrations (Figure 8B)

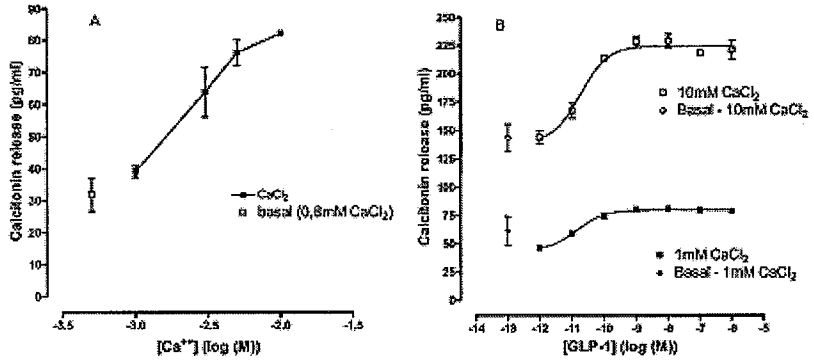


Figure 8 Calcitonin release from MTC 6-23 cells with varying calcium concentrations.

[N000 4.2.3.7.3 P19]

GLP-1R agonists had the same rank order potency of agonist-induced cAMP accumulation (GLP-1(7-37) > exenatide >> liraglutide) in CA-77 cells with similar potencies compared to MTC 6-23 cells (Figure 10). GLP-1R agonists didn't stimulate adenylyl cyclase in human TT cells, but the positive control, forskolin, did. GLP-1(7-37), but not pentagastrin, stimulated calcitonin secretion from CA-77 cells.

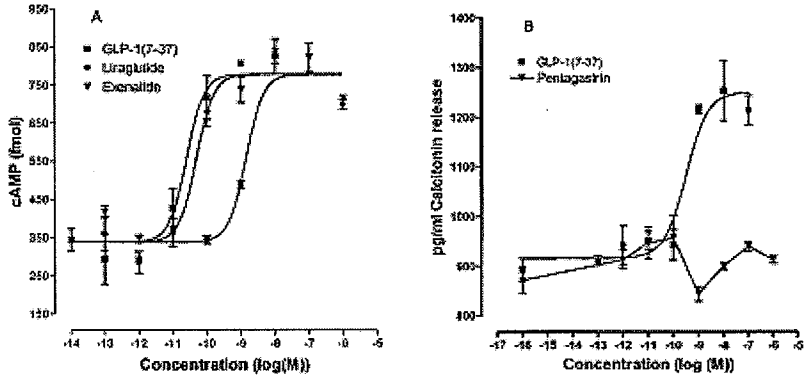


Figure 10 cAMP and calcitonin data from the rat thyroid C-cell line, CA-77.

The data are from experiments 13737-056 and 14725-002.

[N000 4.2.3.7.3 P21]

Forskolin increased cAMP accumulation (not shown) and calcitonin secretion from human TT cells (Figure 12), but GLP-1R agonists GLP-1(7-37), exenatide, or liraglutide did not.

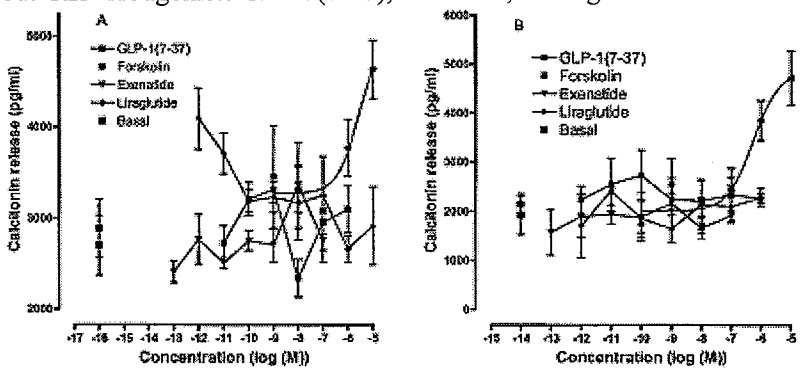


Figure 12 Calcitonin release from human C-cell line, TT.

[N000 4.2.3.7.3 P23]

The rank order potency and absolute potencies of GLP-1R agonists GLP-1, exenatide, and liraglutide to stimulate intracellular cAMP accumulation were similar in a rat pancreatic beta cell line, RIN2A18, and rat thyroid c-cell MTC 6-23 cells (Table 1, below)

	GLP-1	Exenatide	Liraglutide
cAMP EC_{50} (pM) RIN2A18	96±14 n=8	59±27 n=2	1400±500 n=8
cAMP EC_{50} (pM) rMTC 6-23	120±70 n=13	90±40 n=6	5800±2800 n=10
Calcitonin EC_{50} (pM) rMTC 6-23	80±60 n=8 95±3 (10%HS, n=2) 68±52 (5%HS, n=5) 88±73 (1%HS, n=5)	55±26 n=3	5300±2400 n=6 7600±600 (10%HS, n=2) 4200±3600 (5%HS, n=5) 830±270 (1%HS, n=5)

Table 1 Potency for GLP-1, exenatide and liraglutide in rat pancreas and thyroid cell lines.

cAMP experiments were conducted using 0.1% serum albumin and calcitonin release experiments 15% serum (horse serum, HS) when nothing else is indicated.

[N000 4.2.3.7.3 P24]

- 205295 / Investigation of the mitogenic potential of liraglutide in rats and human C-cell lines

The mitogenic potential of liraglutide, human GLP-1 (7-37), and exenatide were determined by [^{3}H]thymidine incorporation into DNA of thyroid c-cell lines rat CA77, rat MTC 6-23, and human TT cells and as a positive control, rat insulinoma INS-1E cells. Although fetal calf serum caused a proliferative response in rat and human c-cell lines, none of the GLP-1R agonists were mitogenic (Figure 5). To demonstrate activity of GLP-1R agonists in this assay, GLP-1 (7-37) was a mitogen in rat INS-1E cells, but only at low glucose concentrations. Gastrin and EGF were previously characterized mitogens in human TT cells, but TT cells used in this assay did not proliferate in response to either and these cells were devoid of gastrin receptors in a functional binding assay.

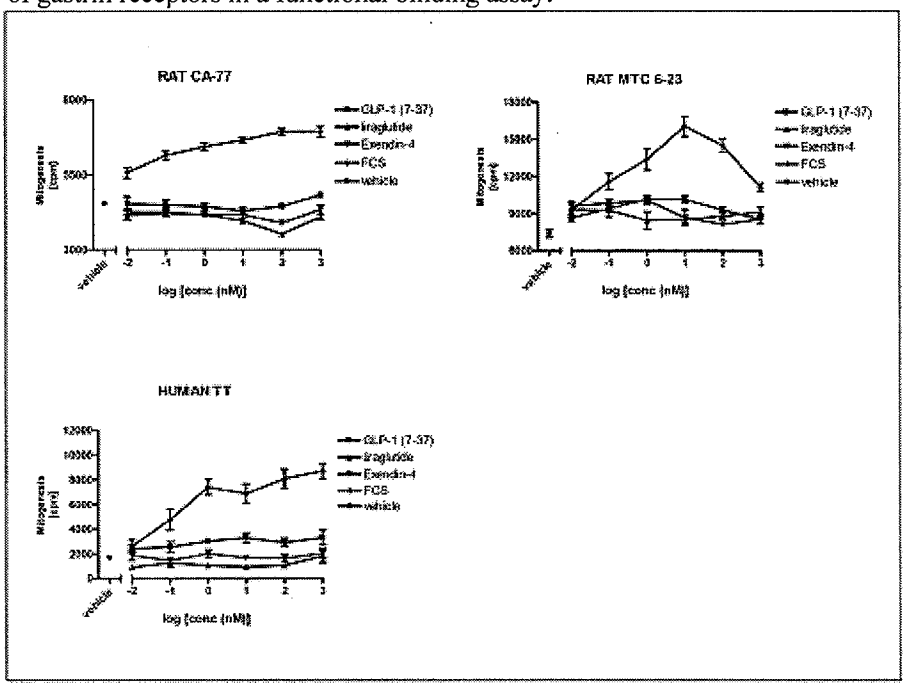


Figure 5 GLP-1 and GLP-1 analogue mediated proliferation of the three C-cell lines.

[N000 4.2.3.7.3 P18]

- 13736-092 / Liraglutide binding to rat gastrin (CCK2R) and bombesin (BB2R) receptors in AR42J cells

Liraglutide doesn't elicit calcitonin secretion from c-cells by activating CCK_2 (gastrin receptor) or bombesin (gastrin releasing peptide receptor) receptors. Varying concentrations up to 1 μM GLP-1 (7-37), exenatide, or liraglutide did not displace specific ^{125}I-CCK-8 or [^{125}I]Tyr4-bombesin binding to cell membranes from rat AR42J cells, a pancreatic acinar cell line endogenously expressing CCK_2 and bombesin receptors, respectively. Both CCK_2 and bombesin receptors mediate cell proliferation through a phospholipase C dependent, Gq/G11-coupled pathway.

Table 1 Result summary

Test item	Gastrin/CCK2 receptor IC_{50}* [95% CI] (nM)	Bombesin/BB2 receptor IC_{50}* [95% CI] (nM)
Liraglutide	> 1,000	> 1,000
Exendin-4	> 1,000	> 1,000
GLP-1	> 1,000	> 1,000
Pentagastrin	0.54 [0.14 – 2.0]	-
Gastrin-17	1.9 [0.09 – 39]	-
Tyr^4-Bombesin	-	0.23 [0.19 – 0.27]
D-(Phe^6,Leu-$NHEt^{13}$,des-Met^{14})Bombesin(6-14)	-	5.4 [2.4 – 13]

*IC_{50} values are calculated from the mean of Log(IC_{50}) from 3 experiments, except for pentagastrin, which is from 4 experiments.

[N000 4.2.3.7.3 P9]

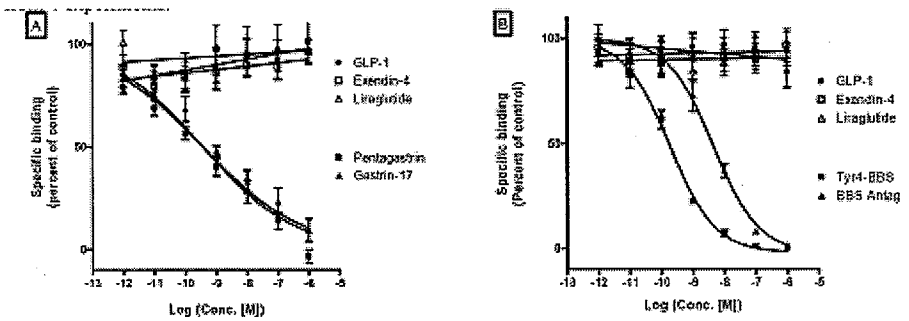


Figure 1 Displacement by GLP-1, exendin-4 and liraglutide of A) ^{125}I-CCK-8 and B) ^{125}I-Tyr^4-bombesin binding to gastrin and bombesin receptors, respectively, from the rat pancreatic acinar cell line AR42J.

[N000 4.2.3.7.3 P9]

RATS

- 203281 / Effects on calcium homeostasis after a single subcutaneous administration to male rats in a fasted condition – Combined evaluation of the in life phase, hormone analysis, statistical analysis, and molecular analysis

AND

- 203282 / Study on acute effects on calcium homeostasis related hormones after single dose subcutaneous administration in fasted and calcium treated rats

In male Sprague Dawley rats, a single subcutaneous dose of 0.75 mg/kg liraglutide transiently increased plasma calcitonin < 2 fold peaking 1 hour after dosing. Increased plasma PTH occurring 6 hours after dosing was considered a counter-regulatory response, but it occurred in the absence of substantial changes in plasma calcium concentrations. Consistent with liraglutide-induced increased calcitonin secretion, liraglutide decreased calcitonin peptide levels in thyroid, but it also decreased thyroid calcitonin mRNA levels within 6 hours of dosing. In male rats calcium loaded by calcium gluconate ip injection, a single sc dose of 0.75 mg/kg liraglutide transiently increased calcitonin secretion < 2 fold above that induced by calcium gluconate alone. Within 6 hours of dosing, calcium loading alone decreased thyroid calcitonin peptide levels and increased calcitonin mRNA levels. In calcium loaded rats, liraglutide increased calcitonin peptide levels in thyroid and increased calcitonin mRNA levels above those elicited by calcium loading alone. These results suggest in calcium loaded rats, liraglutide increased

calcitonin transcription and translation more than it increased calcitonin secretion, and its effects on calcitonin secretion, transcription, and translation were greater than for calcium loading alone.

Plasma calcitonin, PTH, and calcium levels were measured up to 6 hours after administration of a single subcutaneous dose of 0 (vehicle) or 0.75 mg/kg liraglutide (2 mL/kg, neck injection site) in fasted male Sprague Dawley rats (30/dose, fasted 12 – 14 hours prior to dosing, study 203281). A second study used the same study design, except rats were also given a single intraperitoneal injection of 1 mM /kg calcium after vehicle or liraglutide treatment to determine if increased calcium levels affect the response to liraglutide (study 203282). At the end of the study, thyroid calcitonin protein and transcript levels were quantified. Study observations were body weight, ionized calcium, plasma pH, adjusted ionized calcium (pH 7.4), plasma intact PTH, and plasma calcitonin with orbital plexus blood samples taken fromisoflurane/O_2/N_2O anesthetized rats prior to dosing and 0.25, 0.5, 1, 3, or 6 hours after dosing.

There were no treatment-related deaths, clinical signs or body weight changes with or without injected calcium. One rat in the liraglutide + calcium group died during anesthesia, but its death was not attributed to treatment.

In the absence of injected calcium, there were no changes in plasma ionized calcium compared to calcium concentrations prior to dosing (uncorrected or pH corrected calcium, see the summary table below where group 1 is control and group 2 is liraglutide treated). Liraglutide appeared to increase blood pH between 0.5 and 3 hours after dosing (Figure 3).

Blood sampling time point (hours after dosing)	Group mean Ca^{++} as % of levels before dosing		Group mean Ca^{++}(pH 7.4) as % of levels before dosing	
	Group 1	Group 2	Group 1	Group 2
0.25	0 %	0 %	0 %	1 %
0.5	-2 %	-4 %	-3 %	-4 %
1	0 %	-3 %	0 %	-2 %
3	3 %	-2 %	2 %	-1 %
6	2 %	0 %	1 %	-1 %

[N000 4.2.3.7.3 P28]

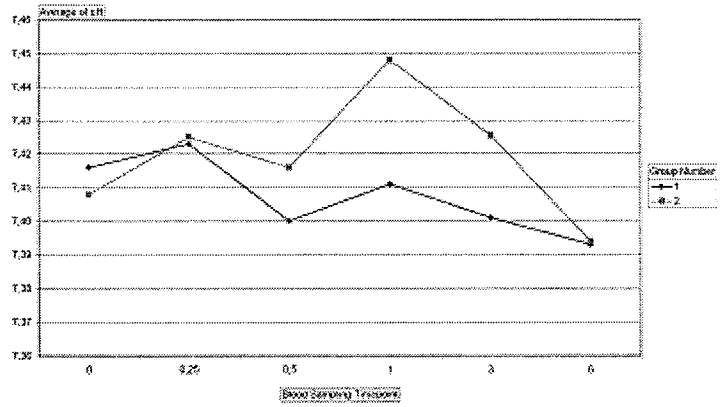


Figure 3 pH of blood

[N000 4.2.3.7.3 P32]

Compared to the control group, plasma calcium levels in the liraglutide treated group trended lower from 0.5 hours onward in rats concurrently administered calcium (below). There were no liraglutide-related changes in pH in rats concurrently injected with calcium (Figure 3, below).

Blood sampling timepoint (hours after dosing)	Group mean Ca^{++} (mmol/L) as % of levels before dosing		Group mean Ca^{++}(pH 7.4) (mmol/L) as % of levels before dosing	
	Group 1	Group 2	Group 1	Group 2
0.25	27 %	33 %	27 %	32 %
0.5	17 %	14 %	16 %	12 %
1	15 %	8 %	12 %	7 %
3	0 %	-3 %	-2 %	-4 %
6	2 %	-2 %	2 %	-3 %

[N000 4.2.3.7.3 P26]

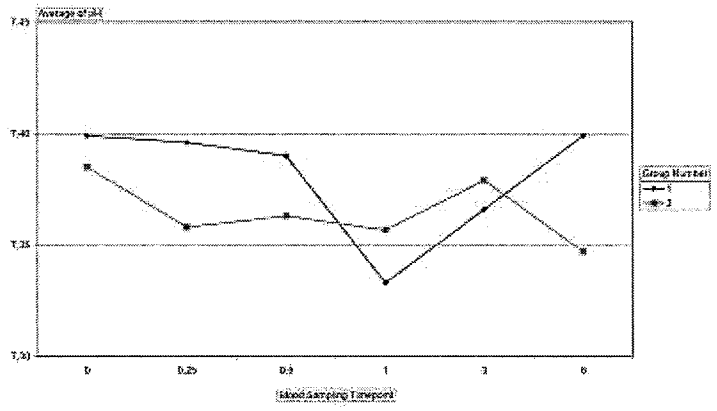


Figure 3 pH of blood

[N000 4.2.3.7.3 P30]

Plasma calcitonin was significantly increased 1 hour after dosing in the 0.75 mg/kg liraglutide treated group, but the increase wasn't sustained, probably because pH adjusted plasma ionized calcium levels were decreased. The sponsor hypothesized decreased plasma calcium was secondary to increased excretion in urine, but this was not demonstrated in this study. Decreased plasma calcium may have countered any sustained effect of liraglutide to increase calcitonin. Increased plasma intact PTH occurring 6 hours after dosing in the liraglutide treated group was consistent with lower plasma calcium.

Reviewer note: The sponsor determined statistical significance of treatment related changes in plasma calcitonin, calcium, and intact PTH using natural log transformed values.

In the liraglutide treated group, plasma calcitonin increased 0.5 and 1 hour after dosing (Figure 2, Table 2). Increased calcitonin levels did not occur at liraglutide's Tmax, which typically occurs 3 – 6 hours after subcutaneous dosing. PTH levels in the liraglutide group were increased 6 hours after dosing. Increased PTH in the liraglutide treated group is consistent with a counter-regulatory response to minimally decreased blood calcium concentration.

Figure 2. Calcitonin Response to Vehicle and Liraglutide (NNC 90-1170) (mean +/- SEM)

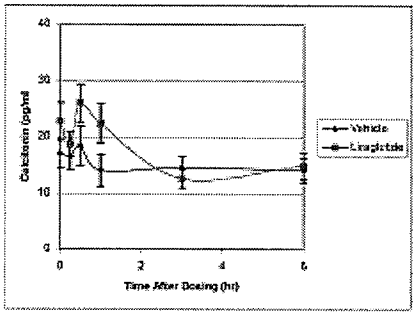
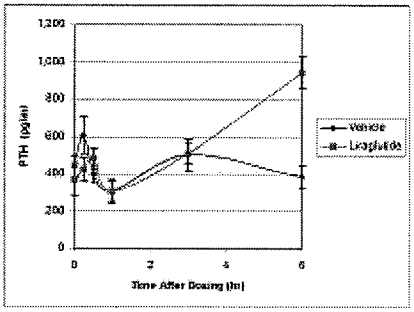


Figure 1. Intact PTH Response to Vehicle and Liraglutide (NNC 90-1170) (mean +/- SEM)



[N000 4.2.3.7.3 P90]

Table 2. Calcitonin and PTH Levels in Vehicle- and Liraglutide (NNC 90-1170)-Treated Animals

Treatment Group	Time Point	Data	Calcitonin	Intact Parathyroid Hormone
	hrs		pg/mL	pg/mL
Vehicle	0	mean	17.60	443.72
		SD	8.29	179.08
		SEM	2.62	56.63
		n	10	10
Vehicle	0.25	mean	16.46	689.75
		SD	7.34	318.53
		SEM	2.32	100.73
		n	10	10
Vehicle	0.5	mean	18.35	399.16
		SD	11.15	157.41
		SEM	3.53	49.78
		n	10	10
Vehicle	1	mean	14.10	305.86
		SD	8.86	198.95
		SEM	2.80	62.91
		n	10	10
Vehicle	3	mean	14.51	502.52
		SD	6.51	272.31
		SEM	2.06	86.11
		n	10	10
Vehicle	6	mean	14.10	383.69
		SD	6.73	195.67
		SEM	2.13	61.84
		n	10	10

Table 2. Calcitonin and PTH Levels in Vehicle- and Liraglutide (NNC 90-1170)-Treated Animals (cont.)

Treatment Group	Time Point	Data	Calcitonin	Intact Parathyroid Hormone
	hrs		pg/mL	pg/mL
Liraglutide	0	mean	22.33	372.08
(NNC 90-1170)		SD	10.62	268.27
		SEM	3.36	84.83
		n	10	10
Liraglutide	0.25	mean	18.64	424.90
(NNC 90-1170)		SD	6.93	206.30
		SEM	2.19	65.17
		n	10	10
Liraglutide	0.5	mean	26.05	482.30
(NNC 90-1170)		SD	10.75	178.34
		SEM	3.40	56.40
		n	10	10
Liraglutide	1	mean	22.39	307.67
(NNC 90-1170)		SD	11.08	161.29
		SEM	3.51	51.00
		n	10	10
Liraglutide	3	mean	12.54	508.66
(NNC 90-1170)		SD	5.07	165.02
		SEM	1.60	52.19
		n	10	10
Liraglutide	6	mean	14.83	945.28
(NNC 90-1170)		SD	8.37	275.45
		SEM	2.65	87.11
		n	10	10

[N000 4.2.3.7.3 P91-92]

In calcium loaded rats, calcitonin levels were markedly increased within 1 hour after calcium loading and returned to baseline levels within 3 hours (Figure 1a, Table 3a, b below). Calcitonin levels in the liraglutide treated group were < 2 fold higher than in the vehicle control group 0.25 hours after dosing, but liraglutide's Tmax after subcutaneous dosing in rats typically occurs 3 – 6 hours after dosing. Six hours after dosing, iPTH was higher in the liraglutide group compared to controls, and increased iPTH was consistent with minimally lower plasma calcium levels in the liraglutide treated group compared to controls starting 3 hours after calcium loading.

Figure 1b Calcitonin Values – All Data (mean +/- SEM)

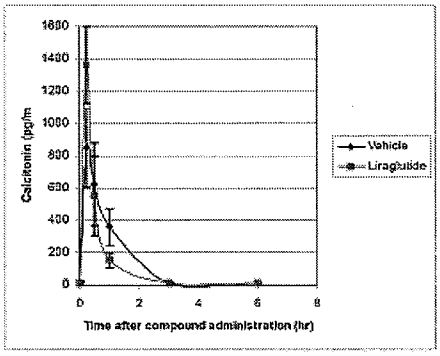
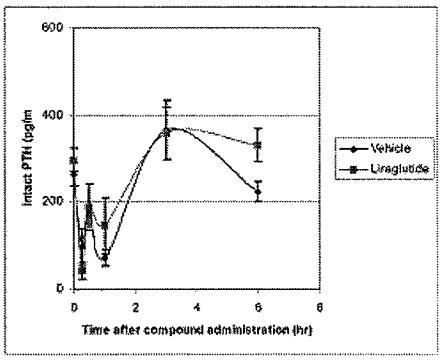


Figure 1a PTH Values – All Data (mean +/- SEM)



[N000 4.2.3.7.3 P88]

Table 3a Summarized Results

Treatment	Time Point hrs	Data	Calcitonin pg/mL	PTH pg/mL
Vehicle	0	mean	71.78	364.80
		SD	15.00	86.01
		SEM	4.74	27.20
		n	10	10
Vehicle	0.25	mean	848.44	97.27
		SD	774.32	123.63
		SEM	244.86	43.71
		n	10	8
Vehicle	0.5	mean	623.13	189.14
		SD	807.59	168.46
		SEM	255.38	53.27
		n	10	10
Vehicle	1	mean	360.43	72.06
		SD	360.60	54.70
		SEM	114.03	18.23
		n	10	9
Vehicle	3	mean	14.92	366.02
		SD	9.35	193.48
		SEM	2.96	68.41
		n	10	8
Vehicle	6	mean	15.35	224.41
		SD	9.83	75.89
		SEM	3.11	24.00
		n	10	10

Table 3b Summarized Results

Treatment	Time Point hrs	Data	Calcitonin pg/mL	PTH pg/mL
Liraglutide (NNC 90-1170)	0	mean	12.41	297.38
		SD	5.02	79.20
		SEM	1.67	26.40
		n	9	9
Liraglutide (NNC 90-1170)	0.25	mean	1364.35	42.07
		SD	748.14	57.50
		SEM	236.58	18.18
		n	10	10
Liraglutide (NNC 90-1170)	0.5	mean	553.31	188.85
		SD	773.90	170.40
		SEM	244.73	53.88
		n	10	10
Liraglutide (NNC 90-1170)	1	mean	154.90	146.59
		SD	139.69	169.17
		SEM	46.56	63.94
		n	9	7
Liraglutide (NNC 90-1170)	3	mean	14.39	358.88
		SD	7.85	156.34
		SEM	2.48	59.09
		n	10	7
Liraglutide (NNC 90-1170)	6	mean	13.92	331.12
		SD	4.95	110.80
		SEM	1.57	36.93
		n	10	9

[N000 4.2.3.7.3 P86-87]

Calcium loading reduced thyroid calcitonin peptide levels in vehicle treated rats, but in liraglutide treated rats, calcium loading increased thyroid calcitonin levels (Table 7). The effect of calcium to decrease thyroid calcitonin levels is consistent with increased calcitonin release, but the effect of liraglutide to increase thyroid calcitonin levels in calcium loaded rats is paradoxical, but to explain this effect, the sponsor suggests liraglutide increases calcitonin translation more than it increases secretion.

Table 7 The effect of calcium-load itself on calcitonin protein level in thyroid tissue from rat.

Time point after treatment	*Vehicle rats*[C]		*Liraglutide-treated rats*[C]	
	Without calcium-load (NN203181)	*Calcium-loaded (NN203182)*	*Without Calcium-load (NN203181)*	*Calcium-loaded (NN203182)*
1 hour	1.0	-1.1[$]	1.0	2.5[$]
3 hours	1.0	-1.6	1.0	1.7
6 hours	1.0	-2.2	1.0	4.7

[C] The effect of calcium-load itself in vehicle rats was expressed as fold up- or downregulation of the level of calcitonin peptide in calcium-loaded vehicle rats (from study *NN203182*) when compared to vehicle rats without calcium-load (from study *NN203181*). [C] Likewise the effect of calcium-load itself in liraglutide-treated rats was expressed as fold up- or downregulation on the level of calcitonin peptide in calcium-loaded and liraglutide-treated rats (*NN203182*) when compared to liraglutide-treated rats without calcium-load (*NN203181*). [$] Positive value = "fold upregulation". Negative value = "fold downregulation".

[N000 4.2.3.7.3 P148]

Compared to vehicle treated fasted rats, liraglutide decreased thyroid calcitonin levels up to 2.7 fold, consistent with it proposed effect to increase calcitonin secretion. In calcium loaded rats, liraglutide treatment increased thyroid calcitonin levels up to 3.8 fold. The difference in the effect of liraglutide on thyroid calcitonin levels in the presence and absence of calcium loading suggests liraglutide increases calcitonin synthesis more than it enhances calcium evoked calcitonin secretion.

Table 6 The effect of liraglutide on the levels of calcitonin peptide in thyroid tissue from rat.

Time point after treatment	*Fasted rats[a] (NN203181)*		*Fasted, calcium-loaded rats[a] (NN203182)*	
	Vehicle	*Liraglutide-treated*	*Vehicle*	*Liraglutide-treated*
1 hour	1.0	-1.4[b]	1.0	1.9
3 hours	1.0	1.5[b]	1.0	1.7
6 hours	1.0	-2.7	1.0	3.8
Pool 1-3-6 hours	1.0	-1.3	1.0	2.3

[a] The effect of liraglutide-treatment was expressed as fold up- or downregulation of the protein level in treated rats when compared to vehicle rats.
[b] Positive value = "fold upregulation"; Negative value = "fold downregulation".

[N000 4.2.3.7.3 P148]

Calcium loading significantly increased thyroid calcitonin mRNA levels in vehicle treated rats 3 and 6 hours after dosing and in liraglutide treated rats at 1, 3, and 6 hours after dosing (Table 5).

Table 5 The effect of calcium-load itself on calcitonin mRNA levels in thyroid tissue from rat.

Time point after treatment	*Vehicle rats[a]*		*Liraglutide-treated rats[a]*	
	Without calcium-load (NN203181)	*Calcium-loaded (NN203182)*	*Without calcium-load (NN203181)*	*Calcium-loaded (NN203182)*
1 hour	1.0	1.1[b]	1.0	6.8**
3 hours	1.0	3.6*	1.0	6.5**
6 hours	1.0	3.5*	1.0	12.9**

[a] The effect of calcium-load itself in vehicle rats was expressed as fold up- or downregulation of the mRNA level of calcitonin in calcium-loaded vehicle rats (from study *NN203182*) when compared to vehicle rats without calcium-load (from study *NN203181*). [a] Likewise, the effect of calcium-load itself in liraglutide-treated rats was expressed as fold up- or downregulation of calcitonin mRNA level in calcium-loaded and liraglutide-treated rats (*NN203182*) when compared to liraglutide-treated rats without calcium-load (*NN203181*). [b] Positive value = "fold upregulation". *,** Significant by students t-test $p<0.05$; $p<0.001$

[N000 4.2.3.7.3 P147]

The effect of liraglutide on thyroid calcitonin mRNA depends on plasma calcium. In fasted rats, liraglutide lowered thyroid calcitonin mRNA levels, but in calcium loaded rats, liraglutide increased calcitonin mRNA levels more than calcium loading alone (Table 4).

Table 4 The effect of liraglutide on calcitonin mRNA expression levels in thyroid tissue from rat.

Time point after treatment	*Fasted rats[a] (NN203181)*		*Fasted, calcium-loaded rats[a] (NN203182)*	
	Vehicle	*Liraglutide-treated*	*Vehicle*	*Liraglutide-treated*
1 hour	1.0	-3.5[b]	1.0	1.8[b]
3 hours	1.0	-1.3	1.0	1.4
6 hours	1.0	-2.5	1.0	1.5
Pool 1-3-6 hours	1.0	-2.2*	1.0	1.5*

[a] The effect of liraglutide-treatment was expressed as fold up- or downregulation of the mRNA level in treated rats when compared to vehicle rats.
[b] Positive value = "fold upregulation"; Negative value = "fold downregulation". *Significant by students t-test $p<0.05$.

[N000 4.2.3.7.3 P147]

- 203258 / The effects on calcium homeostasis after a single subcutaneous administration to male rats in a nonfasted condition – Combined evaluation of the in life phase, hormone analysis, and statistical analysis

The effect of a single subcutaneous dose of liraglutide on calcium homeostasis parameters was determined in non-fasted male Sprague Dawley rats (50/dose) administered 0 (vehicle) or 0.75 mg/kg liraglutide (2 mL/kg). In this study, liraglutide had no effect on plasma calcitonin, but iPTH increased 6 hours after dosing and decreased plasma calcium occurring 1 to 24 hours after dosing were attributed to liraglutide-induced diuresis.

Study parameters were mortality and clinical signs, body weight, plasma ionized calcium, pH, phosphate, albumin, PTH, and calcitonin, and urinalysis (urine volume and total calcium, phosphate, magnesium, sodium, potassium and chloride). There were no treatment-related mortalities, clinical signs, or body weight changes.

Plasma calcium in the liraglutide group (group 2) trended lower than the concurrent control group (group 1) from 1 to 24 hours after dosing (Figure 1) and this decrease was unaffected by adjusting the calcium concentration (Figure 3) for pH (Figure 2). Decreased plasma calcium in the liraglutide treated group was attributed to increased urine excretion during the first 8 hours after dosing.

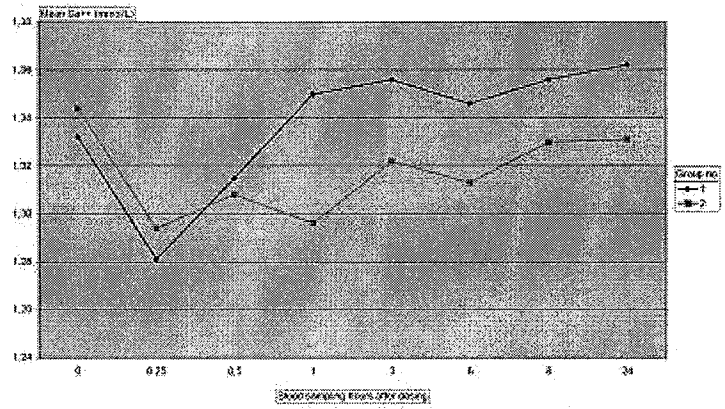


Figure 1 Mean plasma Ca^{++} (mmol/L)

[N000 4.2.3.7.3 P29]

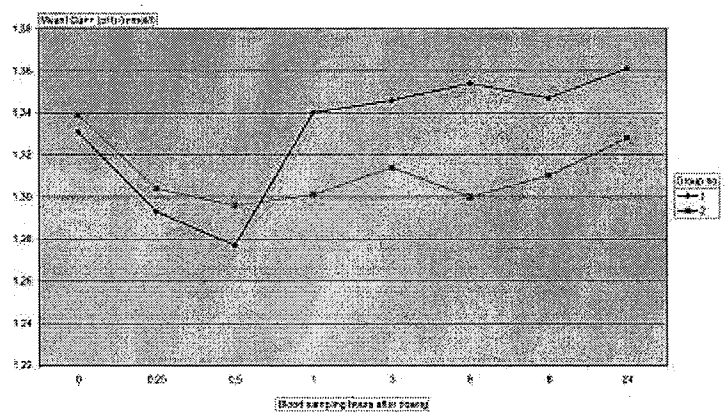


Figure 3 Mean adjusted Ca^{++}(pH 7.4)

[N000 4.2.3.7.3 P31]

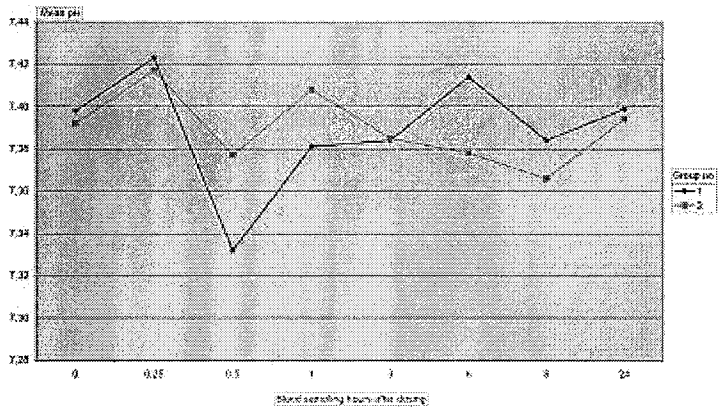


Figure 2 Mean plasma pH

[N000 4.2.3.7.3 P30]

Albumin levels in the liraglutide treated group were higher than the concurrent control from 3 to 24 hours after dosing, and this was attributed to blood volume contraction due to the diuretic effect of liraglutide.

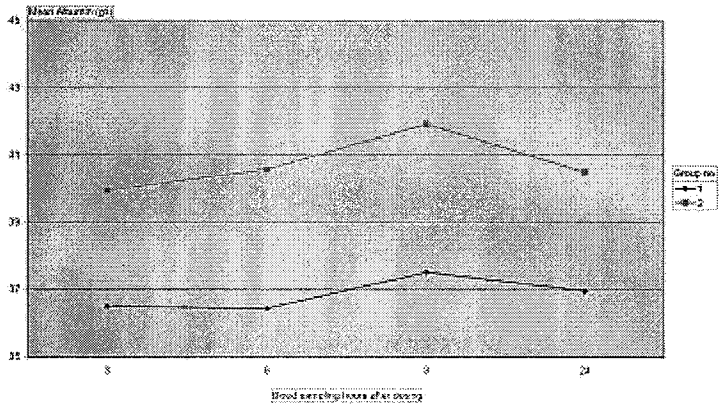


Figure 4 Mean plasma albumin (g/L)

[N000 4.2.3.7.3 P32]

Lower plasma phosphate levels in the liraglutide treated group from 3 to 24 hours after dosing were attributed to increased phosphate excretion in urine.

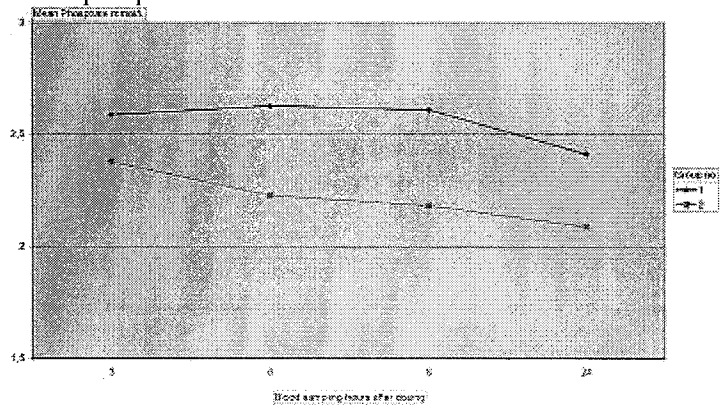


Figure 5 Mean plasma phosphate (mmol/L)

[N000 4.2.3.7.3 P33]

Plasma calcitonin levels were not increased in the liraglutide treated group. Consistent with decreased calcium levels, intact PTH was significantly increased 6 hours after dosing in the liraglutide treated group.

Figure 2 Calcitonin Response to Vehicle and Liraglutide (NNC 90-1170) (mean +/- SEM)

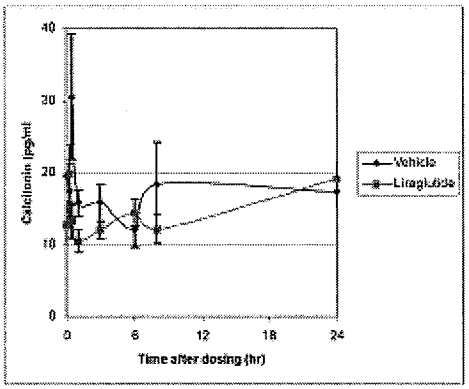
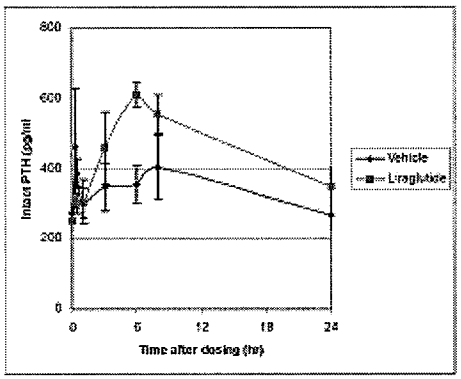


Figure 1 Intact PTH Response to Vehicle and Liraglutide (NNC 90-1170) (mean +/- SEM)



[N000 4.2.3.7.3 P66-7]

Liraglutide had a diuretic effect with significantly increased urine volume from 0 – 8 hours and 0 – 24 hours after dosing, increased total sodium and phosphorus at 0 – 24 hours, and decreased total potassium and chloride at 0 – 24 hours (Table 3). During the 24 hour urine collection period after dosing, there were no changes in total calcium or magnesium excretion. Physiologic levels of calcitonin can increases phosphate and sodium excretion without affecting calcium or magnesium excretion. In the absence of increased plasma calcitonin, diuretic and naturetic effects may be directly attributed to liraglutide.

Table 3 Urine analysis

Urine collected between 0-24 hours

	Group	Sex		Vol (ml)	K^+ (mmol/L)	K^+ (mmol)	Na^+ (mmol/L)	Na^+ (mmol)	CT (mmol/L)	CT (mmol)
Vehicle			Mean	10.40	1287.30	5.15	570.36	2.12	830.00	3.12
0 mg/kg	1	M	Stdev	2.07	166.53	0.53	136.23	0.52	181.24	0.37
			n	10	10	10	10	10	10	10
Liraglutide			Mean	23.46	361.84	2.81	290.96	2.56	237.26	2.16
0.75 mg/kg	2	M	Stdev	7.35	78.78	0.40	58.54	0.64	40.08	0.38
			n	10	9	9	9	9	9	9
			p-level	<0.001	<0.001	<0.001	0.071	<0.001	<0.001	<0.001
			Sign/method	***/SA[1]	***/ST[2]	***/ST	NS[3]/ST	***/ST	***/ST	***/SA

Urine collected between 0-24 hours	Group	Sex		Vol (ml)	Ca^{++} (mmol/L)	Ca^{++} (mmol)	Mg^{++} (mmol/L)	Mg^{++} (mmol)	PO_4^{--} (mmol/L)	PO_4^{--} (mmol)
Vehicle			Mean	10.40	4.26	0.02	17.04	0.07	105.85	0.61
0 mg/kg	1	M	Stdev	2.07	1.13	0.00	1.23	0.01	29.19	0.11
			n	10	10	10	10	10	10	10
Liraglutide			Mean	23.46	4.35	0.03	17.88	0.12	172.02	1.19
0.75 mg/kg	2	M	Stdev	7.35	0.85	0.01	1.49	0.04	48.41	0.17
			n	10	10	10	10	10	9	9
			p-level	<0.001	0.007	0.853	<0.001	0.185	<0.001	<0.002
			Sign/method	***/SA	**/ST	NS/ST	***/ST	NS/ST	***/ST	**/ST

[1]) SA: Satterthwaite's
[2]) ST: Students Test
[3]) NS: Non Significant

[N000 4.2.3.7.3 P38]

Consistent with liraglutide's Tmax, the peak effect on increased sodium and phosphate excretion occurred at 4 – 8 hours after dosing (see Table below). Figure 1 (below) shows liraglutide increased urine volume from 0 – 4 and 4 – 8 hours after dosing, but not at 8 – 24 hours post-dose. Liraglutide increased total urine calcium 0 – 4 and 4 – 8 hours after dosing, and consistent with increased PTH occurring 6 hours after dosing, calcium excretion in the liraglutide group was reduced 8 – 24 hours post-dose. The effects of liraglutide to increase urine volume and excretion of sodium and phosphate are consistent with increased plasma calcitonin, but there was no evidence of increased plasma calcitonin in the liraglutide treated group.

Urine collected between 4-8 hours

	Group	Sex		Vol (ml)	K^+ (mmol/L)	K^+ (mmol)	Na^+ (mmol/L)	Na^+ (mmol)	Cl (mmol/L)	Cl (mmol)
Vehicle			Mean	1.4	374.55	0.5093	203.4	0.2818	295.4	0.4039
0 mg/kg	1	M	Stdev	0.6	58.81	0.1523	65.0	0.1351	80.5	0.1492
			n	10	10	10	10	10	10	10
Liraglutide			Mean	4.8	112.53	0.5844	120.6	0.6202	69.7	0.3661
0.75 mg/kg	2	M	Stdev	2.1	26.43	0.1593	14.9	0.1094	9.7	0.1101
			n	10	9	9	9	9	9	9
			p-level	0.0005	0.3083	<0.0001	<0.0001	0.0029	0.5619	<0.0001
			Signif/method	***/SA	NS/ST	***/ST	***/ST	**/SA	NS/ST	***/SA

	Group	Sex		Vol (ml)	Ca^{++} (mmol/L)	Ca^{++} (mmol)	Mg^{++} (mmol/L)	Mg^{++} (mmol)	PO_4^{--} (mmol/L)	PO_4^{--} (mmol)
Vehicle			Mean	1.4	0.65	0.0009	1.27	0.0018	20.41	0.0317
0 mg/kg	1	M	Stdev	0.6	0.26	0.0006	0.78	0.0011	14.67	0.0279
			n	10	10	10	10	10	10	10
Liraglutide			Mean	4.8	2.71	0.0128	8.40	0.0402	45.02	0.2265
0.75 mg/kg	2	M	Stdev	2.1	1.05	0.0062	6.23	0.0178	19.41	0.1040
			n	10	10	10	10	10	9	9
			p-level	0.0005	0.0002	0.0001	<0.0001	<0.0001	<0.0001	0.0084
			Signif/method	***/SA	***/SA	***/SA	***/SA	***/SA	***/SA	***/SA

[N000 4.2.3.7.3 P40]

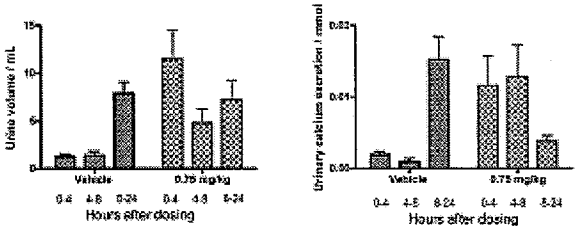


Figure 1 **NN203258. Urine volume and urinary calcium concentration (0-4-8-24 hours) in male rats treated with a single dose of liraglutide (0.75 mg/kg) or vehicle (mean and 95% CI).**
Significant treatment-related increases observed for both parameters ($p<0.01$).

[N000 4.2.3.7.3 P5]

- 203317 / Liraglutide: Effects on calcium homeostasis related parameters and thyroid volume fractions after up to six weeks daily subcutaneous administration followed by a 2 week reversibility period in male rats – Combined evaluation of in life phase, hormone analysis, and statistical analysis

The effect of once daily subcutaneous injections of 0 (vehicle) or 0.75 mg/kg/day NNC 90-1170 (2 mL/kg) on thyroid c-cells and plasma calcitonin was determined in young male Sprague Dawley rats (~2 months old at start of dosing) treated for 6 weeks followed by a 2 week recovery period. The main study group consisted of 65 rats/dose (45 days of treatment followed by a 2 week recovery) and a satellite

toxicokinetic group consisted of 15 rats /dose (28 days of treatment prior to necropsy). On day 45, all main study rats were administered a single intraperitoneal injection of 1 mmol/kg calcium after their final dose. Satellite group rats were administered 3 separate intraperitoneal injections of 50 mg/kg BrdU (10 mL/kg) at 48, 24, and 1 hour prior to scheduled necropsy. Antemortem observations were clinical signs, body weight, food consumption, urinalysis (satellite group, week 4, samples collected 0 – 4, 4 – 8, and 8 – 24 hours after dosing), blood calcium (including pH adjusted, satellite group, orbital blood taken 6 and 24 hours after dosing in weeks 1, 2, and 3), serum vitamin D (satellite group, sampled prior to dosing in week 3), and blood samples from the main study group taken prior to dosing and 0.25, 0.5, 1, 3, 6, 8, and 24 hours after dosing on study days 29 (non-fasting), 38 (fasting), 45 (fasting, calcium treated), and 58 (fasting) for determination of pH corrected calcium, plasma iPTH, and calcitonin. Rats were fasted for up to 6 hours after dosing, and then had free access to food. Main study rats were sacrificed and discarded after taking the last blood sample. Satellite group rats were sacrificed and necropsied with samples of thyroid, liver, and proximal duodenum saved for microscopic examination and/or mRNA analysis.

Injection site wounds, nodules, and swelling occurred in both vehicle control and NNC 90-1170 treated rats, and it was attributed to the vehicle. Decreased body weight, body weight gain, and food consumption in the 0.75 mg/kg/day NNC 90-1170 group was attributed to the GLP-1R mediated pharmacological effects of NNC 90-1170.

Urine output significantly increased 45% at 0 – 4 and 4 – 8 hours after dosing, but not at 8 – 24 hours postdose. Calcium excretion increased, phosphorus excretion decreased, and there were sporadic small decreases in sodium, potassium, and magnesium excretion. Increased urine volume is consistent with diuretic effects of GLP-1 or calcitonin and increased calcium excretion can be a be attributed to increased calcitonin.

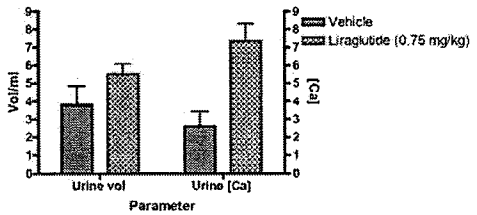


Figure 1 NN203317, Urine volume and urinary calcium concentration values (mean and 95% CI) measured 0-4 hours post-dose in male rats treated with liraglutide (0.75 mg/kg) or vehicle for 4 weeks.
Significant treatment-related increases observed for both parameters ($p<0.05$).

[N000 4.2.3.7.3 P7]

The effect of liraglutide treatment on plasma intact PTH and calcitonin were determined in nonfasting, fasting, and calcium-loaded fasting rats. Values for iPTH or calcitonin below the limit of assay detection were reported (see below).

"

Please note that some samples were diluted prior to calcitonin analysis to obtain sufficient volume to complete the assay. Several of these diluted samples produced a result that was below the LOQ of the assay and were flagged appropriately. However, once the assay result was multiplied by the dilution factor, the final result was above LOQ, when in fact the assay result used to calculate the final result was below LOQ, hence the LOQ flag attached the final result.

The LOD/LOQ values for the rat PTH and Calcitonin assays are, as follows:

PTH: LOD: 11.89 pg/ml
LOQ: 24.00 pg/ml

Calcitonin: LOD: 0.14 pg/ml
LOQ: 2.87 pg/ml

"

[N000 4.2.3.7.3 P129]

In rats treated for 6 weeks, PTH levels trended lower in NNC 90-1170 treated rats (Figure 3a). Calcium loading rapidly decreased PTH in both dose groups, but 3 – 8 hours after dosing, PTH levels rebounded to above baseline in the liraglutide group. Twenty-four hours after dosing, PTH was below baseline in both dose groups. In control and liraglutide treated groups, calcium loading increased calcitonin 35 – 50 fold above baseline, and calcitonin levels rapidly returned to near baseline levels within 3 hours after dosing.

Figure 3a: Effect of Liraglutide (NNC 90-1170) on PTH Under Fasting and Calcium-Loaded Conditions (mean +/- SEM)

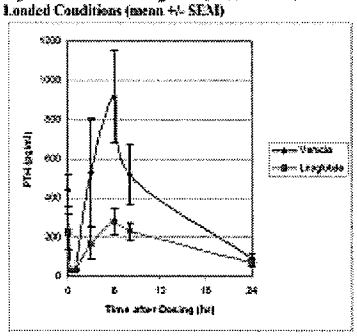
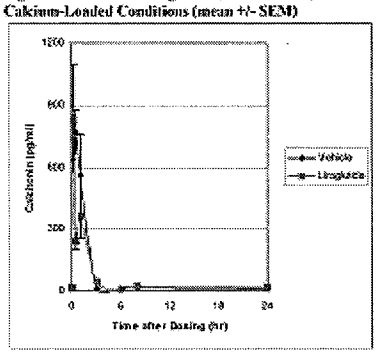


Figure 3b: Effect of Liraglutide (NNC 90-1170) on Calcitonin Under Fasting and Calcium-Loaded Conditions (mean +/- SEM)



[N000 4.2.3.7.3 P141]

In nonfasted rats in week 4, NNC 90-1170 had no discernable effect on PTH levels for up to 24 hours after dosing, but calcitonin levels trended higher (Figures 1a and 1b). The magnitude of any effect of liraglutide on calcitonin levels was small compared to calcium loading.

Figure 1a: Effect of Liraglutide (NNC 90-1170) on PTH Under Non-Fasting Conditions (mean +/- SEM)

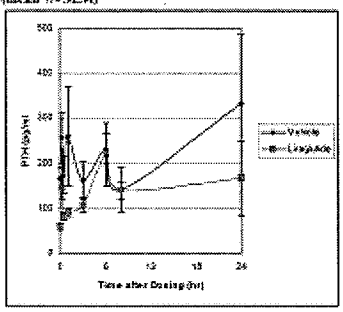
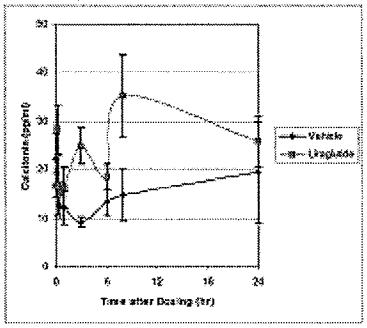


Figure 1b: Effect of Liraglutide (NNC 90-1170) on Calcitonin Under Non-Fasting Conditions (mean +/- SEM)



[N000 4.2.3.7.3 P133]

In fasted rats in week 5, NNC 90-1170 had no discernable effect on PTH levels for up to 24 hours after dosing, but plasma calcitonin levels trended higher (Figures 2a and 2b). PTH levels in both liraglutide and control groups peaked 3 hours after dosing and returned to baseline within 8 hours. Calcitonin levels were increased 6 and 8 hours after dosing.

Figure 2a: Effect of Liraglutide (NNC 90-1170) on PTH Under Fasting Conditions (mean +/- SEM)

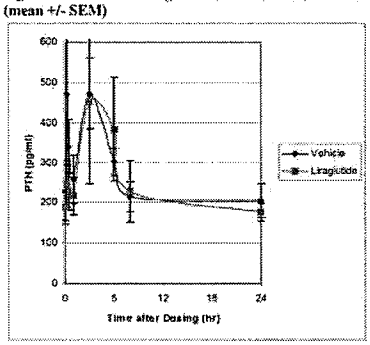
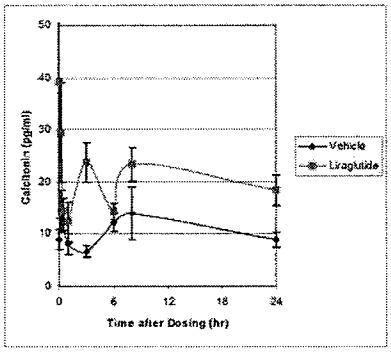


Figure 2b: Effect of Liraglutide (NNC 90-1170) on Calcitonin Under Fasting Conditions (mean +/- SEM)



[N000 4.2.3.7.3 P137]

In fasted rats in week 8, after 6 weeks of treatment followed by a 2 weeks recovery period, PTH levels trended higher and calcitonin levels trended lower in rats previously treated with liraglutide (Figures 2a and 2b). PTH levels in both liraglutide and control groups peaked 3 hours after dosing and returned to baseline within 8 hours. Effects on PTH and calcitonin occurring 6 hours after dosing may be due, at least in part, to feeding.

Figure 4a: PTH After Two-Week Recovery Period from Liraglutide (NNC 90-1170) Treatment (mean +/- SEM)

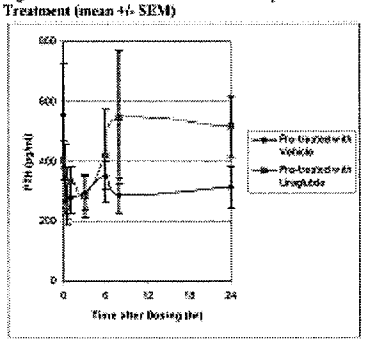
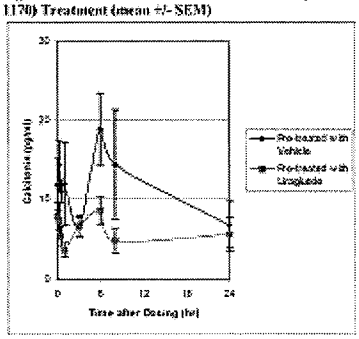


Figure 4b: Calcitonin After Two-Week Recovery Period from Liraglutide (NNC 90-1170) Treatment (mean +/- SEM)



[N000 4.2.3.7.3 P145]

In study week 3, 1,25 dihydroxy vitamin D was reduced in liraglutide treated rats compared to vehicle controls (Table 2).

Table 2. 1,25 Dihydroxyvitamin D levels

Treatment Group	Animal Number	1,25 dihydroxy-vitamin D (pg/ml)	Notes	Treatment Group	Data	1,25 dihydroxy-vitamin D (pg/ml)
Vehicle	70	69.61		Vehicle	mean	76.99
Vehicle	65	53.44			SD	24.85
Vehicle	66	59.03			SEM	6.42
Vehicle	67	82.40			n	15
Vehicle	68	48.02				
Vehicle	69	83.70	e			
Vehicle	71	113.05				
Vehicle	72	49.13				
Vehicle	73	99.68	e			
Vehicle	74	67.82	d			
Vehicle	75	90.00				
Vehicle	76	129.45	e			
Vehicle	77	55.38	e			
Vehicle	78	91.85				
Vehicle	79	69.32				
Liraglutide	157	54.41	e	Liraglutide	mean	54.82
Liraglutide	158	89.83	e		SD	22.04
Liraglutide	144	106.54	e		SEM	5.69
Liraglutide	145	67.96			n	15
Liraglutide	146	61.54				
Liraglutide	147	45.09	e			
Liraglutide	148	43.74				
Liraglutide	149	34.72	e			
Liraglutide	150	45.99				
Liraglutide	151	24.54	e			
Liraglutide	152	25.94				
Liraglutide	153	43.11				
Liraglutide	154	55.56				
Liraglutide	155	62.60	e			
Liraglutide	156	60.67	e			

d: percent CV of duplicate analyses exceeded acceptable range; unable to repeat due to lack of specimen
e: due to insufficient sample volume, sample was run in single

[N000 4.2.3.7.3 P187]

Unexpectedly, 4 weeks of treatment with 0.75 mg/kg/day NNC 90-1170 reduced absolute and relative thyroid weight, but it didn't affect the incidence of BrdU labeled cells, calcitonin immunoreactive cells (c-cells) or double labeled cells (calcitonin + BrdU, Table 2).

Table 2 Group mean values of absolute and relative volume fractions in thyroids of male rats dosed liraglutide for 4 weeks

Parameter		Group 1 (vehicle)	Group 2 (0.75 mg liraglutide/kg/day)
Body weight (g)	Mean	350	330*
	SD	27	20
	N	15	15
Thyroid weight (g)	Mean	0.0077	0.0054***
	SD	0.0020	0.0013
	N	15	15
Relative thyroid weight (g)	Mean	0.0022	0.0016**
	SD	0.0005	0.0004
	N	15	15
BrdU LI (Valve/Ver)	Mean	0.0379	0.0395
	SD	0.0539	0.0342
	N	13	14
Relative volume of follicular cells (Fol) (%)	Mean	37.11	36.71
	SD	5.70	6.38
	N	13	14
Relative volume of colloid (Col) (%)	Mean	21.46	24.96
	SD	4.28	5.93
	N	13	14
Relative volume of C-cells (C) (%)	Mean	1.18	1.00
	SD	0.51	0.50
	N	13	14
Relative volume of double stained C-cells (DSC) (%)	Mean	0.044	0.036
	SD	0.062	0.034
	N	13	14

* Statistically significant different to control at *p<0.05, ** p<0.01 and ***p<0.001 when applying a one way analysis of variance of log transformed values.

[N000 4.2.3.7.3 P217-218]

NNC 90-1170 did not affect relative calcitonin transcript levels in thyroid of rats treated for 4 weeks (Figure 7).

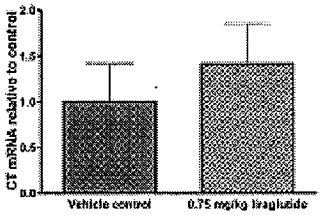


Figure 7 NN2040I8. Calcitonin (CT) mRNA in rats (14/animals/group) dosed with liraglutide for 4 weeks.
Increase in CT mRNA after 4 weeks of dosing. Calcitonin mRNA was quantified relative housekeeping genes (beta actin/GAPDH) and normalised towards mean levels in vehicle controls (mean and 95% CI).

[N000 4.2.3.7.3 P11]

- 204163 / Effects on plasma calcitonin, thyroid C-cell mass, and formation of antibodies after up to 483 days daily subcutaneous administration in young and aged male rats – Combined evaluation of the in life phase including antibody analysis, calcitonin

Liraglutide increased the incidence of age-related focal c-cell hyperplasia, but without accelerating its onset. The increased incidence of thyroid c-cell adenomas was liraglutide treatment duration dependent. Increased plasma calcitonin levels was age-dependent, and did not correlate with liraglutide dose or treatment-duration.

Effects of once daily subcutaneous injections of 0 (vehicle), 0.075, 0.25, or 0.75 mg/kg/day liraglutide (1 mL/kg) on thyroid c-cells and plasma calcitonin were determined in young male Sprague Dawley rats (2 months old at start of dosing, 10/dose/group, 160 rats total) treated for 7, 10, 13, or 16 months and aged male Sprague Dawley rats (8 months old at start of dosing, 10/dose/group, 160 rats total) treated for 1, 4, 7, or 10 months. The design for the in-life portion of the study is depicted in Figure 1. This study was designed to differentiate the effect of liraglutide treatment and age on plasma calcitonin and thyroid c-cell hyperplasia in male rats. Anti-liraglutide antibody formation was determined from orbital plexus blood from a satellite group of 10 male rats/dose with the same doses used in the main study and with blood sampling in weeks 13, 26, and 52 after a 3 day treatment-free period prior to sampling. Because wounds developed at the injection site within the first 3 months of the study in all dose groups, including controls, the vehicle was changed (although specific changes in the composition of the vehicle were not obvious). Study observations were mortality and clinical signs, body weight, fasting orbital venous plexus blood samples (2 rats/dose/time point, isoflurane anesthetized) for determining plasma calcitonin (14 and 28 days prior to starting treatment, then prior to dosing and 3 hours post-dose on treatment days 1, 28; 119; 210; 301; 392 and 483), plasma calcium (prior to starting treatment and 3 hours after dosing on the day of necropsy from all rats to determine uncorrected and pH corrected ionized calcium), anti-liraglutide antibodies (detected by precipitation of protein bound ^{125}I-liraglutide in plasma), and macroscopic and microscopic pathology including staining for calcitonin immunoreactivity in thyroid and BrdU labeling (by administering 3 intraperitoneal injections of 50 mg/kg BrdU (5-bromo-2-deoxyuridine, 10 mL/kg) to all main study group rats approximately 48, 24, or 3 hours prior to necropsy).

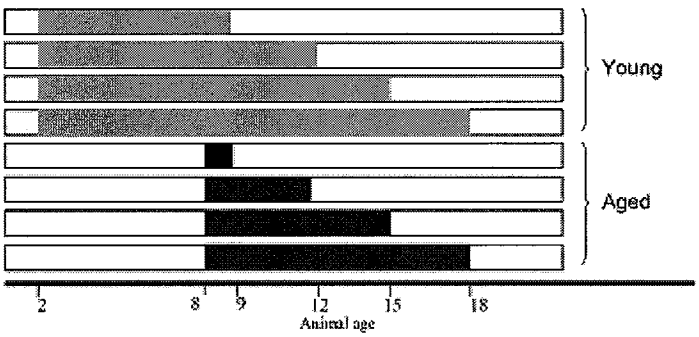


Figure 1 (NN204163) Design of In-life study. The age and duration of dosing (bars) was chosen to ensure same age of young (green) and aged rats (blue) at termination

[N000 4.2.3.7.3 P6]

Protocol deviations that were not considered sufficient to affect the integrity of the study included incorrect dose volumes, incorrect dose concentrations (including administering 0.075 mg/kg liraglutide to 3 control group rats), early removal of food for fasting prior to blood sampling, occasionally withholding treatment on study days 42 – 47 due to wound at or near the injection site on 6 rats, blood samples from 4 rats incorrectly identified and therefore not processed, and 3 decedents not replaced by backup rats at the time of necropsy.

Unscheduled deaths occurred in all dose groups, and rats that died on study were replaced. The incidence of unscheduled deaths was higher than controls at ≥ 0.075 mg/kg/day liraglutide in aged male rats and at ≥ 0.25 mg/kg/day in young male rats, but the increased incidence wasn't dose related. The table below shows the cause of death for rats dying on study, when a cause was identified.

Animal No	Group/subgroup No	Cause of death
63, 89, 118, 169, 170, 179, 300	2b, 2 backup, 3c, 4d, 4d, 4 backup and 7g	Eye problems
326, 344	8E, 8g	Convulsive fits
122	3e	Wounds
45, 86, 134, 297, 359	1 backup, 2 backup, 3 backup, 7h and 8 backup.	Tumour development
219, 310, 370	5h, 7h and 7 satellite	Found dead in the cage, partly eaten by the other rat in the cage.
380	7 satellite	Paralysed leg / Lame
84, 87, 147, 227, 348	2d, 2 backup, 4b, 6e, and 8h	One or more of the following clinical signs: sever weight loss, piloerection, dehydration, weakness, gassive behaviour or respiratory problems.
208, 209, 210	5g	Problems with anaesthesia in connection to blood sampling.

[N000 4.2.3.7.3 P46]

Two rats in the young 0.75 mg/kg/day group convulsed after dosing. After the vehicle associated with wound at the injection was changed, convulsions only occurred in 2 high dose young males that had repeated severe convulsions within 10 – 15 minutes of dosing that resulted in their humane sacrifice. Young high dose rat 326 had convulsions lasting 3 – 15 minutes after dosing from day 85 – 102, and the rat was necropsied on day 103. Young high dose group rat 344 had repeated severe convulsions 5 minutes after dosing on day 223 and it was sacrificed moribund. The incidence of wounds was higher in young rats at 0.75 mg/kg/day liraglutide, and although the sponsor believed the incidence decreased after the vehicle was changed (after up to 3 months of dosing), it was a frequent finding in this group after study day 120. Therefore, wound at the injection site were considered treatment-related at 0.75 mg/kg/day liraglutide in young rats.

Group	Aged				Young			
Liraglutide dose (mg/kg/day)	0	0.075	0.25	0.75	0	0.075	0.25	0.75
Unscheduled Deaths	1	5	3	4	3	2	5	4
Convulsions (related to dosing, # of affected rats)	1	1	0	0	0	2	0	2
Wounds	4	4	6	8	7	4	9	37

Consistent with liraglutide's GLP-1R agonist activity, group mean terminal body weight was significantly lower than controls at ≥ 0.25 mg/kg/day in aged rats and at ≥ 0.075 mg/kg/day in young rats (Figure 2) at all sacrifice times except for the 0.075 mg/kg/day group sacrificed on day 392.

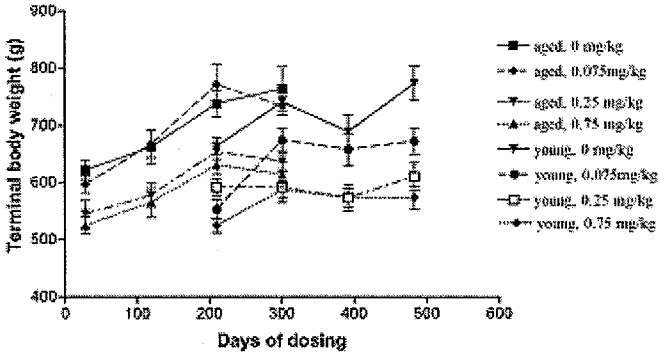


Figure 2 Terminal body weight of aged and young rats dosed liraglutide for various "days of dosing"

[N000 4.2.3.7.3 P659]

There were no liraglutide-related changes in plasma ionized calcium, plasma pH, or pH adjusted ionized calcium. Anti-liraglutide antibodies were not detected in plasma from liraglutide-treated rats. The table below shows plasma calcium, pH, and pH adjusted calcium concentration prior to starting treatment and on study day 301 (after 43 weeks of treatment in young (groups 1 – 4) and aged (groups 5 – 8) rats).

Males

GROUP	Ca++ DAY 301			pH DAY 301			Ca++ (pH 7.4) DAY 301		
	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
1	1.37	0.05	14	7.30	0.07	14	1.31	0.06	14
2	1.39	0.05	11	7.30	0.09	11	1.33	0.05	11
3	1.36	0.05	12	7.34	0.07	12	1.33	0.06	12
4	1.36	0.03	12	7.30	0.05	12	1.31	0.03	12
5	1.36	0.04	10	7.30	0.05	10	1.31	0.05	10
6	1.39	0.05	9	7.31	0.11	9	1.34	0.03	9
7	1.36	0.03	10	7.34	0.07	10	1.32	0.04	10
8	1.35	0.03	10	7.34	0.06	10	1.32	0.04	10

[N000 4.2.3.7.3 P659]

If liraglutide had any physiologically relevant effect on plasma calcitonin, it occurred within the first month of treatment, it was transient, and the magnitude was small. Group mean plasma calcitonin significantly increased ~1.3 – 1.9 fold above concurrent controls at 0.75 mg/kg/day in aged rats on study

28 and prior to dosing on study day 119 (Table 2). In young rats, plasma calcitonin significantly increased ~ 2 fold prior to and after dosing on day 28 at 0.75 mg/kg/day liraglutide, but calcitonin was significantly lower than controls in the 0.75 mg/kg/day group prior to dosing on day 119 and after dosing on days 119 and 210. There were no significant or consistent effects of liraglutide on plasma calcitonin at doses ≤ 0.25 mg/kg/day at any time.

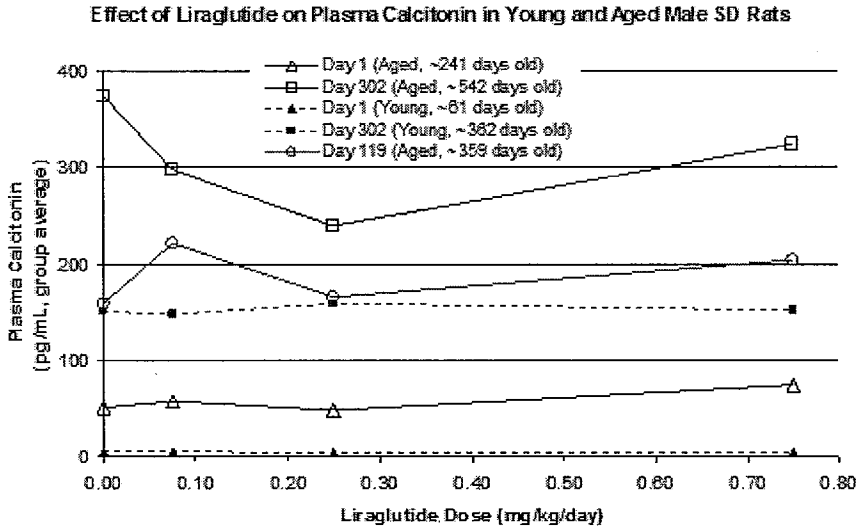
Table 2 Estimated ratio to the control group (C)

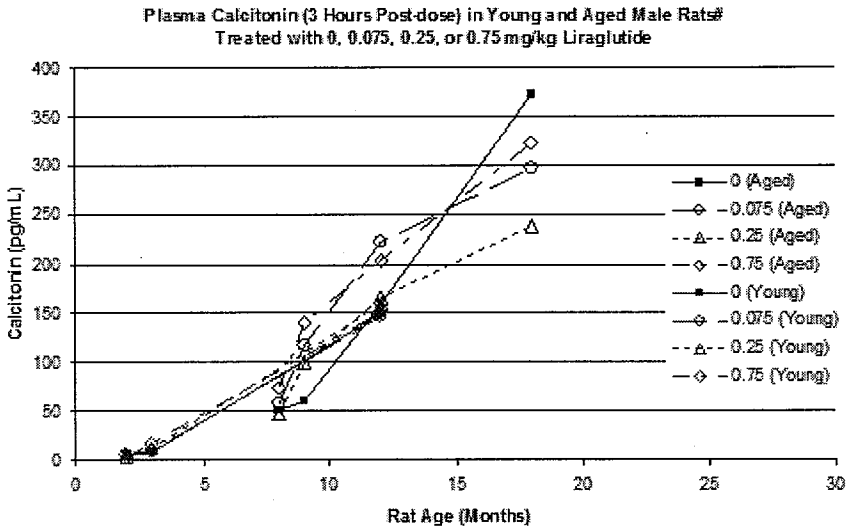
		Comparison											
		0.075 / C				0.25 / C				0.75 / C			
		post		Pre		post		pre		post		pre	
		ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value
Age group	day												
Young	28	1.16	0.303	1.12	0.418	1.52	0.003	1.78	0.000	2.06	0.000	2.08	0.000
	119	0.87	0.129	0.88	0.132	0.78	0.008	0.74	0.001	0.80	0.021	0.78	0.004
	210	0.92	0.459	1.04	0.687	0.77	0.021	0.78	0.028	0.82	0.081	0.79	0.031
	302	0.93	0.632	0.80	0.222	0.96	0.760	0.90	0.569	0.86	0.304	0.92	0.644
	393	0.95	0.782	0.95	0.763	0.87	0.423	1.00	0.984	1.11	0.568	1.20	0.285
	483	0.80	0.280	1.05	0.839	0.87	0.494	0.91	0.671	0.99	0.946	1.20	0.460
Old	28	1.19	0.126	1.22	0.020	1.30	0.024	1.20	0.039	1.87	0.000	1.37	0.000
	119	1.26	0.030	1.15	0.140	0.98	0.883	0.97	0.739	1.27	0.025	1.09	0.346
	210	1.30	0.054	1.31	0.048	1.08	0.552	0.98	0.909	1.30	0.059	1.27	0.082
	302	1.04	0.838	1.02	0.929	0.81	0.300	1.33	0.254	1.23	0.310	0.81	0.419

p-values < 0.05 are highlighted

[N000 4.2.3.7.3 P770]

Plasma calcitonin was not measured during the rat carcinogenicity study, but it was determined in mechanistic studies in male rats. Calcitonin measured in plasma of young male Sprague Dawley rats (2 months old at start of dosing) or aged male rats (8 months old at start of dosing) taken 3 hours after the first dose (day 1) of 0, 0.075, 0.25, or 0.75 mg/kg/day liraglutide and after dosing on day 302 showed increased plasma calcitonin was age dependent, but not liraglutide dose-dependent (see Figure below). Calcitonin levels in young rats treated with liraglutide for 302 days were similar to those in aged rats treated for 119 days, and these rats were the same chronological age at the time of sampling (~ 360 days old). Calcitonin levels were aged dependent, not liraglutide dose or treatment-duration dependent.





#When treatment started, young rats were 2 months old and aged rats were 8 month old.

There were no treatment-related macroscopic pathology findings. Treatment-related histopathology findings, confined to thyroid, were diffuse and focal c-cell hyperplasia and c-cell adenomas. An index combining the severity and incidence of diffuse c-cell hyperplasia suggests there were no treatment related changes in aged rats (Table 8, Figure 3), but diffuse c-cell hyperplasia was significantly lower in "young" rats sacrificed after 16 months (483 days) of treatment with 0.75 mg/kg/day liraglutide (Table 9, Figure 3). At the same dose after 16 months in young rats (0.75 mg/kg/day), the incidence of focal c-cell hyperplasia and adenomas were higher than controls.

Table 9 Summary of thyroid qualitative changes in young male rats dosed liraglutide for various periods

	Days of dosing (age)	210 (9 months)		301 (12 months)		392 (15 months)		483 (18 months)	
	Group (mg/kg)	1 (0)	4 (0.75)	1 (0)	4 (0.75)	1 (0)	4 (0.75)	1 (0)	4 (0.75)
No of animals examined		10	10	8	10	10	10	11	11
No of animals with NAD=			1						
Diffuse C-cell hyperplasiaS	Group mean ± SD	2.17 ± 0.5	1.66 ± 1.0	2.49±0.6	2.03±0.4	2.76 ± 0.7	2.38 ± 0.7	2.96±0.8	2.42±0.6*
Focal C-cell hyperplasiaS									
	Minimal	3	1	2	7	4	6	3	7
	Slight		1	1	1	1	2	2	3
	Total	3	2	3	8	5	8	5	10
C-cell adenoma			1		3	1	3	2	4
Dilated ultimobranchial ducts				1			1		
Dilated follicles		2	3		2	5	4	4	4
Focal interstitial inflammatory cell infiltration									
Focal hyperplasia in parathyroid									1
Focal follicular cell hyperplasia						1			

○ NAD, no abnormalities detected; S For diffuse C-cell hyperplasia a mean score was calculated for each animal as the sum of scores of the evaluation of the individual sections with C-cells present/no of sections with C-cells present. From these individual means a group mean was calculated. For focal C-cell hyperplasia, the highest score for each animal was tabulated. For adenoma and other changes the incidence is given. * statistically different from control p<0.05.

[N000 4.2.3.7.3 P677]

In 0.75 mg/kg/day aged rates, the incidence of minimal to slight focal c-cell hyperplasia was greater than concurrent controls after 28, 119, 210, and 301 days of treatment. At 0.75 mg/kg/day in young rats, the incidence of focal c-cell hyperplasia increased with treatment duration after 301, 392, and 483 days, but not at 210 days. These results suggest focal c-cell hyperplasia occurred after only 1 month of dosing in aged rats and after at least 301 days of dosing in younger rats. These results are consistent with the

absence of focal c-cell hyperplasia in the 6 month chronic repeat dose toxicity study of liraglutide in rats, which used 'young rats'. In 0.75 mg/kg/day groups, the incidence of c-cell adenomas increased with treatment duration after ≥ 210 day in both aged and younger rats. Increased focal c-cell hyperplasia and adenomas did not correlate with increased plasma calcitonin.

Table 8 Summary of thyroid qualitative changes in aged male rats dosed liraglutide for various periods

	Days of dosing (age)	28 (9 months)		119 (12 months)		210 (15 months)		301 (18 months)	
	Group (mg/kg)	1 (0)	4 (0.75)	1 (0)	4 (0.75)	1 (0)	4 (0.75)	1 (0)	4 (0.75)
No of animals examined		9	10	10	9	10	10	14	12
No of animals with NAD¤		2	1						
Diffuse C-cell hyperplasia§	Group mean ± SD	0.66 ± 0.6	1.11 ± 0.6	2.24 ± 0.8	2.16 ± 0.7	2.38± 0.6	2.54 ± 0.6	2.72 ±0.4	2.26 ± 0.4
Focal C-cell hyperplasia§									
	Minimal		2	2	3	1	4	2	2
	Slight				3		3		1
	Total		2	2	6	1	7	2	3
C-cell adenoma							1		3
Dilated ultimobranchial ducts		2	1	1	1			2	1
Dilated follicles				2	2	3	3	3	
Focal interstitial inflammatory cell infiltration							1		
Focal hyperplasia in parathyroid								1	

¤ NAD, no abnormalities detected; § For diffuse C-cell hyperplasia a mean score was calculated for each animal as the sum of scores of the evaluation of the individual sections with C-cells present/no of sections with C-cells present. From these individual means a group mean was calculated. For focal C-cell hyperplasia, the highest score for each animal was tabulated. For adenoma and other changes the incidence is given.

[N000 4.2.3.7.3 P676]

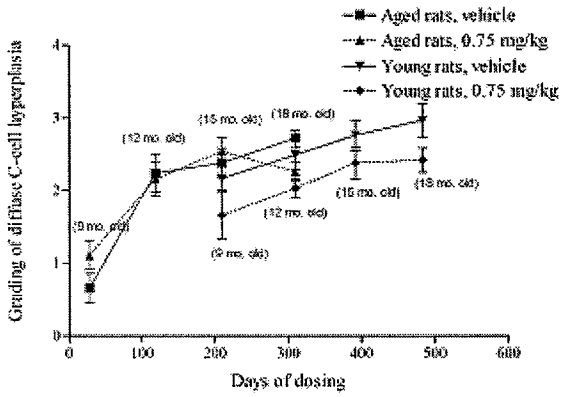


Figure 3 Grading of diffuse C-cell hyperplasia of aged and young rats vs. days of dosing (grade 1 corresponds minimal, grade 2 slight, grade 3 moderate and grade 4 marked diffuse C-cell hyperplasia)

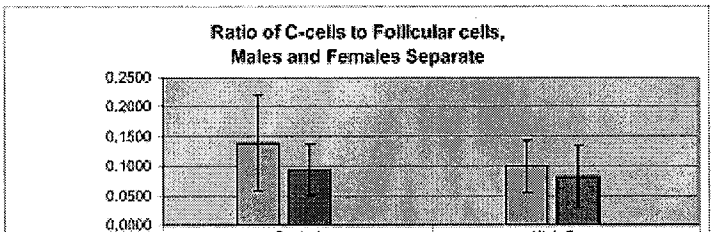
[N000 4.2.3.7.3 P660]

The incidence and severity of focal c-cell hyperplasia was increased by liraglutide treatment at 0.75 mg/kg/day in both aged rats (8 months at the start of treatment) or young rats (2 months at the start of treatment). The appearance of focal c-cell hyperplasia in aged rats treated for 28 days (9 months old) and young rats treated for 210 days (9 months old) with 0.75 mg/kg/day liraglutide and the absence of adenomas in aged or young control groups until rats were at least 12 months old (aged rats) or 15 months old (young rats) suggest liraglutide accelerates the transformation of c-cells from hyperplasia to neoplasms. Treatment-related progression in the severity of diffuse c-cell hyperplasia and it's further progression to focal c-cell hyperplasia did not occur. In fact, in the 0.75 mg/kg/day liraglutide group of aged rats terminated on day 301, the incidence of focal c-cell hyperplasia and the combined incidence of focal c-cell hyperplasia and adenomas was lower then the same dose group terminated on day 210.

- 204021 / Quantification of thyroid C-cells by digital image analysis on histological sections prepared from specimens from Inveresk studies 577863 (cynomolgus monkeys) and 455476 (Crl: CD rats)

C-cell density and proliferation was assessed in preserved thyroid glands from rats in the 26 week repeat dose toxicity study. Quantitative image analysis of thyroid tissue sections stained for calcitonin immunoreactivity and proliferative cell nuclear antigen (PCNA) showed there were no statistically significant differences between male and female control (0 mg/kg/day, vehicle only) and high dose groups (1 mg/kg/day NNC 90-1170) in thyroid c-cell mass or proliferation.

Figure 5 Results for Rats, Males and Females Separate (Arithmetic Means ± Standard Deviation)



	Control	High Dose
Males	0.1397	0.0899
Standard Deviation	0.0813	0.0442
Females	0.0941	0.0827
Standard Deviation	0.0430	0.0533

Note: This chart shows arithmetic means and is for illustration only. Adjusted mean values have been used for statistical analysis.

[N000 4.2.3.7.3 P45]

MICE

Liraglutide Effects on Thyroid C-cell Parameters In Vivo

- 205106 / NNC 90-1170 single dose study in mice with subcutaneous administration

NNC 90-1170 toxicokinetic parameters and calcitonin levels were determined in CD-1 mice (3/sex/dose/time point, except for 0 mg/kg group, 10/sex/dose at time 0) administered a single subcutaneous dose of 0 (vehicle), 0.03, 0.2, 1, or 3 mg/kg/day NNC 90-1170 (5 mL/kg), the same doses used in the mouse carcinogenicity study. Blood sample for NNC 90-1170 toxicokinetic analysis and calcitonin levels were taken prior to dosing, then 0.5, 1.5, 3, 6, 12, 24, and 36 hours after dosing.

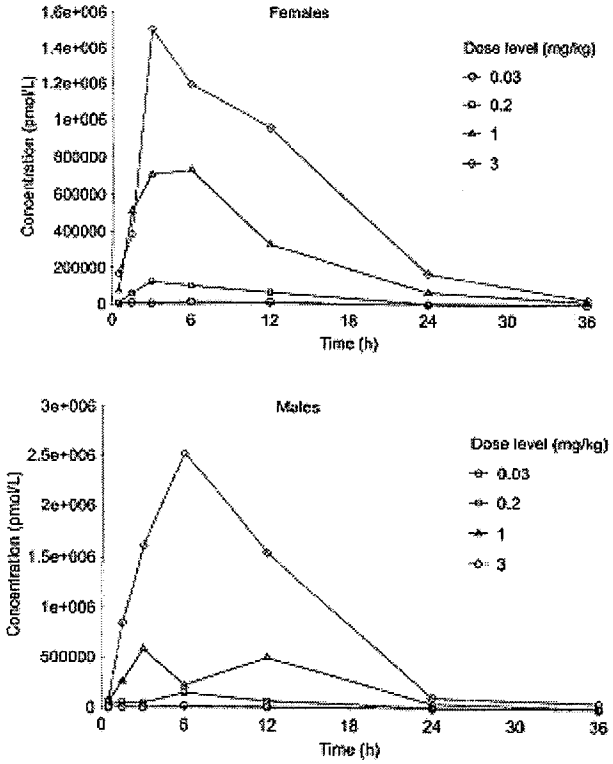
NNC 90-1170 plasma levels were determined using a validated ELISA assay (dilution and incubation in human plasma to remove endogenous GLP-1, overnight incubation with an anti-liraglutide capture antibody, incubation with a second biotin labeled anti-liraglutide antibody, incubation with streptavidin-labeled peroxidase, then color development after addition of TMB and H_3PO_4). Toxicokinetic parameters are summarized in the table below. AUC is $AUC_{0\text{-}inf}$.

Nominal Dose (mg/kg)	Sex	Group	C_{max} (pmol/L)	t_{max} (hr)	AUC (hr*pmol/L)
0.030	Females	2	14380	6.0	170100
	Males	2	18860	6.0	240500
		Mean	16620	6.0	205300
0.20	Females	3	123900	3.0	1343000
	Males	3	147100	6.0	1559000
		Mean	135500	4.5	1451000
1.0	Females	4	732900	6.0	8933000
	Males	4	577900	3.0	6579000
		Mean	655400	4.5	7756000
3.0	Females	5	1503000	3.0	18800000
	Males	5	2521000	6.0	28290000
		Mean	2012000	4.5	23540000

C_{max} and AUC values rounded to 3-4 significant figures

[N000 4.2.3.7.3.1 P12]

Figure 1 Mean (arithmetic, n=3/timepoint) plasma concentration–time profiles of liraglutide in mice.



[N000 4.2.3.7.3.1 P65]

Both peak and total ($AUC_{0\text{-}inf}$) plasma NNC 90-1170 immunoreactivity increased across the dose range with Tmax ranging from 3 to 6 hours after dosing.

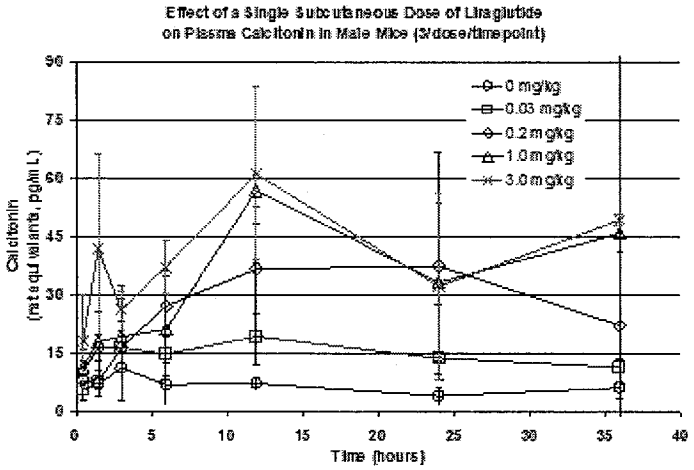
Mouse plasma calcitonin was measured using a rat calcitonin immunoradiometric assay from Immutopics (catalog # 50-5000, bead-immobilized anti-calcitonin monoclonal antibody for capture and ^{125}I-labeled anti-rat calcitonin polyclonal antibody for labeling). The cross reactivity of mouse calcitonin with the rat calcitonin assay was not established. The graphs below show the time course of plasma calcitonin levels after dosing with vehicle or liraglutide (error bars show the range of data for each mean value).

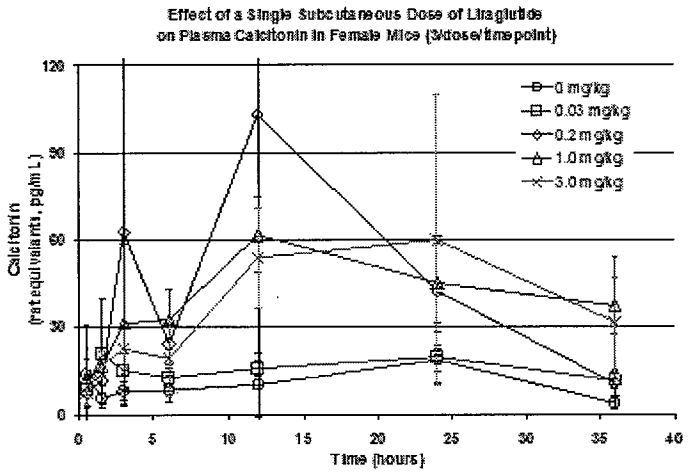
Plasma calcitonin increased at ≥ 1 mg/kg liraglutide in males and females for up to 36 hours after dosing (Table 5 and graphs below). The sponsor considered calcitonin levels increased at ≥ 0.2 mg/kg liraglutide. In males, control group plasma calcitonin levels ranged from 2 to ~ 19 pg/mL. Although average calcitonin levels were above 19 pg/mL in the 0.2 mg/kg group 36 hours after dosing, in the 1 mg/kg group from 6 to 36 hours after dosing and in the 3 mg/kg group from 1.5 to 36 hours after dosing, there were non-responding mice. In females, the maximum plasma calcitonin level in the control group females was 63 pg/mL, about 3 fold higher than in males. All NNC 90-1170 treated groups had at least one mouse within the control group range, and increased group mean calcitonin levels was not dose related.

Table 5 Mean calcitonin values

		group				
		1	2	3	4	5
		mcalc	mcalc	mcalc	mcalc	mcalc
time	sex					
0.5	Females	13.64	8.39	7.51	13.16	7.26
	Males	7.88	5.51	10.29	11.47	18.15
1.5	Females	5.76	21.04	12.01	16.42	17.38
	Males	6.86	7.33	16.33	18.06	41.92
3	Females	8.21	15.05	62.85	31.31	22.76
	Males	11.07	16.29	16.45	19.37	26.09
6	Females	8.59	12.74	24.24	32.67	19.57
	Males	6.84	14.85	27.15	21.09	37.30
12	Females	10.23	15.91	103.10	61.78	53.99
	Males	7.03	19.14	36.66	56.98	61.35
24	Females	18.57	19.63	42.51	45.28	59.91
	Males	3.85	13.73	37.43	33.31	32.17
36	Females	3.94	11.76	10.65	37.41	31.76
	Males	6.19	11.43	22.04	45.82	49.45

[N000 4.2.3.7.3.1 P107]





The following summary of the analysis of NNC 90-1170 effects on plasma calcitonin was provided by the sponsor.

Calcitonin analysis:

Three way and pair wise interactions between treatment, time and sex were not significant. The effect of sex was not significant, whereas the effect of treatment and time was significant. The ratio of liraglutide treated groups to the Control group was 1.09 ([0.66;1.79], P>0.50), 1.83([1.11;3.01], P = 0.02), 2.36 ([1.43; 3.88], P<0.001), 2.43 ([1.47; 4.00] , P<0.001) for Groups 2, 3, 4 and 5 respectively, i.e all dose groups except the low dose had significantly higher calcitonin levels than the Control and the increase in calcitonin levels increased with dose. Comparisons between neighbouring Groups 2, 3, 4 and 5 showed significant difference between groups 2 and 3, and non-significant differences between groups 3 and 4, 4 and 5, i.e. the calcitonin level in the low dose group was significantly lower than in the Intermediate I group and the levels in the three highest dose groups were not significantly different.

[N000 4.2.3.7.3.1 P12-13]

- 204268 / A 9 week exploratory study with reversibility in mice – Combined evaluation of the in life phase, hormone analysis, molecular analysis, pathology, and statistical analysis

To determine the time course of liraglutide induce increased calcitonin and thyroid c-cell hyperplasia in CD-1 mice, males and females were treated with 0, 0.2, or 5 mg/kg/day liraglutide (5 mL/kg) for 2 or 9 weeks. Reversibility of increased plasma calcitonin and thyroid c-cell hyperplasia that occurred in mice treated for 9 weeks with 5 mg/kg/day liraglutide was evaluated after 6 or 15 week recovery periods. This study includes the following study reports

Table 1 Studies included:

Novo Nordisk Study number	NN204268	NN204315	Doc.id.409, SqA, 2022	NN204413	NN204338
Phase	In life phase	Hormone analysis	Statistical analysis	Molecular analysis	Pathology
Test facility	Scanbox A/S	SkeleTech Inc.	Novo Nordisk A/S	Novo Nordisk A/S	Inveresk
GLP-status	GLP	GLP	Non-GLP	Non-GLP	GLP

[N000 4.2.3.7.3.1 P12-13]

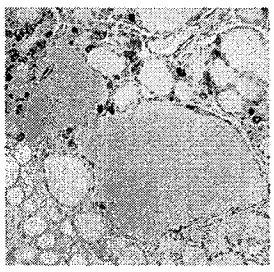
The following table shows the number of mice in each dose group denoted as groups 1 (0 mg/kg/day, vehicle), 2 (0.2 mg/kg/day liraglutide), or 3 (5 mg/kg/day liraglutide). Subgroup a was terminated after 2 weeks of treatment (30 mice/sex/dose), subgroup b was terminated after 9 weeks of treatment (25 mice/sex/dose), subgroup c was terminated after 9 weeks of treatment followed by a 6 week recovery period (17 mice/sex/dose), and subgroup d was terminated after 9 weeks of treatment followed by a 15 week recovery period (17 mice/sex/dose).

Group	Animal No		Sub-group	Sub-group	Day of termination
	Male	Female	males	females	
1	1 - 80	81 - 160	a) 1-30	a) 81-110	14
			b) 31-46	b) 111-126	69
			c) 47-63	c) 127-143	105
			d) 64-80	d) 144-160	175
2	161 - 240	241 - 320	a) 161-190	a) 241-270	14
			b) 191-206	b) 271-286	69
			c) 207-223	c) 287-303	105
			d) 224-240	d) 304-320	175
3	321 - 400	401 - 480	a) 321-350	a) 401-430	14
			b) 351-366	b) 431-446	69
			c) 367-383	c) 447-463	105
			d) 384-400	d) 464-480	175

[N000 4.2.3.7.3.1 P23]

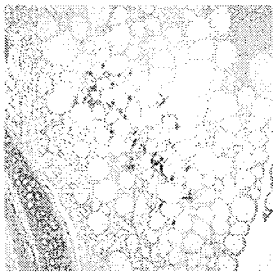
Study observations were mortality and clinical signs, body weight, blood sampling (orbital blood taken prior to sacrifice) for plasma calcitonin levels (RIA assay) and liraglutide toxicokinetics (ELISA assay), and examinations of the thyroid including histopathology (stained immunohistochemically for calcitonin and counterstained with hematoxylin), calcitonin and GLP-1R mRNA levels (quantitative PCR with levels normalized to beta-actin and GAPDH), transmission electron microscopy, and analysis of cell proliferation in mice intraperitoneally injected with 50 mg/kg BrdU 3, 24, or 48 hours prior to sacrifice (BrdU labeled samples of thyroid were not examined). For mice terminated after 2 weeks of treatment, blood samples were taken prior to dosing and 0.5 and 3 hours after dosing to determine calcitonin levels. The following definitions were used to characterize thyroid C-cell related findings (copy of the sponsor's text, modified for brevity).

> *Ultimobranchial ducts* was used to describe an unusual follicle with flattened epithelium, increased in size and associated with C-cells (see Plates 2 & 4 in Appendix 5). This was graded as minimal if there were only one or two ducts, as mild if there were three to five, and as moderate if more than five.



[N000 4.2.3.7.3.1 P386]

> *Localized C-cells* was used to describe the normal variation in the localized distribution of C-cells in the mid-zone area of the thyroid lobe.
>
> grade 1 - C-cells were distributed individually, around the periphery of occasional follicles and in clusters of up to 5 cells (see Plate 1 in Appendix 5).

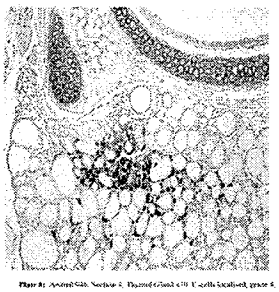


[N000 4.2.3.7.3.1 P385]

grade 2 - C-cells formed several clusters of between 5 and 10 cells and/or formed an almost continuous layer around the periphery of several follicles (see Plates 3 & 4 in Appendix 5).

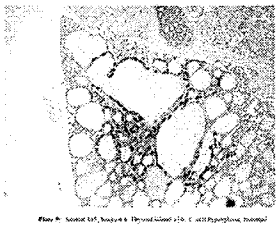
grade 3 - C-cells formed clusters of more than 10 cells and/or formed an almost continuous layer around the periphery of many follicles (see Plates 5, 6 & 7 in Appendix 5).

grade 4 - C-cells formed several coalescing clusters of more than 10 cells and/or formed a continuous single or double layer around the periphery of many follicles (see Plate 8 in Appendix 5).



[N000 4.2.3.7.3.1 P392]

C-cell hyperplasia described an increase in the number of C-cells forming aggregates of less than five average follicles in diameter with or without displacement of individual thyroid follicles (see Plates 9 & 10 in Appendix 5).



[N000 4.2.3.7.3.1 P393]

C-cell hyperplasia was considered the part of a continuum of c-cell proliferation (localized c-cells grade 1 < grade 2 < grade 3 < grade 4 < minimal hyperplasia < mild hyperplasia). Otherwise only standard pathological terminology was used.

The following mortalities were reported:

Group 1 #17 sacrificed moribund on day 11 due to a large wound

#64 sacrificed moribund on day 105 due to general ill health
#80 sacrificed moribund on day 18 due to a large wound

Group 2 #208 sacrificed moribund on day 78 due to general ill health
#215 died from a dosing error on day 55
#305 sacrificed moribund on day 141 due to general ill health

Group 3 #391 sacrificed moribund on day 18 due to a large wound
#396 died from a dosing error on day 33
#399 sacrificed moribund on day 11 due to a large wound
#450 went missing on day 64
#465 sacrificed moribund on day 130 due to general ill health
#472 sacrificed moribund on day 137 due to general ill health

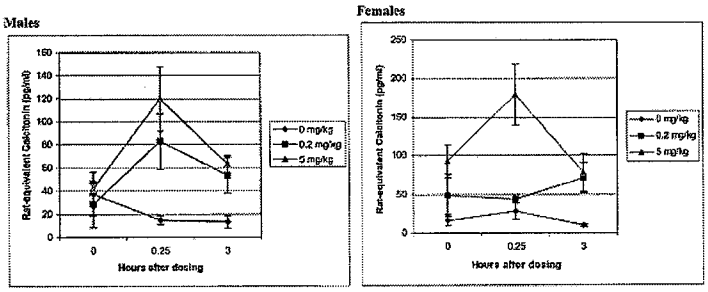
Wounds occurring at the injection within the first 16 days of treatment were diminished after the vehicle was changed from batch No PQ 50297 to batch No 433-04-115 (used from days 1 – 16) on Day 17 (and used until termination). These wounds were considered related to treatment with vehicle batch PQ 50297.

There was no treatment-related effect on body weight.

Plasma calcitonin was measured at the end of the treatment period on days 14 and 63, then at the end of recovery on days 105 and 175. The average calcitonin level in control groups was 14.8 +/- 25.8 pg/mL in males (values ranging from 0.3 – 195.2) and 39.6 +/- 69.3 pg/mL in females (values ranging from 0.8 – 311.0).

On day 14, there were no significant differences in plasma calcitonin levels between treatment groups prior to dosing in males, but calcitonin was significantly higher than concurrent controls 0.5 and 3 hours after dosing with 0.25 or 5 mg/kg liraglutide (Figure 1, the x-axis is mislabeled 0.25 hours for the 0.5 hour time point). In females, plasma calcitonin in the 5 mg/kg group was significantly higher than controls prior to dosing and 0.5 and 3 hours after dosing. Plasma calcitonin in the 0.2 mg/kg group was not significantly different from control prior to dosing and 0.5 hours after, but it was significantly higher 3 hours after dosing.

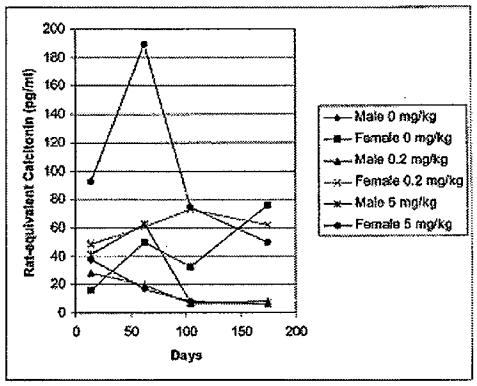
Figure 1. Calcitonin levels at multiple time points after dosing on Day 14



[N000 4.2.3.7.3.1 P111]

Calcitonin levels were determined prior to terminal sacrifice at the end of treatment on days 14 and 63 and at the end of recovery on days 105 and 175 (Figure 2, Table 2). Calcitonin was significantly elevated ($p < 0.05$, unpaired Student's t-test) in 5 mg/kg females at the end of 14 and 63 week treatment periods, but calcitonin levels declined to control group levels during recovery. In males, calcitonin was statistically significantly higher than the concurrent control group at 5 mg/kg on day 63 only, but not at the end of 6 and 15 week recovery periods.

Figure 2. Calcitonin levels at a single time point on Days 14, 63, 105 and 175



[N000 4.2.3.7.3.1 P114]

The calcitonin assay was highly variable, so the biological significance of statistically significantly elevated plasma calcitonin in 5 mg/kg/day males and females is unknown.

Table 2. Calcitonin levels at a single time point on Days 14, 63, 105 and 175

Treatment Group	Gender	Data	Rat-equivalent Calcitonin (pg/ml)			
			Day 14	Day 63	Day 105	Day 175
1	M	mean	37.37	16.55	8.28	5.49
0 mg/kg		SD	59.15	18.98	12.18	4.41
		SEM	18.70	4.90	2.95	1.14
		n	10	15	17	15
1	F	mean	16.22	49.96	32.37	75.89
0 mg/kg		SD	22.50	71.39	60.91	108.73
		SEM	7.12	18.43	14.77	26.37
		n	10	15	17	17
2	M	mean	28.26	19.49	6.48	7.98
0.2 mg/kg		SD	63.04	13.05	5.06	8.92
		SEM	19.94	3.26	1.31	2.23
		n	10	16	15	16
2	F	mean	48.34	60.87	72.70	61.91
0.2 mg/kg		SD	85.97	70.51	94.01	78.10
		SEM	27.19	17.63	22.80	18.94
		n	10	16	17	17
3	M	mean	41.48	62.97	7.46	6.52
5 mg/kg		SD	15.67	31.09	8.56	4.99
		SEM	4.95	7.77	2.08	1.33
		n	10	16	17	14
3	F	mean	92.49	189.32	74.03	49.84
5 mg/kg		SD	68.68	131.31	69.82	88.74
		SEM	21.72	32.83	17.46	22.18
		n	10	16	16	16

[N000 4.2.3.7.3.1 P115]

The sponsor's analysis of liraglutide effects on calcitonin levels yielded somewhat different results. The sponsor contends there were no significant differences between males and females for liraglutide-induced changes in plasma calcitonin, so they combined data within each dose group and each time point to increase the number of samples/time point, and used the natural log of plasma calcitonin concentrations for statistical analysis. From this analysis, 5 mg/kg liraglutide significantly increased plasma calcitonin prior to dosing and at 0.5 and 3 hours after while treatment with 0.2 mg/kg liraglutide significantly increased calcitonin 0.5 and 3 hours after dosing, but not prior to dosing on day 14. Results are shown in Figure 1. The differences between the reviewer and sponsor analysis on day 14 were the effect in males prior to dosing at 5 mg/kg (according to reviewer, no difference from control in males) and the effect in females 0.5 hours after dosing with 0.25 mg/kg (according to reviewer, no difference from control).

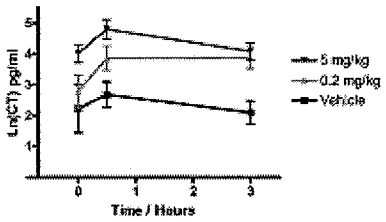


Figure 1 NN204315. Plasma CT values (natural logarithm transformed, mean and 95% CI) in CD-1 mice treated with liraglutide or vehicle for 2 weeks (20/group/time, sexes combined).
CT in liraglutide treated animals significantly elevated pre- (5 mg/kg group) and post-dose (5 mg/kg and 0.2 mg/kg group) ($p<0.001$).

[N000 4.2.3.7.3.1 P6]

Using the same analysis by combining data from both sexes and analyzing statistical differences in the natural log of the mean calcitonin concentration in each dose group, the sponsor concluded calcitonin was significantly increased in the 5 mg/kg group at the end of the 9 week treatment period, and that elevated calcitonin returned to control group levels at the end of 6 or 15 week recovery periods (Figure 2). There were no substantive differences between the reviewer and sponsor analysis of plasma calcitonin levels at the end of 9 week treatment and 6 or 15 week recovery periods.

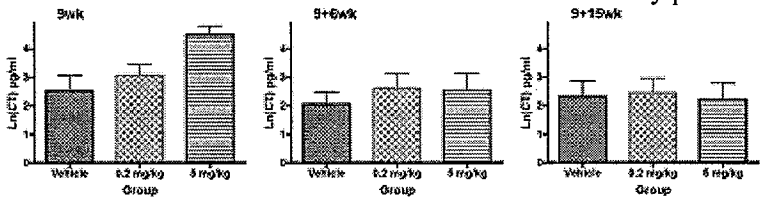


Figure 2 NN204315. Plasma CT values (natural logarithm transformed, mean and 95% CI) in CD-1 mice treated with liraglutide or vehicle for 9 weeks (9wk) followed by a 6 week (9+6wk) or 15 week (9+15wk) recovery period (30-34/group/time, sexes combined).
CT in liraglutide treated animals significantly elevated in the high-dose group after 9 weeks ($p<0.001$) but reverting to the level in controls after recovery for 6 and 15 weeks.

[N000 4.2.3.7.3.1 P7]

After 2 weeks of treatment with 5 mg/kg/day liraglutide, quantitative assessment of immunohistochemically stained thyroid cells in mice showed there were no treatment-related differences in the density of follicular cells, c-cells, or the ratio of c-cells to follicular cells in males or females. There was no treatment related qualitative difference between 5 mg/kg treated or control group mice in the incidence or severity of localized c-cells or dilatation of the ultimobranchial duct.

Table 1 Quantitative Evaluation, at the 2 Week Interim Kill, Group Results

		Control		High Dose	
		Male	Female	Male	Female
C-cells/mm²	Number	15	15	15	15
	Mean	218.52	214.33	250.08	224.13
	SD	63.768	60.556	105.587	83.150
Follicular cells/mm²	Number	15	15	15	15
	Mean	5502.09	5170.97	5261.47	5238.79
	SD	981.145	509.295	721.686	586.813
Ratio C-cells / follicular cells	Number	15	15	15	15
	Mean	0.042	0.042	0.048	0.042
	SD	0.013	0.014	0.017	0.014

[N000 4.2.3.7.3.1 P242]

After 9 weeks, the incidence of minimal to mild thyroid c-cell hyperplasia significantly increased at 5 mg/kg/day liraglutide in females. A low incidence of c-cell hyperplasia occurred at 0.25 mg/kg in males and females and at 5 mg/kg in males.

Table 3 Summary of Histological Findings: 9 Week Terminal Kill

HISTOLOGICAL FINDINGS	GROUP TOTALS					
	Males			Females		
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 2 0.2 mg/kg /day	Grp 3 5.0 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 0.2 mg/kg /day	Grp 3 5.0 mg/kg /day
ENDOCRINE SYSTEM						
THYROID GLAND	(16)	(16)	(16)	(16)	(16)	(16)
C-cells, localised, grade 1	8	3	0	8	3	3
C-cells, localised, grade 2	5	8	7	7	5	4
C-cells, localised, grade 3	1	3	7	1	4	3
C-cells, localised, grade 4	2	1	1	0	3	0
C-cell hyperplasia						
minimal	0	1	1	0	1	3
mild	0	0	0	0	0	3
Total Incidence	0	1	1	0	1	6
Ultimobranchial duct, dilated						
minimal	10	11	9	10	7	10
mild	2	3	6	4	7	4
moderate	1	0	0	0	1	1
Total Incidence	13	14	15	14	15	15
Inflammatory cell infiltration, histiocytic, lymphocytic, diffuse	0	1	0	0	0	0

Figures in brackets represent the number of animals from which this tissue was examined microscopically

[N000 4.2.3.7.3.1 P248]

C-cell hyperplasia was reversed after a 15 week recovery period. Minimal c-cell hyperplasia also occurred after 6 weeks of recovery in 5 mg/kg females (5/16), but 15 weeks after treatment was stopped, the incidence decreased to 1/16. However, the incidence of grade 3/4 localized c-cells was notably higher than controls with an increased incidence and severity at $\geq$ 0.2 mg/kg in both males and females. Because c-cell hyperplasia in mice is rare, it's persistence after a 15 week recovery period suggests it may be a neoplasm.

Table 5 Summary of Histological Findings: 15 Week Recovery Kill

HISTOLOGICAL FINDINGS	GROUP TOTALS					
	Males			Females		
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 2 0.2 mg/kg /day	Grp 3 5.0 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 0.2 mg/kg /day	Grp 3 5.0 mg/kg /day
ENDOCRINE SYSTEM						
THYROID GLAND	(15)	(17)	(14)	(17)	(17)	(16)
C-cells, localised, grade 1	6	2	1	8	3	0
C-cells, localised, grade 2	8	10	3	7	4	1
C-cells, localised, grade 3	2	5	9	2	6	9
C-cells, localised, grade 4	0	0	1	0	4	5
C-cell hyperplasia						
minimal	0	0	0	0	0	1
Total Incidence	0	0	0	0	0	1
Ultimobranchial duct, dilated						
minimal	8	8	8	8	13	7
mild	5	6	5	9	1	6
moderate	1	1	0	0	0	0
Total Incidence	14	15	13	17	14	13

Figures in brackets represent the number of animals from which this tissue was examined microscopically

[N000 4.2.3.7.3.1 P249]

The summary table below shows the incidence of plasma calcitonin levels considered above the control group along with the incidence of focal thyroid c-cell hyperplasia. Plasma calcitonin was

considered elevated if the value was above the overall average + 1 SD for calcitonin levels in male and female control groups (average and standard deviation calculated using data from all time points); 41 pg/mL calcitonin in males and 109 pg/mL in females. On day 14, the incidence of elevated calcitonin was greater than controls at 0.5 and 3 hours after dosing with 0.2 mg/kg in males, prior to dosing and 0.5 and 3 hours after dosing with 5 mg/kg in males, but only 0.5 hours after dosing with 5 mg/kg in females. Thyroid c-cell hyperplasia did not occur after 14 days of treatment. On day 63 in males, the incidence of elevated calcitonin was higher than controls at 5 mg/kg, but the low, non-significance incidence of c-cell hyperplasia was the same in both groups (6.3%), despite the absence of increased plasma calcitonin at 0.2 mg/kg. At the end of 6 or 15 week recovery periods, elevated calcitonin or c-cell hyperplasia didn't occur at any dose in males. At the end of the 9 week treatment period, the incidence of elevated calcitonin and c-cell hyperplasia were both increased at 5 mg/kg in females, but not at 0.2 mg/kg. The incidence of elevated plasma calcitonin was increased in 0.2 mg/kg recovery group females at the end of both 6 and 15 week recovery periods, despite the absence of c-cell hyperplasia. In 5 mg/kg recovery group females, plasma calcitonin and c-cell hyperplasia were elevated at the end of the 6 week recovery period, but not at the end of recovery week 15.

Liraglutide Dose (mg/kg/day)	Study Day	Sample Time[1]	Males				Females			
			Plasma Calcitonin			Incidence of minimal / mild focal C-cell hyperplasia	Plasma Calcitonin			Incidence of minimal / mild focal C-cell hyperplasia
			Average	SD	% of values >41 pg/mL		Average	SD	% of values > 109 pg/mL	
0	14, 63, 105, 175		14.8	25.8	9.2% (7/76)	0%	39.6	69.3	10.1% (8/79)	0%
0.2	14	P	28.3	63.0	10% (1/10)		48.3	86.0	10% (1/10)	
		0.5	82.7	76.3	70% (7/10)		44.4	15.0	0%	
		3	53.0	48.8	70% (7/10)	0%	71.4	62.7	10% (1/10)	0%
	63	P	19.49	13.05	12.5% (2/16)	6.3% (1/16)	60.87	70.51	18.8% (3/16)	6.3% (1/16)
	105 (recovery)	P	6.48	5.06	0%	0% (0/15)	72.7	94	23.5% (4/17)	0% (0/17)
	175 (recovery)	P	7.98	8.92	0% (0/17)	0% (0/17)	61.91	78.1	31.3% (5/16)	0% (0/17)
5	14	P	41.5	15.7	40% (4/10)		92.5	68.7	20% (2/10)	
		0.5	119.6	88.2	90% (9/10)		179.2	123.9	60% (6/10)	
		3	63.1	23.8	90% (9/10)	0%	77.6	78.6	10% (1/10)	0%
	63	P	62.97	31.09	68.8% (11/16)	6.3% (1/16)	189.32	131.31	62.5% (10/16)	37.5% (6/16)
	105 (recovery)	P	7.46	8.56	0% (0/17)	0% (0/17)	74.03	69.82	25% (4/16)	31.3% (5/17)
	175 (recovery)	P	6.52	4.99	0% (0/14)	0% (0/14)	49.84	88.74	13.3% (2/15)	6.3% (1/16)

[1]Sample times were predose (P) and 0.5 or 3 hours after dosing.

The table below shows calcitonin levels in mice diagnosed with thyroid c-cell hyperplasia to determine if the 2 findings are coincident. In 2 males with c-cell hyperplasia after the 9 week treatment period, calcitonin was below or slightly above the maximum control group value of 41 pg/mL. In females with the finding of c-cell hyperplasia on day 63, all but one had calcitonin levels elevated above the maximum control group value of 109 pg/mL. However all but one female mice with c-cell hyperplasia persisting in recovery periods had calcitonin levels within the control group range. Results in females suggest elevated calcitonin levels were related to liraglutide treatment, not c-cell hyperplasia. At the end of the treatment period (day 63), plasma calcitonin was elevated in nearly all females with c-cell hyperplasia, but at the end of a 6 week recovery period (day 105) plasma calcitonin in high dose females was within a normal range in nearly all female mice, despite the persistence of hyperplasia.

Mice with minimal / mild thyroid C-cell hyperplasia

Liraglutide Dose (mg/kg/day)	Study Day	Sex	Mouse#	Plasma Calcitonin (pg/mL)
0.2	63	M	194	14.7*
		F	275	188.5
5	63	M	355	45.1
		F	431	257.3
			432	151.7
			438	134.6
			439	323.4
			444	366.0
			445	82.3*
	105 (recovery)	F	448	14.7*
			451	207.8
			453	44.1*
			454	31.8*
			457	22.4*
	175 (recovery)	F	476	3.5*

*Value at or below the control group mean + 1 sd (41 pg/mL calcitonin in males, 109 pg/mL in females).

The sponsor's analysis of thyroid histopathology combined c-cell hyperplasia findings from male and female mice to generate Table 2. This analysis suggests focal c-cell hyperplasia dose-dependently increases at ≥ 0.2 mg/kg/day liraglutide in males and females and it was partially reversed during a 6 week recovery period and fully reversed after 15 weeks. The sponsor's analysis is substantially different from the reviewer's which shows a significant dose-related increase in c-cell hyperplasia only occurred at 3 mg/kg/day in females, but there a trend of increased c-cell hyperplasia in both males and females at ≥ 0.2 mg/kg. There was no substantial difference in the assessment of reversibility.

Table 2 NN2043338. Incidence focal C-cell hyperplasia in mice dosed with liraglutide for 9 weeks followed by a 6 week (9+6wk) or 15 week (9+15wk) recovery period (30-34/group/time, sexes combined).

Time point	No. of animals with focal C-cell hyperplasia / total number in group		
Dose mg/kg/day	0	0.2	5
9 weeks dosing	0 / 32	2 / 32	7 / 32
9 + 6 week recovery	0 / 34	0 / 32	5 / 33
9 + 15 week recovery	0 / 32	0 / 34	1 / 30

The treatment-related increase is seen in focal C-cell hyperplasia after 9 weeks of dosing shows reversibility after 6 and 15 weeks of recovery.

[N000 4.2.3.7.3.1 P7]

Relative calcitonin and GLP-1R transcript levels in thyroid from mice treated with vehicle or liraglutide for 9 weeks were measured by real-time quantitative RT-PCR incorporating fluorescent primers into amplified cDNA and normalizing calcitonin or GLP-1 levels to transcript levels encoding housekeeping proteins GAPDH or beta-actin. Summary results in Table 2 show relative levels of calcitonin transcript in thyroid (males and females in the same dose groups combined) increased 2 fold at 0.2 mg/kg liraglutide and 3.9 fold at 5 mg/kg, but due to variability in transcript levels, the difference was only statistically significant from vehicle control treated mice at 5 mg/kg. The standard deviation of the mean for vehicle control and 0.2 mg/kg groups show the range of values in these groups include values < 0. Although the increase appears to be dose-related and although it was statistically significant at 5 mg/kg, the biologic relevance of increased calcitonin transcript at 5 mg/kg is questionable given the variability in the assay.

Table 2 The effect of liraglutide on calcitonin mRNA expression levels in thyroid tissue from mice.

Dose level	*N° of samples*	$2^{-\Delta C_T}$ *Mean*	$2^{-\Delta C_T}$ *Standard deviation*	*Effect of treatment*[a]	*p-value*
Vehicle	18	0.207	0.305	1.0	n.a.
0.2 mg/kg/day	23	0.420	0.523	2.0	0.134
5.0 mg/kg/day	29	0.808	0.556	3.9	0.000124

[a] The effect of liraglutide treatment on the levels of mRNAs encoding calcitonin was expressed as fold upregulation of the mRNA level in liraglutide-treated mice when compared to vehicle-treated mice.

[N000 4.2.3.7.3.1 P202]

Although there was a trend of liraglutide to increased thyroid GLP-1 transcript levels, the 1.6 fold increase at 0.2 and 5 mg/kg liraglutide didn't reach statistical significance and the increase wasn't dose-related.

Table 3 The effect of liraglutide on GLP-1R mRNA expression levels in thyroid tissue from mice.

Dose level	*N° of samples*	$2^{-\Delta C_T}$ *Mean*	$2^{-\Delta C_T}$ *Standard deviation*	*Effect of treatment*[a]	*p-value*
Vehicle	18	0.001	0.001	1.0	n.a.
0.2 mg/kg/day	23	0.002	0.001	1.6	0.116
5.0 mg/kg/day	29	0.002	0.001	1.6	0.060

[a] The effect of liraglutide treatment on the levels of mRNAs encoding GLP-1R was expressed as fold upregulation of the mRNA level in liraglutide-treated mice when compared to vehicle-treated mice.

[N000 4.2.3.7.3.1 P203]

Reviewer note: The sponsor should determine if PCR efficiencies for target and reference genes are similar or if the assay is valid. A validation experiment using cDNA sample concentrations spanning 6 orders of magnitude (including 3 replicates of each standard curve point, running singleplex reactions with target and endogenous control in separate wells and primer concentrations of 900 pM and probe concentrations of 250 nM) is necessary to determine if the $\Delta\Delta C_T$ calculation in valid (the slope of the log [cDNA or RNA] vs ΔC_T plot should be 0 - < 0.1). Given sex differences in calcitonin levels and differences in the incidence of thyroid c-cell hyperplasia, the sponsor should not combine results from both sexes when analyzing thyroid calcitonin and GLP-1R transcript levels.

- 203261 / 204288 / NNC 90-1170: A 4 week toxicity study in mice with subcutaneous administration

In a 4 week repeat dose study of 0, 0.1, 0.5, 1, or 5 mg/kg/day NNC 90-1170 administered subcutaneously once a day in CD-1 mice, minimal / mild thyroid c-cell hyperplasia (confirmed by immunohistochemical staining with an anti-calcitonin antibody) occurred in 1 male in the 1 mg/kg/day group and in 2 females in the 5 mg/kg/day group. The single incidence at 1 mg/kg in males was considered incidental because none occurred at 5 mg/kg/day.

Sex	Male					Female				
Doses, mk/d	0	0.1	0.5	1.0	5.0	0	0.1	0.5	1.0	5.0
Thyroid gland No. Examined	10	10	10	10	10	10	10	10	10	10
Focal C-cell hyperplasia, unilateral										
Minimal	0	0	0	1	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	0	0	1
Total incidence	0	0	0	1	0	0	0	0	0	2
Follicular dilatation	0	0	0	0	0	0	1	1	1	1

[IND 61,040 Pharm/Tox Review 3 10/25/04 P13]

- 204082 / 204289 / NNC 90-1170: 13 week toxicity study in mice with subcutaneous administration

In a 13 week repeat dose toxicity study of 0, 0.2, 1, or 5 mg/kg/day NNC 90-1170, the incidence of minimal to mild focal thyroid c-cell hyperplasia increased with dose at ≥ 0.2 mg/kg/day in males and females (main study and satellite TK group combined).

Main study animals:

Sex		Male				Female			
Doses, mMol/d		0	0.2	1.0	5.0	0	0.2	1.0	5.0
Thyroid	No. Examined	10	10	10	10	10	10	10	10
	Follicular cyst	0	4	7	5	2	5	1	1
	Focal C-cell hyperplasia								
	minimal	0	5*	5*	5*	0	2	1	2
	mild	0	0	0	2	0	0	0	2
	Total incidence	0	5*	5*	7**	0	2	1	4

* p≤0.05; ** p≤0.01.

Satellite study animals:

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS Males Grp 1 0 mg/kg /day	Grp 2 0.2 mg/kg /day	Grp 3 1 mg/kg /day	Grp 4 5 mg/kg /day	Females Grp 1 0 mg/kg /day	Grp 2 0.2 mg/kg /day	Grp 3 1 mg/kg /day	Grp 4 5 mg/kg /day
ENDOCRINE SYSTEM									
THYROID GLAND		(13)	(14)	(14)	(14)	(13)	(13)	(14)	(14)
No abnormality detected		12	8	7*	2**	11	6	7	3**
Follicular cyst		1	2	2	5*	2	4	2	5
Focal C-cell hyperplasia									
minimal		0	4	5*	8**	0	5*	7**	6*
mild		0	0	0	2	0	0	0	3
Total incidence		0	4	5*	10**	0	5*	7**	9***

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a number indicates that the lesion specified was not identified

[IND 61,040 Pharm/Tox Review 3 10/25/04 P20]

To determine the effect of liraglutide on plasma calcitonin (report 204289), CD-1 mice were subcutaneously injected with 0, 0.2, 1, or 5 mg/kg/day liraglutide (5 mL/kg) for 13 weeks and blood samples were collected prior to dosing and 1, 2, 4, 6, 8 and 24 hours after dosing on the first day of dosing and in week 13 (2 mice/sex/dose/time point). Calcitonin was measured using a two-site immunoradiometric assay (IRMA) for rat plasma calcitonin that partially cross-reacts with mouse calcitonin (plasma calcitonin capture using a bead-tethered monoclonal antibody, then the captured calcitonin was radiolabeled using a ^{125}I labeled goat anti-rat calcitonin polyclonal antibody. Alignment of the amino acid sequences for mouse, rat, and human calcitonin is shown below.

Calcitonin Amino Acid Sequence Alignment

```
Mouse:    CGNLSTCMLGTYTQDLNKFHTFPQTSIGVEAP
Rat:      CGNLSTCMLGTYTQDLNKFHTFPQTSIGVGAP
Human:    CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP
```

Despite only 2 difference in the amino acid sequence of rat and human calcitonin, the IRMA for rat calcitonin only has 12.5% cross reactivity with human calcitonin (technical information for Immutopics rat calcitonin IRMA kit, cat# 50-5000).

Reviewer note: The sponsor did not validate the assay for mouse plasma calcitonin using the Immutopics rat calcitonin IRMA kit. The assay was standardized using rat calcitonin.

The sponsor states plasma calcitonin levels were higher than controls in all liraglutide treated groups in males and females on day 1 and in week 13. Liraglutide increased plasma calcitonin at all doses on both days, but without a definitive relation to dose. Peak calcitonin levels in liraglutide treated mice were ~7 fold in all dose groups in week 13 compared to day 1, including female controls. In week 13, increased pre-dose and liraglutide-stimulated calcitonin secretion in liraglutide treated groups was consistent with treatment-related c-cell hyperplasia.

Figure 1a Average Calcitonin Values in Female Animals on Day 1

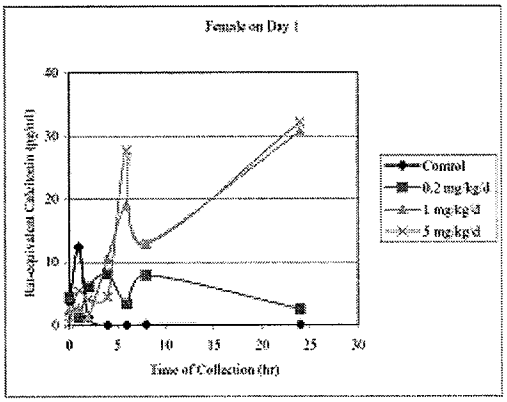
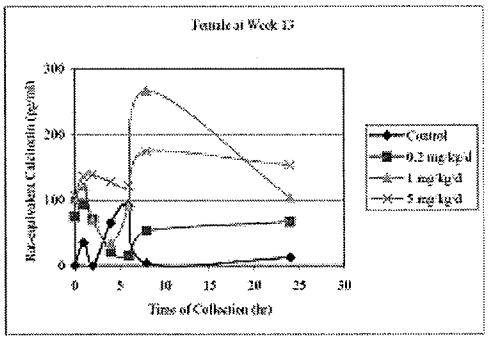


Figure 1b Average Calcitonin Values in Female Animals at Week 13



[N000 4.2.3.7.3.1 P8]

Figure 2a Average Calcitonin Values in Male Animals on Day 1

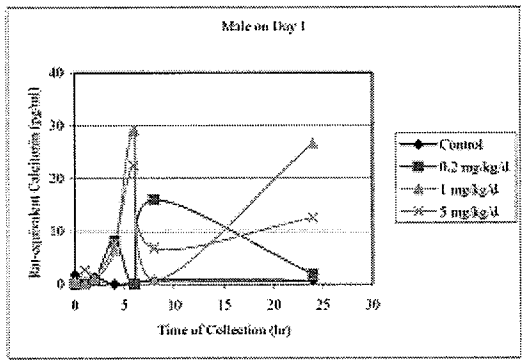
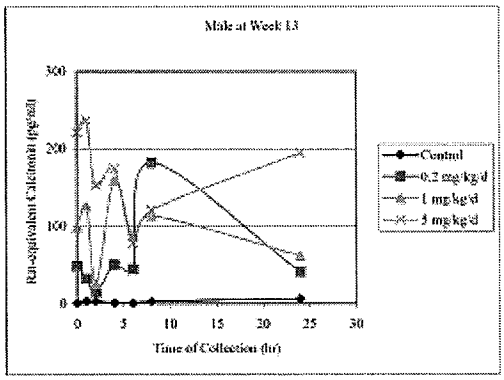


Figure 2b Average Calcitonin Values in Male Animals at Week 13



[N000 4.2.3.7.3.1 P9]

Exenatide Effects on Thyroid C-cell Parameters In Vivo

- 205050 / NNC 0113-0000-0000 and liraglutide. Study on calcitonin and toxicokinetics after 3–days of subcutaneous administration in fasted male mice

This study compared the effects of 3 days of treatment with 0.9% saline (injected every 12 hours, negative control), NNC-0113-0000-0000 (exenatide) (0.06 or 0.25 mg/kg injected subcutaneously once a day or 0.03 or 0.125 mg/kg injected twice a day), or liraglutide (0.06 or 0.25 mg/kg injected once a day) on clinical signs, body weight, and plasma calcitonin in male CD-1 mice (40/dose). Orbital plexus blood samples were taken from fasted mice prior to dosing and 1, 3, 6, and 12 hours after the last dose (8 mice/dose/time point). Calcitonin was quantified using the rat calcitonin IRMA assay (assay kit from Immutopics).

Group	Compound	Dose interval	Dose level (mg/kg/dose)	Total daily dose (mg/kg/day)
1	0.9 % NaCl	12 hours	0	0
2	NNC 0113-0000-0000	24 hours	0.06	0.06
3		12 hours	0.03	0.06
4		24 hours	0.25	0.25
5		12 hours	0.125	0.25
6	Liraglutide	24 hours	0.06	0.06
7		24 hours	0.25	0.25

[N000 4.2.3.7.3.1 P20]

Reviewer note: The standard curve and controls use rat calcitonin (12 – 1,800 pg/mL rat calcitonin for the standard curve), and mouse calcitonin cross-reactivity with this assay was not established.

Analysis of the dosing formulations showed exenatide concentrations were slightly higher than the nominal concentration (108 – 112%) and liraglutide concentrations were substantially lower (62 – 86%). However, since toxicokinetic parameters were determined after the last dose, drug exposure was confirmed. The actual doses of liraglutide were 0.04 mg/kg and 0.22 mg/kg.

Compound	Group	Expected concentration mg/ml	Achieved concentration mg/ml	Deviation (% of expected concentration)
NNC 0113-0000-0000	2	0.012	0.013	108
	3	0.006	0.0067	112
	4	0.05	0.054	108
	5	0.025	0.027	108
Liraglutide	6	0.012	0.0074[A]	62
	7	0.05	0.0429	86

[A] According to standard operating procedure (SOP 434-1018) the recommended amount for analysis is 10-100 µg, and thus the analysis is less accurate when the amount analysed is below this range (i.e. less than 0.02 mg/ml).

[N000 4.2.3.7.3.1 P26]

Four mice (see Table 4 below) were sacrificed moribund due to severe bite wounds and clinical signs of subdued behavior and piloerection. Two mice in group 2 were lost prior to initiating treatment (either completely cannibalized or escaped from the cage). There were no treatment-related clinical signs, but bite wounds on the hind part of the back or tail occurred in 19% of the mice, including 17.5% of mice in the saline control group.

Table 4 Number of pre-scheduled terminations

Part	Day	Group		
		2	3	7
A	Day 1	1		1
B	Day 4	1		
	Day 5		1	

[N000 4.2.3.7.3.1 P27]

Body weight on day 3 was lower than day 1 in all dose groups, including controls (group 1). Decreased body weight gain was greater in exenatide treated groups (group 2 – 3) than in liraglutide treated groups (groups 6-7). In exenatide treated groups, dosing every 12 hours (groups 4 - 5) had a greater effect on decreasing body weight gain compared to dosing once a day (groups 2 - 3).

Table 5 Body weight gain

Group	Body weight gain (1st day of dosing – 3rd day of dosing)	
	g	% of control
1	-0.17	
2	-0.90	539
3	-0.94	560
4	-1.56	933
5	-1.58	942
6	-0.33	197
7	-0.71	426

[N000 4.2.3.7.3.1 P28]

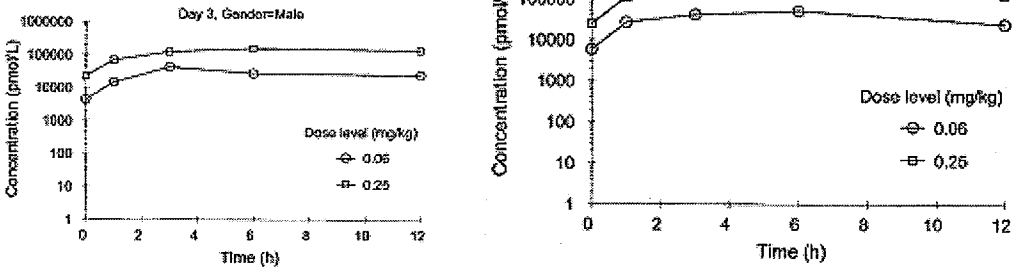
Mice treated with exenatide or liraglutide were exposed, but plasma toxicokinetic parameters could only be calculated for liraglutide (see Table 7 below) because of the short elimination half life of exenatide (~ 30 minutes in mice) and the ≥ 1 hour interval between blood sampling times. Liraglutide plasma concentration versus time profile is show in Figure 2. Consistent with a long elimination half life, liraglutide was detected in predose blood samples. Both peak and total liraglutide exposures increased with dose on both days. Tmax occurred 3 to 6 hours after dosing.

Table 7 Toxicokinetic parameters for liraglutide

Period	Dose (mg/kg)	C_{max} (pmol/L)	t_{max} (hr)	AUC_{0-12h} (hr*pmol/L)	AUC_{∞} (hr*pmol/L)
Day 3	0.06	42 600	3	326 000	576 000
Day 5	0.06	51 000	6	435 000	580 000
Day 3	0.25	156 000	6	1 530 000	2 920 000
Day 5	0.25	268 000	3	1 860 000	3 040 000

[N000 4.2.3.7.3.1 P32]

Figure 2 Mean (semilog) plasma concentration-time profiles (Day 3 and Day 5) following dose of liraglutide in male mice.



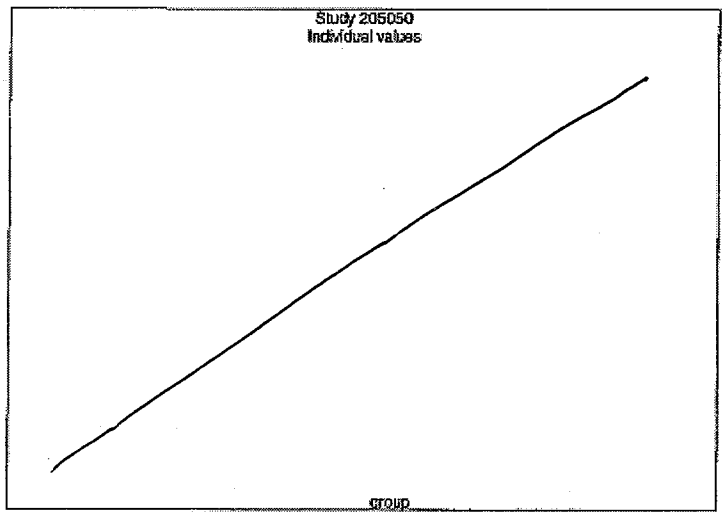
[N000 4.2.3.7.3.1 P142]

Consistent with its short half-life, exenatide was not detected in day 3 predose blood samples or at 6 or 12 hours post-dose on days 3 or 5 (LOQ 45 pM using an RIA). The following table show mean exenatide plasma concentration 1 and 3 hours after dosing.

Exenatide Dose				Plasma Exenatide (pM)		
				Hours Post-dose		
Group	mg/kg/injection	Interval	mg/kg/day	Sample Day	1	3
2	0.06	24	0.06	3	7950	267
				5	12730	59
3	0.03	12	0.06	3	4166	63
				5	5655	75
4	0.25	24	0.25	3	37400	1145
				5	40700	408
5	0.13	12	0.25	3	26470	582
				5	18000	376

Mouse plasma calcitonin was quantified with a rat calcitonin immunoradiometric assay kit from Immutopics using a standard curve based on dilutions of rat calcitonin (ranging from 12 to 1800 pg/mL) and controls consisting of known concentrations of rat calcitonin (included in the kit) and calcitonin in pooled rat plasma. Statistical significance was determined using the natural log of calcitonin concentrations

In the saline control group (group 1), calcitonin levels ranged from 2.8 to 163.1 pg/mL. Individual plasma calcitonin values in each dose group (transformed to the natural log of plasma calcitonin concentrations) are shown in the graph below. There was significant overlap between control and all exenatide or liraglutide treated groups.



b(4)

[N000 4.2.3.7.3.1 P91]

Statistical analysis of day 3 pre-dose plasma calcitonin showed levels were significantly higher than controls (group 1) in exenatide treated groups 3,4, and 5 and liraglutide treated groups 6 and 7 (statistical analysis comparing natural log of plasma calcitonin concentrations). Group 1 predose values ranged from 4.5 to 94.1 pg/mL calcitonin (Figure 1, below). The geometric mean of all treated groups was within this range.

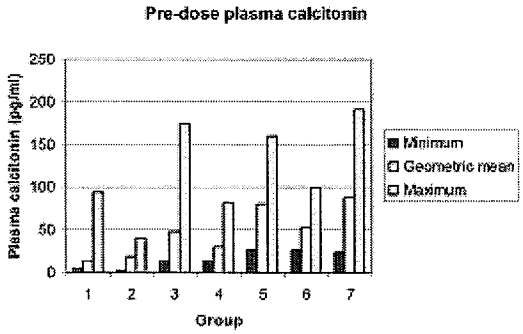


Figure 1 Pre-dose plasma calcitonin

Geometric group mean post-dose plasma calcitonin (pg/ml)

[N000 4.2.3.7.3.1 P29]

The sponsor stated calcitonin levels in all exenatide or liraglutide treated groups measured 1, 3, 6, and 12 hours after dosing were significantly higher than controls (Figure 2). However, the geometric mean values were all within the range of the control group. For exenatide treated groups, the sponsor concludes day 3 predose calcitonin levels were higher in mice treated every 12 hours (groups 3 & 5) compared to mice treated once a day (groups 2 & 4), but there was no significant difference between 0.06 or 0.25 mg/kg/day doses. After dosing, there was no significant dose-response, except 12 hours post-dose when calcitonin levels in the 0.25 mg/kg exenatide groups were significantly higher than in the 0.06 mg/kg exenatide group. Calcitonin levels in liraglutide treated groups (groups 6 & 7) prior to and after dosing on day 3 were significantly higher than the control group and the effect was dose-related with higher plasma calcitonin at 0.25 mg/kg/day (group 7). Day 3 predose calcitonin levels were significantly higher in 0.25 mg/kg liraglutide treated mice (group 7) compared to 0.25 mg/kg exenatide administered once a day, but there was no difference if the same dose of exenatide was administered twice a day (0.125 mg/kg/q12h). Day 3 predose plasma calcitonin levels in the 0.06 mg/kg liraglutide group (group 6) was not significantly different from 0.06 mg/kg/day exenatide (0.03 mg/kg administered every 12 hours, group 3), but calcitonin levels in the 0.06 mg/kg exenatide administered once a day (group 2) was not different from controls (group 1). After dosing on day 3, plasma calcitonin levels at 0.25 mg/kg liraglutide (group 7) were significantly higher than all other group, including once or twice daily 0.25 mg/kg exenatide (groups 4 and 5). Day 3 post-dose calcitonin levels at 0.06 mg/kg liraglutide (group 6) were significantly higher than 0.06 mg/kg exenatide (administered once or twice a day, groups 2 and 3) at 12 hours after dosing, but it wasn't significantly different from exenatide treated group (groups 2 – 5) at any other time.

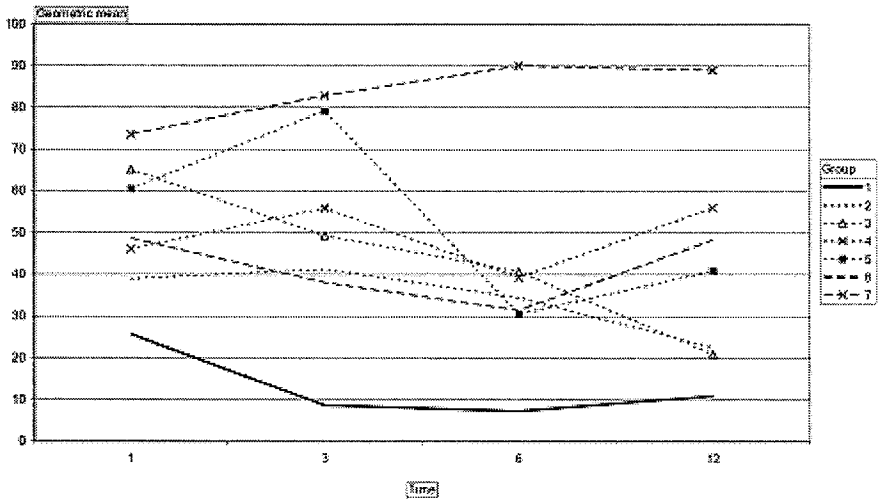


Figure 2 Post-dose plasma calcitonin levels
Geometric group mean post-dose plasma calcitonin (pg/ml)

[N000 4.2.3.7.3.1 P30]

Group 7 mice had the highest geometric mean plasma calcitonin levels. The table below shows plasma calcitonin levels measured in individual mice treated with saline (group 1, injected twice a day) or 0.25 mg/kg/day liraglutide (group 7, injected once a day). These results show all but 3 plasma calcitonin values in group 7 treated mice (mice # 258, 268, and 276, column labeled "calc" with values expressed as pg/mL rat plasma calcitonin equivalents) were below the highest value of 163.1 pg/mL in the control group (group 1, mouse 10). In fact, group 7 mice 257, 259, and 267 may not have responded to liraglutide because their calcitonin levels were < 50 pg/mL.

Obs	group	animal	time	day	calc
1	1	1	0	3	
2	1	2	0	3	
3	1	3	0	3	
4	1	4	0	3	
9	1	9	1	3	
10	1	10	1	3	
11	1	11	1	3	
12	1	12	1	3	
17	1	17	3	3	
18	1	18	3	3	
19	1	19	3	3	
20	1	20	3	3	
25	1	25	6	3	
26	1	26	6	3	
27	1	27	6	3	
28	1	28	6	3	
33	1	33	12	3	
34	1	34	12	3	
35	1	35	12	3	
36	1	36	12	3	

Obs	group	animal	time	day	calc
241	7	241	0	3	
242	7	242	0	3	
243	7	243	0	3	
244	7	244	0	3	
249	7	249	1	3	
250	7	250	1	3	
251	7	251	1	3	
252	7	252	1	3	
257	7	257	3	3	
258	7	258	3	3	
259	7	259	3	3	
260	7	260	3	3	
265	7	265	6	3	
266	7	266	6	3	
267	7	267	6	3	
268	7	268	6	3	
273	7	273	12	3	
274	7	274	12	3	
275	7	275	12	3	
276	7	276	12	3	

b(4)

[Complied from data in Appendix A at N000 4.2.3.7.3.1 P96, 101-102]

- 2005 005 / Modeling of pharmacokinetics and effect on plasma calcitonin after once daily dose administration of liraglutide

The sponsor performed nonlinear mixed-effects modeling of liraglutide pharmacokinetics and pharmacodynamics (liraglutide induced increased plasma calcitonin) in mice using data from a single dose study and a 3 day repeat dose study (reports 205106 and 205050, respectively). Modeling exenatide pharmacokinetics and pharmacodynamics was performed in a separate study (report 2005 001).

The pharmacokinetic model as described by the sponsor follows.

The final model was a one-compartment model with absorption lag-time, linear absorption and elimination and with random effects on clearance and absorption rate constant:

$$C(t) = \frac{Dose \cdot F \cdot k_a}{V \cdot (k_a - CL/V)} \left[e^{-(t-t_{lag}) \cdot CL/V} - e^{-(t-t_{lag}) \cdot k_a} \right] \quad for\ t > t_{lag}$$

The concentration is 0 if $t \leq t_{lag}$.

No effect of dose was statistical significant but for sex there was a statistically significant effect (p=0.12%) on CL/F with males having about 13% reduced clearance compared to females. However, the estimation procedure of NONMEM did not succesfully complete the covariance step thus the effect was not included in the final model.

The additive part of the error model came out with a variance close to 0, so the final error model was given by $f^0 \cdot \varepsilon$.

Parameter estimates are given in Table 1 where V/F and CL/F are given since CL, V, and F can not be calculated when only subcutaneous data are available. These parameter estimates are in line with non-compartmental PK parameters calculated in (4). In NONMEM (see Appendix A) the model was parameterised in terms of log-parameters in order to ensure positive pharmacokinetic parameters and random effects also appear on the log-scale.

[N000 4.2.3.7.3.1 P9]

Pharmacokinetic parameters determined from pharmacokinetic modeling of subcutaneously administered liraglutide in mice are shown in Table 1 (below). The 95% confidence interval of estimated plasma liraglutide concentrations on day 3 after once daily dosing with 0.225 mg/kg dose (60 nmol/kg) liraglutide administered subcutaneously is shown in Figure 7.

Table 1 Parameter estimates for the one-compartment model with lag-time.

Parameter	Estimate	CV(%)	Between subject variation CV(%)	Between subject Correlation (log-scale)
CL/F (L/kg/h)	0.033	6.4	1.7	
V/F (L/kg)	0.24	6.2	-	-0.94
K_a (1/h)	0.33	16	16	
t_{lag} (h)	0.35	13	-	
θ	0.98	2.7	-	
σ^2	0.27	[redacted]		

[N000 4.2.3.7.3.1 P10]

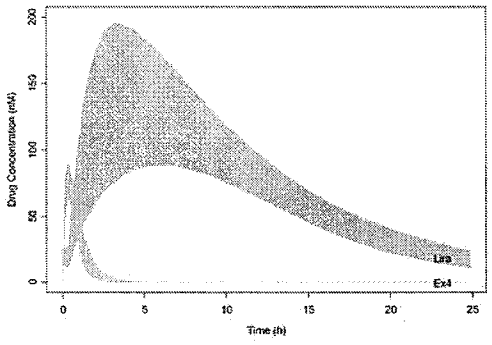


Figure 7 95% CI (day 3) for concentrations of liraglutide and exendin-4 after once daily dosing with 60 nmol/kg for three days.

[N000 4.2.3.7.3.1 P16]

The pharmacokinetic model (liraglutide induced increased plasma calcitonin) as described by the sponsor follows.

The final model was an indirect response model with ratio of CT to control as response and population predictions of liraglutide concentration as stimulatory input to the build up of calcitonin response. The E_{max} depended on whether a single dose (SD=1) or multiple doses (SD=0) had been administered, though this factor was confounded with which of the two studies the data originated from. No effects of sex could be detected. The equations defining the structural calcitonin response model are given below:

$$\frac{dR}{dt} = k_{out} \cdot (S - R)$$

$$S = \frac{E_{max} \cdot C}{EC_{50} + C}$$

$$E_{max} = (SD \cdot E_{max1} + (1 - SD) \cdot E_{max2}) \cdot \exp(b \cdot CT_{control})$$

$$\frac{CT}{CT_{control}} = 1 + R$$

[N000 4.2.3.7.3.1 P12-13]

Pharmacodynamic parameters estimated from modeling plasma calcitonin response to a single dose and 3 days of repeat dosing are summarized in table 2. Emax, the maximum fold liraglutide induced increase in plasma calcitonin over concurrent control ($CT/CT_{control}$), varied between single (Emax1) and repeat dose (Emax 2) data with the difference attributed to differences in standards used in the calcitonin assay. The EC_{50} was estimated at 11.8 – 44.3 nM liraglutide, which was in good agreement with the in vitro EC_{50} of 91 – 108 nM for liraglutide induced increased cAMP in plasma membranes of recombinant BHK 467-12A cells expressing the human GLP-1R (in the presence of human serum). The coefficient of variation was > 30% for most parameter estimates.

Table 2 Parameter estimates for the indirect response model on $CT/CT_{control}$

Parameter	Estimate	CV(%)
E_{max1}	7.8	31
EC_{50} (nM)	22.9	33
b	-0.062	66
k_{out} (1/h)	0.29	7.9
E_{max2}	19.5	37
Residual variance (σ^2)	2.3	-

[N000 4.2.3.7.3.1 P13]

Figure 5 shows the dependence of plasma calcitonin response on plasma liraglutide concentration assuming a constant control level of 10 pg/mL calcitonin.

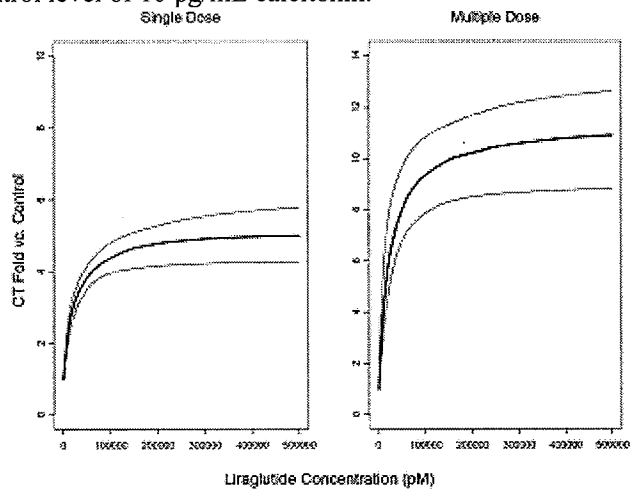


Figure 5 Simulated build-up of $CT/CT_{control}$ response (thick line) as function of liraglutide concentration, assuming constant CT control level=10 pg/mL. Outer lines mark the 95% CI. SD and MD profiles may not be directly comparable because they are based on measurements using different assays.

[N000 4.2.3.7.3.1 P14]

Modeling liraglutide and exenatide pharmacodynamic effects showed differences in the persistence of elevated serum calcitonin. Liraglutide elevated calcitonin persists for 24 hours after dosing while the effect of exenatide diminished (figure 8, below). These pharmacodynamic differences can be explained by pharmacokinetic differences where exenatide is eliminated from plasma at a much higher rate than liraglutide (see Figure 7, above).

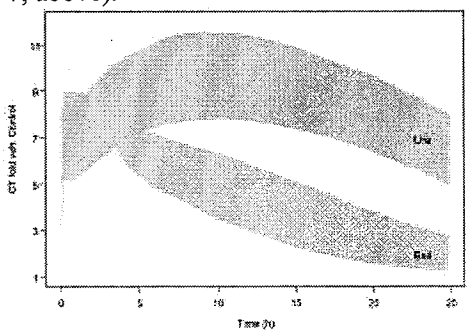


Figure 8 - Simulation of 95% CI (day 3) of calcitonin to constant control (=10 pg/ml) ratio in mice after once daily administration of 60 nmol/kg liraglutide and exendin-4, respectively.

[N000 4.2.3.7.3.1 P17]

Based on the pharmacodynamic model, the sponsor predicts on the third day of repeat dosing, continuous infusion of 60 pmol/kg exenatide (0.225 mcg/kg) will yield plasma calcitonin levels similar to those elicited by a single daily dose of 60 nmol/kg/day liraglutide (0.225 mg/kg) (Figure 9).

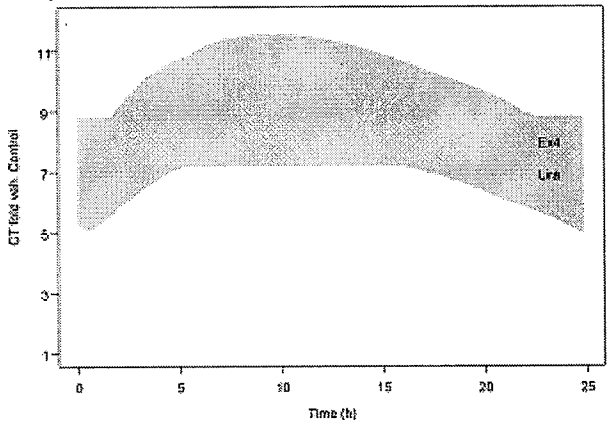


Figure 9 Simulation of 95% CI (day 3) of calcitonin ratio to constant control (=10 pg/ml) in mice after once daily administration of 60 nmol/kg liraglutide and continuous infusion of 60 nmol/kg exendin-4 per day, respectively.

[N000 4.2.3.7.3.1 P18]

Effects of Exenatide on Thyroid C-cell Parameters In Vivo

- 204402 / Study on the acute effects on calcitonin and toxicokinetics after single dose subcutaneous administration in fasted mice

Single doses of 0.25, 1, or 5 mg/kg exenatide increased plasma calcitonin in CD-1 mice, but the effect wasn't dose-related. Based on the fold increase calcitonin compared to the concurrent control at each time point for each sex and statistical analysis of the natural log transformed calcitonin plasma levels, the sponsor contends there was a treatment-related, but not dose-related, increase in plasma calcitonin with the most pronounce effect occurring 6 hours after dosing (the time point with the lowest control group mean calcitonin levels in both males and females, but not the time point with the highest calcitonin levels in any exenatide treated group).

Five groups of fasted CD-1 mice (5 / sex/dose/time point) were administered a single subcutaneous dose of 0, 0.25, 1, or 5 mg/kg exenatide (NNC 0113-0000-0000, 5 mL/kg) or a single intraperitoneal injection of 1 mmol/kg calcium to elicit calcitonin release. Study observations were clinical signs, exenatide toxicokinetics, and plasma calcitonin (prior to dosing and 0.25, 0.5, 1, 3, 6, and 24 hours after dosing).

There were no treatment-related mortalities or clinical signs.

Toxicokinetic parameters are summarized in Table 6. Both peak and total plasma exenatide plasma concentrations increased with dose with a less than dose proportional increase in Cmax and a dose proportional increase in $AUC_{0\text{-}inf}$. The elimination half life was < 1 hour in all dose groups except in males administered 5 mg/kg exenatide, the half life was 6.97 hours, primarily due to anomalously high exenatide levels in one male 24 house after dosing.

Table 6 Summary of TK parameter estimates

Group	NNC 0113-0000-0000 (mg/kg)	Gender	Tmax (hr)	Cmax (pmol/L)	AUC (hr*pmol/L)	$t_{1/2}$ (hr)
2	0.25	Female	0.50	76300	67500	0.32
		Male	0.50	96900	79000	0.30
		Mean	0.500	86600	73200	0.31[a]
3	1.0	Female	0.25	333000	254000	0.56
		Male	0.50	278000	266000	0.57
		Mean	0.375	306000	260000	0.57[a]
4	5.0	Female	0.25	1150000	1080000	0.52
		Male	0.25	1280000	1610000	6.97*
		Mean	0.250	1210000	1340000	0.97[a]

[a]Harmonic mean
*One animal (24 h after dosing) had a remarkably high plasma concentration of NNC 0113-0000-0000 compared to the other animals at the same time point (which all were below limit of quantification) and the 1½ at 5.0 mg/kg should be interpreted with caution.

[N000 4.2.3.7.3.1 P24]

Mouse plasma calcitonin was measured using a rat calcitonin IRMA (immunoradiometric assay, Immutopics kit, performed at [redacted] validated using rat calcitonin controls supplied with the kit. In vehicle treated control groups, mean calcitonin concentrations ranged from 7.6 – 72.5 pg/mL in males and 13.7 – 91.7 pg/mL from 0.25 to 24 hours after dosing (Table 5). In males, the group mean value was above the control range 0.25 hours after dosing with 1 mg/kg exenatide. In females, group mean plasma calcitonin levels were above the control range in the 0.25 mg/kg group (0.5 and 3 hours after dosing), the 1 mg/kg group (24 hours after dosing), and 5 mg/kg group (1 and 3 hours after dosing). Plasma calcitonin levels or time of peak calcitonin after dosing were not dose-related in either males or females. The magnitude of calcitonin increase elicited by NNC 90-1170 was much smaller than that elicited by calcium loading (intraperitoneally injected calcium (Table 4)). b(4

Table 5 Mean calcitonin values (pg/ml)*

Time	Group and sex							
	1		2		3		4	
	female	male	female	male	female	male	female	male
0.25	27.82	18.96	55.61	36.69	30.65	116.49	32.96	56.76
0.5	91.69	59.83	113.01	58.73	70.83	41.40	63.19	46.63
1	70.50	16.58	42.08	46.98	69.87	53.91	123.11	45.69
3	30.52	12.57	110.13	28.20	80.52	31.83	108.11	43.04
6	13.69	7.63	49.17	35.94	67.38	48.42	63.45	43.02
24	51.68	72.46	37.45	43.40	128.56	56.07	65.71	45.37

*Due to differences in the CT assay technique these figures are not directly comparable with those obtained in previous studies with liraglutide.

[N000 4.2.3.7.3.1 P23]

In the positive control group in which mice were intraperitoneally injected with calcium, plasma calcitonin increased 15 minutes after dosing in males and females. Table 4 shows the range and mean plasma calcitonin levels in group 5 with results from both sexes combined.

Table 4 Average of calcitonin 15 minutes after dosing

	Calcitonin (pg/ml)	
	Group 5 (9 mg Ca/ml) 15 min. after dosing	Group 1: Pre-dose
Range	13.49-1564.84	5.91-210.40
Mean	917.02	89.85

[N000 4.2.3.7.3.1 P23]

Calcitonin levels in individual group 5 mice are shown in Tables 1 and 2 below. Two mice were non-responsive to calcium injection: male 127 and female 330. It's unclear if these mice didn't receive the

proper dose of calcium, didn't respond to it, or if there was an error in sampling or the calcitonin assay for these mice.

Table 1 Calcitonin results for male mice

Group no	Male no	Dose	Comments	Assay ID	Dilution	Measured pg/ml if diluted	Calcitonin pg/ml
9 mg Ca/ml 5	126	15 min		02 05-Jan-18.01	-	-	
5	127	15 min	Haemolysed	02 05-Jan-18.01	175+25 µl	82	
5	128	15 min	Haemolysed Sample vol.seems <200 µl	02 05-Jan-18.01	-	-	
5	129	15 min	-	02 05-Jan-18.01	-	-	
5	130	15 min	-	02 05-Jan-18.01	-	-	

[Compiled from Table 1at N000 4.2.3.7.3.1 P68 - 71]

b(4)

Table 2 Calcitonin results for female mice

Group no	Female no	Dose	Comments	Assay ID	Dilution	Measured pg/ml if diluted	Calcitonin pg/ml
9 mg Ca/ml 5	326	15 min	-	04 05-Jan-18.01	-	-	
5	327	15 min	-	04 05-Jan-18.01	"	"	
5	328	15 min	-	04 05-Jan-18.01	-	-	
5	329	15 min	-	04 05-Jan-18.01	-	"	
5	330	15 min	-	04 05-Jan-18.01	"	"	

[Compiled from Table 2at N000 4.2.3.7.3.1 P71- 74]

- 205074 / In vivo study with administration of NNC 0113-0000-0000 by subcutaneous administration as bolus injections (once, twice, three times daily) or continuous infusion in female mice

Continuous infusion of 0.25 mg/kg exenatide resulted in sustained elevated plasma calcitonin, but bolus subcutaneous injection of the same dose, either once, twice, or three times daily, did not. The effect of 0.25 mg/kg/day exenatide subcutaneously administered once a day (group 2), twice a day (0.125 mg/kg/injection, group 3), or 3 times a day (0.083 mg/kg/injection, group 4) for 2 days was compared to vehicle treated mice (injected 3 times a day, group 1). Mice in groups 5 and 6 were continuously administered 0 (vehicle, group 5) or 0.25 mg/kg/day exenatide (group 6) for 2 days using an AZLET osmotic minipump. The study objective was to determine the effect of the frequency of exenatide administration on plasma calcitonin levels. Other study parameters were clinical signs, body weight (daily), and exenatide toxicokinetics. Mice were fasted 7 – 9.25 hours and anesthetized with isoflurane prior to retro-orbital sinus blood sampling for plasma calcitonin and exenatide taken 1, 3, 6, 12, 18, and 24 hours after the first dose on the second day of dosing. After sample collection, mice were euthanized by exsanguination and cervical dislocation.

Table 1 Details of subcutaneous dosing

Group	Compound	Dose Conc. (mg/ml)	Dose regimen	Dose per regimen (mg/kg)	Daily dose (mg/kg)	Dose Volume (ml/kg)
1	Vehicle	0	8 h	0	0	5
2	NNC 0113 -0000-0000	0.050	24 h	0.25	0.25	5
3	NNC 0113 -0000-0000	0.025	12 h	0.125	0.25	5
4	NNC 0113 -0000-0000	0.017	8 h	0.083	0.25	5

[N000 4.2.3.7.3.1 P21]

Table 5 Details of continuous dosing

Group	Compound	Dose concentration (mg/ml)	Dose regimen (µg/kg/h)	Daily dose (mg/kg)
5	Vehicle	0	0	0
6	NNC 0113-0000-0002	0.297	10.4	0.25

[N000 4.2.3.7.3.1 P22]

There were no unscheduled deaths. Exenatide formulations were within 16% of the nominal concentrations in all dose groups. Analysis of exenatide solution recovered from implanted osmotic minipumps after 48 hours showed stability of exenatide in the osmotic pumps was compromised by phosphate ——with a significant contamination of an unidentified compound (may be exenatide-related based on its HPLC retention time, Figures 1 and 2 below). Exenatide plasma levels were demonstrated in mice administered the drug by subcutaneously implanted AZLET minipump. b(4)

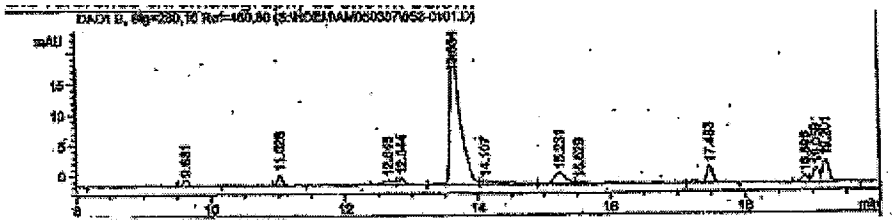


Figure 1. Reference sample of Ex-4

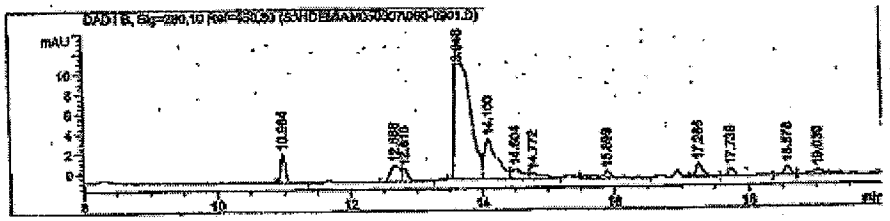


Figure 2. Ex-4 recovered from osmotic pump (group 6F) after 48 h incubation in rat

[N000 4.2.3.7.3.1 P77]

A GLP-1R agonist assay was performed on the recovered material to assess its biologic activity, and it was active (but the results presented by the sponsor were uninterpretable, see Appendix B, Figure 3).

Figure 3. Below is shown the results from the GLP-1 radio-receptor assay.

[N000 4.2.3.7.3.1 P77]

Plasma exenatide concentrations (natural log of exenatide concentrations) versus time graph after the first dose is shown in Figure 1 (below). Although exenatide was administered twice a day (group 2) or 3 times a day (group 3), plasma exposure was probably underestimated in these groups because of the short plasma elimination half life of exenatide and blood sampling intervals.

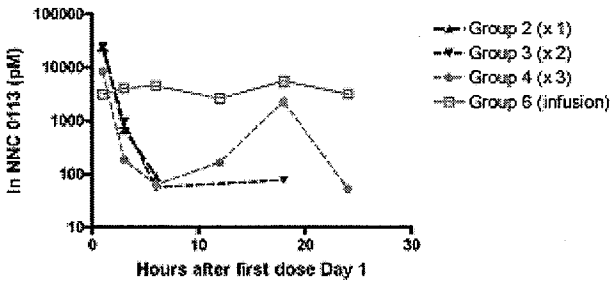


Figure 1 Plasma levels of NNC 0113-0000-0000 (pM) of group 2, 3, 4 and 6.
Groups 2-4 was administrated a total daily dose of 0.25 mg/kg/day NNC 0113-0000-0000 by subcutaneous bolus injections either once daily (0.25 mg/kg, group 2), twice daily (0.125 mg/kg, group 3) or three times daily (0.083 mg/kg, group 4). Group 6 was dosed a daily dose 0.25 mg/kg by continuous infusion (10.4 µg/kg/h). Only plasma levels above limit of detection (45 pM) is shown in the figure. Blood samples were taken 1-24 h after first dosing Day 1.

[N000 4.2.3.7.3.1 P28]

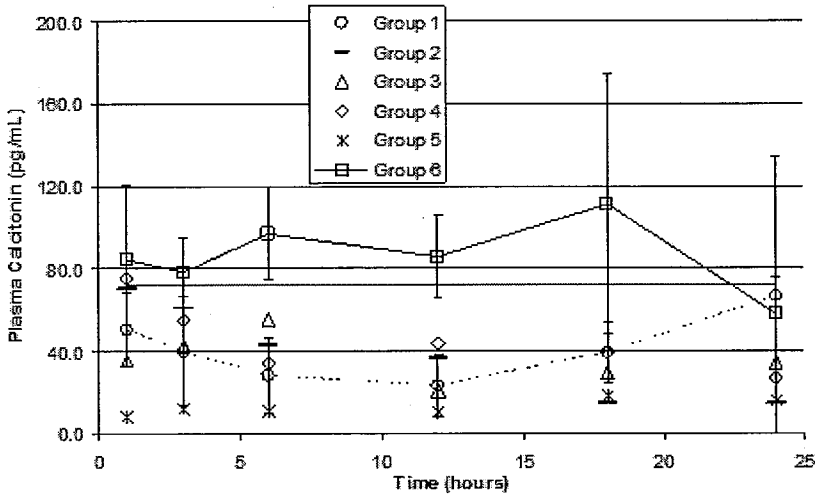
One hour after dosing, a low level of exenatide occurred in the control group that the sponsor attributes to contamination of the dosing solution or blood/plasma samples, but a definitive cause of control group contamination was not determined and it didn't occur at any other time point in group 1. In AZLET minipump control group, exenatide levels were above the 45 pg/mL lower limit of detection 28 hours after dosing in one mouse, but this was considered an incidental finding because it only occurred in one mouse and only 18 hours after dosing.

Table 18 Summary of plasma content of NNC 0113-0000-0000

Group No	1 Hrs	3 Hrs	6 Hrs	12 Hrs	18 Hrs	24 Hrs
1	271	0	0	0	0	0
	127	0	0	0	0	0
	321	0	0	0	0	0
	208	0	0	0	0	0
	171	0	0	0	0	0
Mean	220	0	0	0	0	0
Std.dev	77	0	0	0	0	0
5	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	58	0
Mean	0	0	0	0	12	0
Std.dev	0	0	0	0	26	0

[N000 4.2.3.7.3.1 P58-59]

The control group average plasma calcitonin level was 41.2 +/- 31.1 pg/mL (n = 30, all time points), so group mean values < 72.3 pg/mL (the average + 1 s.d for the control) were considered within the control group range. In group 6 (continuous infusion of 0.25 mg/kg exenatide), plasma calcitonin was statistically significantly higher than concurrent controls 3, 6, 12 and 18 hours after the first dose (first dose administered to control group (group 1) and groups 2, 3, & 4) on study day 2. The graph below shows group mean plasma calcitonin levels. Plasma calcitonin levels in groups subcutaneously injected with exenatide once, twice, or 3 times daily were independent of plasma exenatide concentration.



Group mean plasma calcitonin levels versus time on study day 2 after the first dose. Error bars represent standard deviations of the mean for data in groups 1 and 6. Plasma calcitonin levels were significantly higher than concurrently controls (unpaired t-test, two-tailed $p < 0.05$) at hours 3, 6, 12 and 18. The red line at 72.3 pg/mL calcitonin is the value of mean + 1 sd of all plasma calcitonin measurements from control group 1.

Figure 2 (below) is a plasma calcitonin (Ln of plasma calcitonin) versus time graph on study day 1 (the second day of dosing). The sponsor concluded exenatide elevated plasma calcitonin levels in all groups, but continuous infusion of exenatide resulted in sustained plasma exenatide levels and elevated plasma calcitonin.

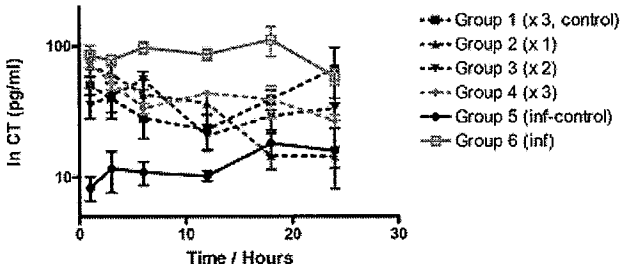


Figure 2 Plasma levels of calcitonin (pg/ml) of groups 1-6.
Group 1 was administrated vehicle three times daily. Groups 2-4 was administrated a total daily dose of 0.25 mg/kg/day NNC 0113-0000-0000 by subcutaneous bolus injections either once daily (0.25 mg/kg, group 2), twice daily (0.125 mg/kg, group 3) or three times daily (0.083 mg/kg, group 4). Group 5 and 6 was administrated vehicle or 0.25 mg/kg/day NNC 0113-0000-0000 (10.4 µg/kg/h) by continuous infusion by osmotic mini pumps. Blood samples were taken 1-24 h after first dosing Day 1.

[N000 4.2.3.7.3.1 P30]

- 205025 / Preliminary investigative study by subcutaneous administration (3 times a day) to CD-1 mice for 2 or 13 weeks – Combined evaluation of the in life phase including hormone analysis and C-cell pathology of the thyroid gland and molecular analysis

Subcutaneous bolus injections of exenatide (0.33 mg/kg/injection 3 times daily for 8 days, then 1 mg/kg/injection 3 times daily for 12 weeks) increased week 13 plasma calcitonin in male CD-1 mice, but not females, and caused distended gall bladder in females. Subcutaneous injections of 0.25, 1, or 5

mg/kg/day exenatide once a day for 2 weeks dose-dependently increased calcitonin mRNA in thyroid, but did affect thyroid GLP-1R mRNA levels.

The effect of subcutaneously injected exenatide on plasma calcitonin, thyroid c-cells, and thyroid GLP-1R and calcitonin mRNA levels was determined in CD-1 mice after dosing with 0 (vehicle), 0.25, 1, or 5 mg/kg/day exenatide injected 3 times daily (0, 0.083, 0.33, or 1.67 mg/kg/injection) for 2 weeks (6/sex/dose) or in a 13 week study of 21 mice/sex/dose, 0 mg/kg (vehicle) for 13 weeks or 1 mg/kg/day (0.33 mg/kg/injection) for 8 days followed by 3 mg/kg/day for 12 weeks (13 week total treatment time). Study parameters were clinical signs, body weight, food consumption, plasma calcitonin, anti-liraglutide antibody analysis, macroscopic pathology, and thyroid histopathology including a quantitative analysis of c-cells.

There were no treatment-related mortalities. Weight loss and decreased food consumption occurred at all exenatide doses on day 1. Decreased food consumption persisted to day 2 for males and to day 5 for females treated with 1 or 5 mg/kg exenatide. Food conversion efficiency trended higher in all exenatide dose groups in the 2 week study and in the 1 / 3 mg/kg group in the 13 week study. Anti-liraglutide antibodies were not detected.

Reviewer note: A "radioimmunoassay" was described in which the sponsor added ^{125}I exenatide to a sample (sample not described in the methods section of Annex 5, antibody determination SOP referenced, but not included in report), incubating it, then quantifying radioactivity in protein precipitant using a gamma counter. The sensitivity of the assay is unknown.

After 2 weeks, group mean calcitonin was statistically significantly higher in all exenatide treated groups compared to concurrent controls (unpaired t-test, $p< 0.05$, Table 1) with calcitonin increased up to 6.2 fold in males and up to 8.1 fold in females.

Table 1 N, mean, and standard deviation of calcitonin values in two week groups

	sex					
	F			M		
	Mean calc	Std calc	Nobs	Mean calc	Std calc	Nobs
group						
1	9.07	2.72	6	8.35	1.65	6
3	48.30	20.38	6	36.85	11.80	5
4	33.24	17.40	6	67.78	31.95	6
5	56.57	24.02	6	62.94	31.91	6

[N000 4.2.3.7.3.1 P279-80]

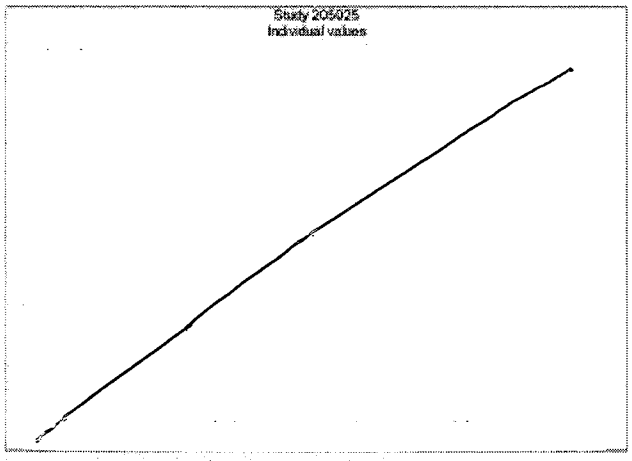
At the end of 13 weeks, group mean calcitonin was significantly increased in 1 / 3 mg/kg/day treated males, but not in females. In 1 / 3 mg/kg males, plasma calcitonin was 3.8 fold higher than controls.

Table 5 N, mean, and standard deviation of calcitonin values for 13 week groups

	group					
	2			6		
	Mean calc	Std calc	Nobs	Mean calc	Std calc	Nobs
sex						
F	45.90	56.47	21	80.41	99.21	21
M	13.19	5.90	20	50.53	23.00	21

[N000 4.2.3.7.3.1 P281]

Figure 2 shows the natural log of plasma calcitonin concentrations from each dose group in the 13 week study. Most of the values in treated females were within the control group range (compare control group 2F to treated group 6F).



b(4)

Figure 2 Individual ln transformed calcitonin values for 13 week groups

[N000 4.2.3.7.3.1 P281]

After 2 weeks, no macroscopic pathology findings considered treatment related occurred. After 13 weeks, the incidence of distended gall bladder was significantly increased in females in the 1 / 3 mg/kg/day group.

Group	:	1	2	3	4	5	6	
Compound	:	Control	Control	- NNC0113-0000-0000 -				
Dosage (mg/kg/day)	:	0	0	0.25	1.0	5.0	1.0/3.0	
						--- N U M B E R - O F		
					SEX:	--MALE--		-FEMALE-
					GROUP:	-2-	-6-	-2- -6-
ORGAN AND KEYWORD(S) OR PHRASE					NUMBER:	20	21	21 21
** TOP OF LIST **								
COLON				NUMBER EXAMINED:		20	21	21 21
YELLOW COLOURATION						1	0	0 0
GALL BLADDER				NUMBER EXAMINED:		20	21	21 21
DISTENDED						0	0	0 10 b
LN MESENTERIC				NUMBER EXAMINED:		20	21	21 21
CONGESTED						0	1	0 0
LUNGS & BRONCHI				NUMBER EXAMINED:		20	21	21 21
CONGESTED						0	0	1 0
OVARIES				NUMBER EXAMINED:		0	0	21 21
CYST(S)						0	0	4 5
CYSTIC						0	0	1 2
SEMINAL VESICLES				NUMBER EXAMINED:		20	21	0 0
DARK AREA(S)						0	1	0 0

Significant when compared with Group 1: a - p<0.05; b - p<0.01

[N000 4.2.3.7.3.1 P432]

Only thyroids were examined microscopically from mice sacrificed after 2 or 13 weeks of exenatide treatment. Thyroid tissue sections were immunohistochemically stained for calcitonin to identify c-cells. The only treatment-related qualitative microscopic finding was increased incidence of prominent ultimobranchial ducts in males treated for 13 weeks with 1 / 3 mg/kg/day liraglutide.

Text-table 4
Histopathology – group distribution of qualitative findings after 13 weeks of treatment

Group Dosage (mg/kg/day)	2M 0	6M 1.0/3.0	2F 0	6F 1.0/3.0
Number of animals examined	21	21	21	21
Thyroids (left and right lobes)				
Prominent ultimobranchial follicle (s) (ducts)	3	10	7	5
Ectopic C-cells	1	1	0	0
Ectopic thymus	1	0	2	3
No C-cells present	0	1	0	0

[N000 4.2.3.7.3.1 P38]

Thyroid c-cells were quantified by examining anti-calcitonin stained slides of the left thyroid lobe from mice treated for 2 or 13 weeks. C-cell counts / mm^2 thyroid were highly variable and significantly decreased compared to the concurrent control in 2 week 0.25 mg/kg treated females and significantly higher in 13 week 1 / 3 mg/kg treated females. Given the variability in the response, and the fact that both decreased and increased c-cells occurred in exenatide treated groups, the biological significance of these changes are equivocal. The sponsor considers increased c-cells in group 6 females a biologically significant adaptive response, despite the significant decrease seen in group 3 females and the absence of qualitative c-cell findings including c-cell hyperplasia or adenoma.

TABLE 5

Quantitative C-cell evaluation - group mean values (C-cells/ mm^2)

Group	1	2	3	4	5	6
Compound	Control	Control	.....	NNC01	13-0000-0000	.....
Dosage (mg/kg t.i.d)	0	0	0.083	0.33	1.67	0.33/1.0
Dosage (mg/kg/day)	0	0	0.25	1.0	5.0	1.0/3.0

Group /sex	Dosage (mg/kg/day)	Duration of treatment weeks	Number of animals +	Mean C-cells/mm^2	SD
1M	Control	2	5	198	111
3M	0.25	2	5	154	40
4M	1.0	2	5	140	43
5M	5.0	2	6	200	89
1F	Control	2	6	215	105
3F *	0.25	2	5	87 a	44
4F	1.0	2	6	155	98
5F	5.0	2	6	329	114
2M	Control	13	18	193	70
6M	1/3	13	19	196	104
2F **	Control	13	20	151	77
6F	1/3	13	21	230 b	82

\+ Number of animals with at least one level with C-cells
SD Standard deviation
a Significant when compared with group 1 $p < 0.05$; b – significant when compared with group 2 $p < 0.01$
* Animal 99 (a female in group 3) was excluded
** Animal 74 (a female in group 2) was excluded

[N000 4.2.3.7.3.1 P75]

Relative calcitonin and GLP-1R mRNA levels in thyroid from mice treated with exenatide for 2 weeks was measured by real time quantitative PCR incorporating fluorescent primers in amplified cDNAs and normalizing target transcript levels to transcript levels encoding housekeeping protein GAPDH and beta actin. Summary results are shown in Table 2 (below) and Figure 2 (below). Exenatide significantly and dose-dependently increased thyroid calcitonin mRNA up to 4.8 fold at $\geq$ 0.25 mg/kg.

Table 2 The effect of Exendin-4 on calcitonin mRNA expression levels in thyroid tissue from mice.

Dose level	*N° of samples*	$2^{-\Delta Ct}$ *Mean*	$2^{-\Delta Ct}$ *Standard deviation*	*Effect of treatment**	*p-value*
Vehicle	11	0.444	0.466	1.0	n.a.
0.25 mg/kg/day	8	1.017	0.352	2.3	0.010
1.0 mg/kg/day	8	1.770	1.147	4.0	0.003
5.0 mg/kg/day	6	2.148	1.617	4.8	0.005

* The effect of Exendin-4 treatment on the levels of mRNAs encoding calcitonin was expressed as fold upregulation of the mRNA level in Exendin-4-treated mice when compared to vehicle-treated mice.

[N000 4.2.3.7.3.1 P335]

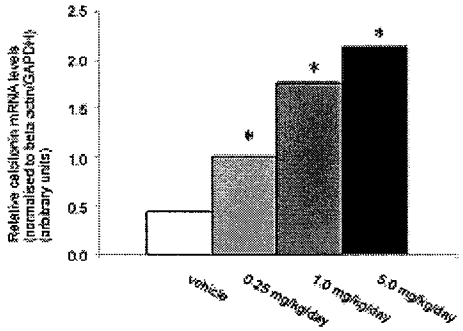


Figure 2 Levels of calcitonin mRNA in mouse thyroid after Exendin-4 treatment

Relative calcitonin mRNA levels in mouse thyroid glands were determined by normalisation to beta actin/GAPDH, and expressed as $2^{-\Delta Ct}$ (arbitrary units). The columns represent mean $2^{-\Delta Ct}$ values within each treatment group (Table 2). Compared to the vehicle group, a dose-dependent increase in the levels of calcitonin mRNA could be observed after two weeks of Exendin-4-treatment. * The upregulated calcitonin mRNA levels in Exendin-4 treated animals were statistically significant, compared to vehicle-treated animals ($p<0.01$, student's t-test).

[N000 4.2.3.7.3.1 P335]

Exenatide did not affect thyroid GLP-1R mRNA levels (Table 3).

Table 3 The effect of Exendin-4 on GLP-1R mRNA expression levels in thyroid tissue from mice.

Dose level	*N° of samples*	$2^{-\Delta Ct}$ *Mean*	$2^{-\Delta Ct}$ *Standard deviation*	*Effect of treatment**	*p-value*
Vehicle	11	0.003	0.005	1.0	n.a.
0.25 mg/kg/day	8	0.004	0.003	1.3	0.697
1.0 mg/kg/day	8	0.002	0.001	0.7	0.664
5.0 mg/kg/day	6	0.002	0.001	0.9	0.897

* The effect of Exendin-4 treatment on the levels of mRNAs encoding GLP-1R was expressed as fold upregulation of the mRNA level in Exendin-4-treated mice when compared to vehicle-treated mice.

[N000 4.2.3.7.3.1 P336]

- 205205 / Investigatory toxicity study by osmotic minipump subcutaneous administration to CD-1 mice for 12 or 16 weeks

Subcutaneous infusion of 0.25 or 1 mg/kg/day exenatide in CD-1 mice increased plasma calcitonin within 4 weeks of treatment and caused focal c-cell hyperplasia in thyroid after 12 weeks.

CD-1 mice were treated with exenatide by continuous subcutaneous infusion (0, 0.25, or 1 mg/kg/day, groups 1 – 3) or once daily subcutaneous injection (0 or 0.25 mg/kg/day, groups 4 – 5) for 12

or 16 weeks. Treatment groups are shown in Text-table 1, below. For continuous infusions, AZLET minipumps were replaced every 4 weeks and removed 24 hours prior to sacrifice. The dose volume of subcutaneous injections was 5 mL/kg in week 1 and 1 mL/kg thereafter. Half of each treatment group was sacrificed in week 12 with surviving mice sacrificed at the end of week 16. Study parameters were clinical signs, body weight, food consumption, fasted blood samples (8 – 16 hours prior to collection) every 4 weeks from groups 1 – 3 and prior to termination for quantifying exenatide exposure, plasma calcitonin, and anti-exenatide antibodies, gross necropsy, and histopathology of thyroid with anti-calcitonin antibody staining to identify c-cells. In-life retro-orbital blood samples were obtained in weeks 4 and 8 from isoflurane anesthetized mice. Terminal vena cava blood samples were obtained in weeks 12 and 16 from isoflurane anesthetized mice.

Text-table 1 – Identity of treatment groups

Group	Treatment	Route	Dosage (mg/kg/day) #	Number of animals		Cage and animal numbers +	
				Male	Female	Male	Female
1	Control	Mini-pump	0	36	36	1-36	157-192
2 *	NNC 0113-0000-0000	Mini-pump	0.25	36	36	37-72	193-228
3 *	NNC 0113-0000-0000	Mini-pump	1.0	36	36	73-108	229-264
4	Control	Subcutaneous injection	0	24	24	109-132	265-288
5 *	NNC 0113-0000-0000	Subcutaneous injection	0.25	24	24	133-156	289-312

Expressed in terms of the active ingredient. A conversion factor of 1.1 for Batch 0555903 and 1.12 for Batch 2580234 was applied to convert the amount of test material as received to that in terms of the active ingredient
* NNC 0113-0000-0000 (exenatide); + Animals singly housed.

[N000 4.2.3.7.3.1 P15]

Reviewer note: The following definition of c-cell hyperplasia was used in this study:

"C-cell hyperplasia is an increase in the number of C-cells to form an aggregate of less than five average follicles in diameter with or without displacement of individual thyroid follicles. This corresponds to Grade 3 in the 5-point scale mentioned above [N000 4.2.3.7.3.1 P336]."

Exenatide concentrations in dosing formulas were within limits (-15% to + 5% of nominal concentration). After the subcutaneous injection dose volume was reduced to 1 mL/kg in week 2, the exenatide concentration was below acceptable limits (-27.8% of nominal concentration), but within acceptable limits by week 8 (-10.2%). To verify the stability of exenatide in implanted minipumps, its concentration was measured after recovery of the dosing formulation from minipumps removed prior to sacrifice. After 4 weeks, exenatide concentrations within minipumps were within acceptable limits for the 1 mg/kg/day high dose (-.4 to -13.1%), but not for the 0.25 mg/kg/day low dose (-31.8% to +6.2%).

Eight unscheduled deaths occurred, but none of the deaths were considered treatment related by the sponsor.

Unscheduled Deaths

Minipump
Group 1 (0 mg/kg): 1 male, 1 female
Group 2 (0.25 mg/kg): 1 male, 1 female
Group 3 (1 mg/kg): 1 male, 2 females

Subcutaneous injection
Group 4 (0 mg/kg): 1 male

A female in group 3 was sacrificed moribund with clinical signs of pallor. Morbidity and mortality of other decedents in groups 1 – 3 was attributed to damage / trauma at the site of minipump implantation. The decedent in group 4 was sacrificed moribund due to trauma of the pinnae and abrasions on the skin.

There were no clinical signs considered treatment-related, but scabs, abrasions, and areas of reddening were associated with surgical implantations and occasional clipper damage. Both doses of continuously infused exenatide decreased body weight and body weight gain compared to controls, but subcutaneously injected 0.25 mg/kg exenatide increased body weight (group 5 compared to group 4). In mice treated by continuous infusion of exenatide, food consumption was decreased up to week 10 in males and for the entire treatment period in females. Subcutaneously injected exenatide had no effect on food consumption.

Text-table 9 - Bodyweight gain during the 16-week treatment period

Gain period	Sex	Group	N[1]	Mean[2]	SD[3]
Week 0 - 11	Males	1	35	7.9	2.2
		2	35	5.9	1.4
		3	35	5.6	1.6
		4	24	5.6	1.8
		5	24	6.8	2.7
	Females	1	35	6.9	2.8
		2	35	5.8	2.0
		3	35	5.9	1.9
		4	24	4.5	2.9
		5	24	5.7	1.5
Week 0 - 15	Males	1	17	9.0	2.4
		2	17	6.3	1.5
		3	17	6.2	1.7
		4	11	6.5	2.6
		5	12	9.1	4.3
	Females	1	17	8.0	3.4
		2	17	6.5	2.3
		3	16	6.6	2.1
		4	12	5.2	2.9
		5	12	7.1	1.6

1 - Number of animals; 2 - Mean of gain; 3 - Standard deviation

[N000 4.2.3.7.3.1 P36]

Reviewer note: Group mean body weight of subcutaneously injected control group mice (group 4) was statistically significantly lower (unpaired t-test, $p < 0.05$) than minipump implanted control group mice (group 1) in both males and females in both study periods (0 – 11 weeks or 0 – 15 weeks). This lower body weight in control group 4 accounts for higher body weight in group 5.

In an anti-exenatide antibody screening assay, anti-exenatide antibodies were detected in 5 of 302 samples by precipitating protein bound ^{125}I-exenatide from mouse plasma after addition and incubation with the radioligand. Antibody positive mice were from the 0.25 mg/kg/day exenatide infusion group (group 3, 2 males and 3 females). Exenatide neutralizing potency was assessed for samples testing positive in the screening assay. All 5 samples inhibited < 30% of exenatide stimulated cAMP accumulation in BHK cells with the human GLP-1R and were therefore considered negative in the neutralization assay.

Reviewer note: The anti-exenatide antibody screening assay was not validated for exenatide, but a similar validated assay is used for anti-liraglutide antibody screening (SOP 878-LP-08006, doc no 022751).

In study weeks 4 and 8, plasma levels of exenatide were determined in mice subcutaneously administered exenatide by continuous infusion (groups 1 – 3). Group mean plasma levels in weeks 4 and 8 (summarized in the table below) increased with dose. Plasma levels ranged from 823 to 8,320 pM in the 0.25 mg/kg group and 2,030 to 25,800 in the 1 mg/kg group. Plasma exenatide was near of below the

limit of detection 24 hours after the minipump was removed (prior to sacrifice). Plasma exenatide was below the limit of detection 24 hours after the last subcutaneously injected dose.

Infusion Dose (mg/kg/day)	Week	Plasma Exenatide (pM)			
		Male		Female	
		Mean	SD	Mean	SD
0	4	BLQ		BLQ	
	8	BLQ		BLQ	
0.25	4	4,245	2,362	2,313	1,098
	8	2,615	1,207	4,939	1,994
1	4	11,692	7,802	9,778	4,143
	8	15,539	3,401	15,194	3,314

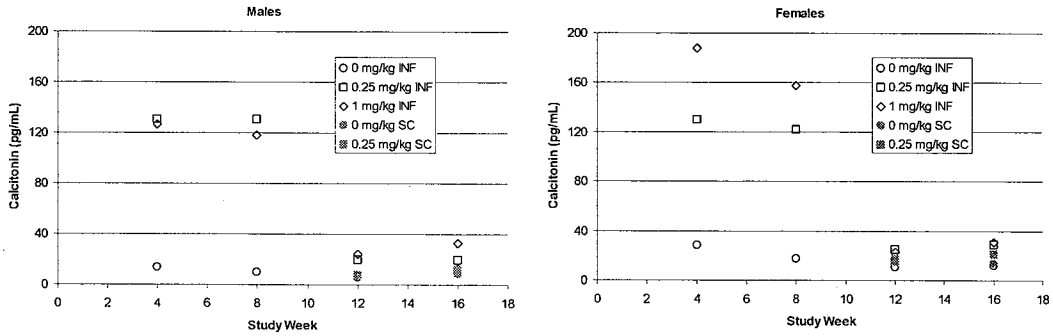
Plasma calcitonin levels were determined from orbital sinus blood collected during weeks 4 and 8 and from vena cava blood collected prior to terminal sacrifice in weeks 12 and 16. Group mean plasma calcitonin concentrations were summarized in Table 8. In mice treated by subcutaneous infusion, calcitonin was significantly higher in 0.25 or 1 mg/kg/day groups compared to concurrent controls in both sexes in weeks 4, 8, 12, and 16. Compared to concurrent controls, group mean calcitonin levels were not significantly increased in mice subcutaneously injected once a day with 0.25 mg/kg/day exenatide for 12 or 16 weeks.

Table 8 Mean and standard deviation (std) of calcitonin measurements

		week							
		4		8		12		16	
		calcitonin		calcitonin		calcitonin		calcitonin	
		Mean	Std	Mean	Std	Mean	Std	Mean	Std
sex	group								
F	1	27.95	46.16	17.57	9.07	10.53	8.64	12.35	10.70
	2	130.19	65.81	122.20	35.00	24.99	13.40	28.80	10.83
	3	188.20	84.63	157.69	58.09	23.16	16.61	31.22	19.43
	4	.	.	.	.	18.27	41.16	13.32	4.57
	5	.	.	.	.	15.06	10.59	21.07	9.93
M	1	13.60	8.11	10.04	9.91	6.07	2.35	12.57	14.25
	2	130.22	33.62	130.40	77.26	19.67	14.65	19.88	15.95
	3	126.73	31.03	117.72	56.71	24.03	20.25	32.48	24.74
	4	.	.	.	.	6.33	2.87	8.21	3.42
	5	.	.	.	.	7.23	3.35	11.64	6.09

[N000 4.2.3.7.3.1 P36]

There was a large difference in calcitonin levels determined in weeks 4 and 8 compared to weeks 12 and 16 (Table 8 and graph below). During blood collection in weeks 4 and 8 treatment was continued, but in weeks 12 and 16, treatment was stopped 24 hours prior to collection.



Gross and histopathology findings after 12 or 16 weeks of exenatide treatment by continuous subcutaneous infusion (0, 0.25, or 1 mg/kg/day) or once daily subcutaneous injection (0 or 0.25 mg/kg/day) are summarized in the table below. Pathology findings were mainly confined to mice

administered exenatide by subcutaneous infusion. A dose-related increased incidence of distended gall bladder occurred at 0.25 and 1 mg/kg exenatide in both males and females. At the end of 16 weeks, distended gall bladder occurred at 0.25 and 1 mg/kg in females, but it only occurred at 1 mg/kg in males. Dose-related increased thyroid c-cell hyperplasia occurred after 12 weeks subcutaneous infusion with 0.25 or 1 mg/kg exenatide. After 16 weeks, increased thyroid c-cell hyperplasia occurred at 0.25 and 1 mg/kg/day exenatide infusion in males and females, but the increased incidence was only dose-related in females.

Organ	Finding	Week	Incidence	Males					Females				
				Infusion			SC		Infusion			SC	
				0	0.25	1.00	0	0.25	0	0.25	1.00	0	0.25
Gall bladder	distension	12	#	0	1	3	0	0	0	2	5	0	0
			%	0	3	8	0	0	0	6	14	0	0
		16	#	0	0	1	0	0	0	3	5	0	0
			%	0	0	3	0	0	0	8	14	0	0
Thyroid	c-cell hyperplasia	12	#	1	6	9	0	0	0	5	11	0	2
			%	6	33	50	0	0	0	28	58	0	17
		16	#	1	7	5	0	0	0	6	10	1	0
			%	6	39	28	0	0	0	33	53	8	0
	dilated ultimobranchial ducts	12	#	5	2	5	2	2	4	3	5	1	2
			%	29	12	29	17	17	24	18	31	8	17
		16	#	5	5	4	2	2	2	5	3	0	1
			%	29	29	24	17	17	12	29	19	0	8

- 2005 001 / Modeling of exendin-4 concentration and effect on plasma calcitonin in mice

The sponsor performed non-linear mixed effect modeling of exenatide pharmacokinetics and pharmacodynamics (exenatide induced increased plasma calcitonin) in mice. The effect of subcutaneous injected exenatide on plasma calcitonin in mice was modeled based on results from a study of single dose subcutaneous injections of 0, 0.25, 1, or 5 mg/kg exenatide (report 204402), a 3 day repeat dose subcutaneous injections of 0, 0.03, or 0.125 mg/kg injected every 12 hours or 0.06 or 0.25 mg/kg injected every 24 hours (report 205050), and a 2 day study of 0.25 administered once a day, twice a day (0.125 mg/kg/injection) or 3 times a day (0.083 mg/kg/injection) or by continuous infusion (AZLET minipump) (report 205074). The sponsor states due to the absence of vehicle control groups using the same dosing frequency, data from administration of 0.06 mg/kg and 0.25 mg/kg once a day in study 205050 and 0.25 mg/kg once a day and 0.125 mg/kg twice a day in study 205074 were excluded from the modeling dataset.

The 2 compartment PK model is described below.

The final PK model was a two compartment model (Figure 4) with random effects on K_a and CL/F, and dose effect on K_a, CL/F and Q/F. Only K_a, CL/F, Q/F, V_1/F, and V_2/F can be calculated from s.c. data alone.

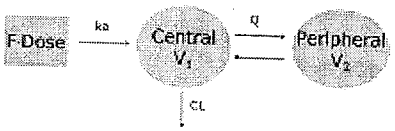


Figure 4 Final two compartment model for exendin-4 concentration. Here only the fraction F of the dose is being absorbed.

[N000 4.2.3.7.3.1 P13]

Dose-dependency of the PK parameters were modeled using the following equations:

$$CL/F = CL_0 \cdot \exp\left(\frac{\theta_6 \cdot dose^2}{\theta_7^2 + dose^2} + \eta\right)$$

$$Ka = K_{a,0} \cdot \exp\left(-\frac{\theta_6 \cdot dose^2}{\theta_7^2 + dose^2} - \eta\right)$$

$$Q/F = Q_0 \cdot \exp(\theta_9 \cdot dose)$$

[N000 4.2.3.7.3.1 P14]

Pharmacokinetic parameters estimates from the model are shown in Table 1 (below).

Table 1 **Parameter estimates for the final PK model**

Parameter	Estimate	CV (%)
CL_0 (L/kg/h)	0.64	9.0
V_2/F (L/kg)	0.18	20
$K_{a,0}$ (1/h)	2.3	12
V_3/F (L/kg)	0.064	11
Q_0 (L/kg/h)	1.1	17
θ_6	0.31	32
θ_7	73	49
θ_8	0.93	1.2
θ_9	-0.00039	24
ω^2	0.013	-
σ^2	0.26	-

[N000 4.2.3.7.3.1 P14]

A direct response pharmacodynamic model based on the ratio of plasma calcitonin levels elicited by active treatment compared to control is shown below.

$$R = \frac{E_{max} \cdot C}{EC_{50} + C}$$

$$E_{max} = (SD \cdot E_{max1} + (1 - SD) \cdot E_{max2}) \cdot e^{\theta_1 \cdot CT_{control}}$$

$$\frac{CT_{treated}}{CT_{control}} = 1 + R$$

The model fit to data in terms of ratio of (geometric) mean to control is shown in Figure 8 and Figure 9. These indicate that the model captures the main features of data within the high level of variation. CI (95%) for the ratio of geometric means are calculated as exp[diff +/- 2STD] where diff is the difference in mean log(CT) and mean log($CT_{control}$), STD is the standard deviation of diff.

[N000 4.2.3.7.3.1 P18]

Table 2 shows parameter estimates for the plasma calcitonin response to exenatide.

Table 2 Parameter estimates for the CT model.

Parameter	Estimate	CV (%)
E_{max1}	7.0	16
EC_{50} (nM)	0.030	40
θ_1	-0.088	13
E_{max2}	16.9	14
σ_1^2	1.03	-

[N000 4.2.3.7.3.1 P21]

Figure 10 shows the dose-dependence of exenatide to increase in plasma calcitonin levels after a single dose and after multiple doses.

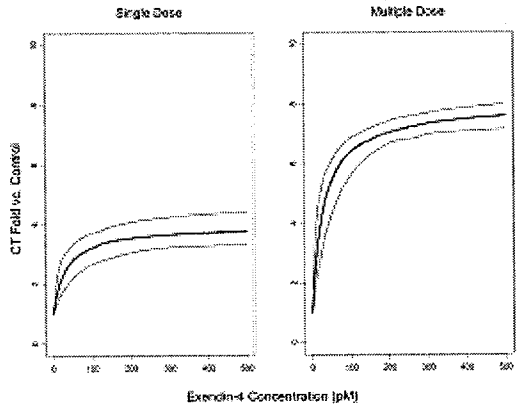


Figure 10 Estimated ratio of $CT/CT_{control}$ response in mice as function of Ex4 concentration for a constant CT control of 10 pg/ml. Ex-4 plasma concentration is in pM here. The outer lines mark the limits of the 95% CI.

[N000 4.2.3.7.3.1 P21]

Exenatide has an estimated EC_{90} of 270 pM (repeat dosing) for increasing plasma calcitonin. Based on the plasma EC_{90} and exenatide pharmacokinetic modeling, the amount of time exenatide levels were above the EC_{90} are shown for different dosing regimens in Table 4. These results suggest a continuous subcutaneous infusion of 0.25 mg/kg/day exenatide yields exenatide plasma levels above the EC_{90} for 100% of the time during treatment, but subcutaneous injections once or 3 times daily do not, even at higher total daily doses.

Table 4 Simulation of % of time above EC_{90} from various dose scenarios single day.

Dose Schedule (mg/kg/day)	Estimate Time above EC_{90} (%)	Lower 95% CI	Upper 95% CI
0.25	15.3	11.8	22.2
1	25.0	16.7	47.3
5	64.2	31.9	91.0
3x0.083	37.5	29.2	54.5
3x0.33	54.5	40.1	88.6
3x1.67	97.9	55.8	100
0.25 – infusion	100	100	100

[N000 4.2.3.7.3.1 P25]

CYNOMOLGUS MONKEYS

- 205121 / Characterization of the distribution of C-cells in thyroids from cynomolgus monkeys

The distribution of c-cells in thyroid from untreated cynomolgus monkeys (5/sex) was characterized in fixed paraffin embedded glands sectioned longitudinally (right lobe) or transversely (left lobe). Sections were stained with hematoxylin – eosin or immunohistochemically stained for calcitonin using a polyclonal rabbit anti-human calcitonin antibody.

Figure 1 Sampling Method

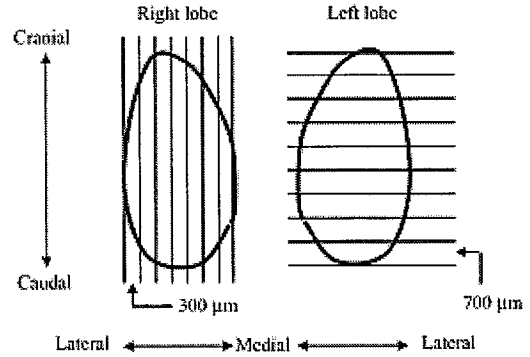


Figure 1. Method for the Sectioning of the Thyroid Gland. Vertical and horizontal lines represent the equidistant levels where sections were cut and collected.

[P11]

In thyroid tissue sections, calcitonin immunoreactive c-cells occurred in clusters or as solitary cells surrounding the epithelial lining (Figure 3). C-cells were only identifiable in HE stained tissue sections when they were arranged in clusters (Figure not shown).

Figure 3 C-cells

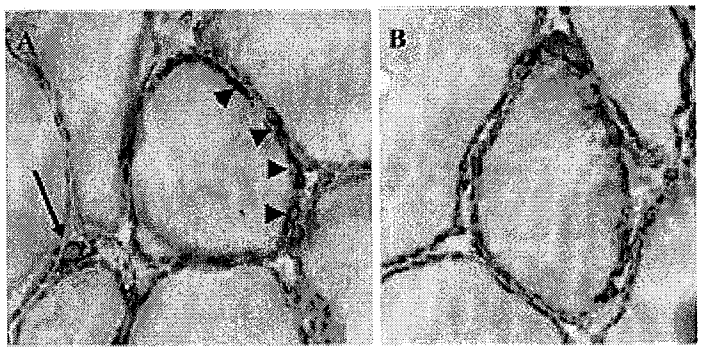


Figure 3. Calcitonin positive C-cells in the cynomolgus monkey thyroid tissue (female, animal 252265). A. C-cells appear either in a parafollicular position (arrow) or are closely attached to the follicular epithelium (arrowheads). B. C-cells lining the epithelium of a medium-sized thyroid follicle. Magnification A and B (x40).

[P18]

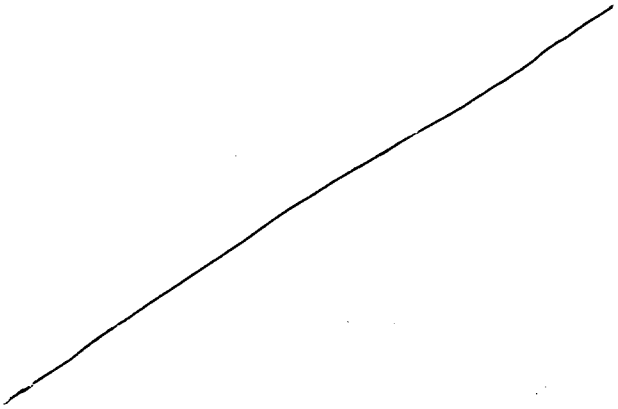
Most c-cells occurred in clusters of up to 10 cells (Table 2) primarily in the middle third of the thyroid lobe (Figure 5).

Table 2 C-cell Appearance

C-cell Appearance	Females		Males	
	Left Lobe	Right Lobe	Left Lobe	Right Lobe
Number of animals examined	5	5	4	4
>20 C-cells in 1 or more sections	4	3	3	3
C-cell cluster (more than 10 cells)	1	0	0	1
C-cell cluster (6-10 cells)	3	2	2	2
C-cell cluster (2-5 cells)	3	4	4	4
No C-cell cluster	0	0	0	0

[P20]

Figure 5 C-cell Distribution



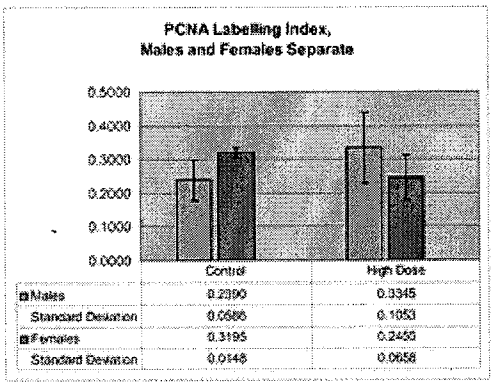
b(4)

[P20]

- 204021 / Quantification of thyroid C-cells by digital image analysis on histological sections prepared from specimens from Inveresk studies 577863 (cynomolgus monkeys) and 455476 (Crl: CD rats)

C-cell density and proliferation was assessed in preserved thyroid glands from monkeys treated with liraglutide for 52 weeks. Quantitative image analysis of thyroid tissue sections stained for calcitonin immunoreactivity and proliferative cell nuclear antigen (PCNA) showed there were no statistically significant differences between male and female control (0 mg/kg/day, vehicle only) and high dose groups (1 mg/kg/day NNC 90-1170) in thyroid c-cell mass or proliferation.

Figure 2 Results for Cynomolgus Monkeys, Males and Females Separate (Arithmetic Means ± Standard Deviation)



	Control	High Dose
Males	0.2890	0.3345
Standard Deviation	0.0586	0.1053
Females	0.3195	0.2455
Standard Deviation	0.0148	0.0658

Note: This chart shows arithmetic means and is for illustration only. Adjusted mean values have been used for statistical analysis.

[N000 4.2.3.7.3 P46]

- 203262 / Liraglutide: Investigative subcutaneous toxicity study in cynomolgus monkeys – combined analysis of the in life phase including C-cell pathology of the thyroid gland and hormone analysis.

Single or repeat dosing with 0.25 or 5 mg/kg/day NNC 90-1170 in cynomolgus monkeys for up to 87 weeks had no effect on plasma calcium, iPTH or calcitonin, and after 87 weeks of treatment, no effect on thyroid C-cell proliferation. Although anti-NNC 90-1170 antibodies were confirmed in 5

monkeys, high plasma levels of NNC 90-1170 could have interfered with anti-NNC 90 1170 screening and did interfere with the assay for neutralizing antibodies.

Cynomolgus monkeys (5/sex/dose) were treated with 0, 0.25, or 5 mg/kg liraglutide (1 – 1.5 mL/kg) injected subcutaneously once a day. The study consisted of 3 single dose phases using nonfasted, fasted, calcium-loaded fasted (0.33 mmol/kg Ca^{+2} IM) monkeys, and a 4th repeat dose phase lasting 87 weeks. There was a 9 – 14 day washout period between single dose phases and prior to repeat dosing. Blood and urine samples (weeks 4, 11, and 12 of repeat dose phase) were taken during the treatment period to determine calcium homeostasis parameters. Plasma PTH (quantified by a 2 antibody, bead-based immunoradiometric assay (IRMA) only to week 61 because the assay kit became unavailable after that), calcitonin (quantified by PEG-precipitation based RIA), and calcium (pH adjusted) were determined in nonfasted, fasted, and calcium-loaded fasted monkeys during the single dose studies and in fasted monkeys throughout the 87 week repeat dose treatment period except in weeks 4 (nonfasted) and 8 (calcium-loaded fasted). Plasma vitamin D (quantified by a RIA after separation from metabolites) was determined in single dose phase 3 and in week 6. Other in-life study parameters were clinical signs, body weight, food consumption, ECG recording (prior to calcium loading and 10, 30, and 60 minutes after), serum chemistry (venous blood; calcium, pH, pH 7.4 adjusted calcium, iPTH, calcitonin, vitamin D), urinalysis (urine volume and concentrations of Ca, PO_4, Mg, Na, Cl), presence and characterization of anti-liraglutide antibodies (sampled during after suspending treatment for 3 days in weeks 59/60, 72/73, and 87/88), and liraglutide plasma toxicokinetics. At the end of treatment, monkeys were sacrificed and thyroid glands were examined microscopically specifically to determine the effects on C-cell mass and proliferative lesions. C-cells were identified by staining with a rabbit anti-human calcitonin antibody. The following study reports were included as appendices to report 203262:

Table 1 Studies included:

Novo Nordisk Study number	NN203262	NN204033	NN204098	Doc.id.504, SqA, 2009	Doc.id.409, SqA,2026
Phase	In life phase	Hormone analysis	Hormone analysis	Statistical analysis	Statistical analysis
Test facility	[redacted]			Novo Nordisk A/S	Novo Nordisk A/S
GLP-status	GLP	GLP	GLP	Non-GLP	Non-GLP

b(4)

[N000 4.2.3.7.3 P3]

Single Dose Phase

During 3 single dose phases (calcium loaded, fasted, and non-fasted), liraglutide had no effect on clinical signs, body weight, or plasma calcium concentrations, except calcium loading increased plasma calcium (corrected) at 0.25 and 1 hour after loading. Liraglutide had no effect on pH corrected calcium concentrations or PTH levels.

Single subcutaneous doses of 0.25 or 5 mg/kg liraglutide had no effect on plasma calcitonin in fasted monkeys up to 24 hours after dosing (Figure 1).

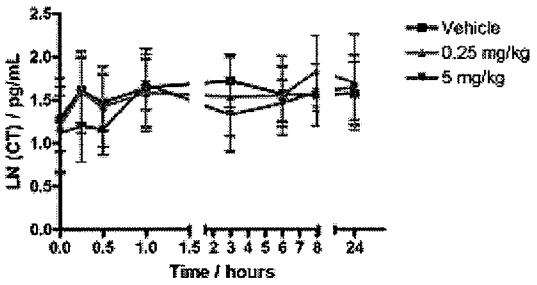
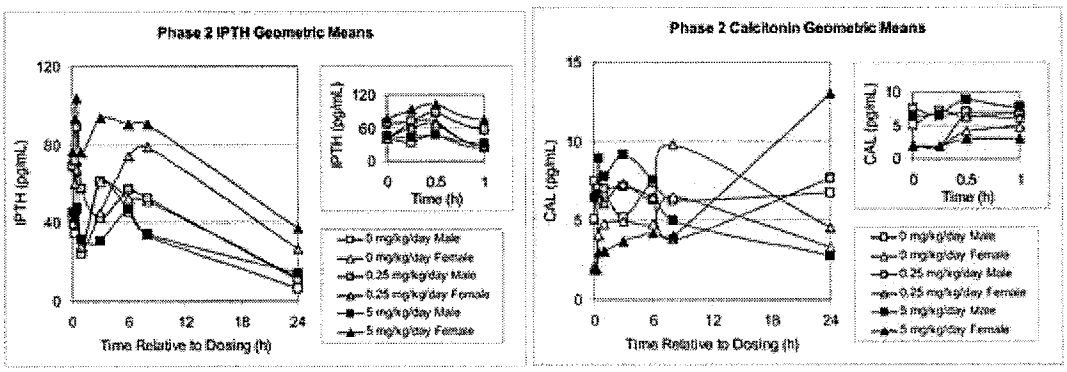


Figure 1 **Plasma CT (natural logarithm transformed) values (mean and 95% CI) in fasted cynomolgus monkeys (5 males and 5 females/group) treated with liraglutide (5.0 or 0.25 mg/kg) or vehicle as a single dose.**

CT in liraglutide treated animals did not show any effect upon the treatment over the 24 hour dosing interval.

[N000 study 203262 P5]

10.4 IPTH & Calcitonin Results - Single Dose Phase 2 (Fasting)



[study 203262 P634]

Table 5 Blood Gas Analysis: Phase 1: Group Mean Values: Males

Group / Dose Level (mg/kg)		Timepoint after Dosing: Predose			15 min			30 min			1 h			3 h		
		Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.35	1.18	7.34	1.24	1.10	7.33	1.24	1.21	7.38	1.23	1.21	7.33	1.30	1.29	7.30
	SD	0.05	0.06	0.11	0.06	0.06	0.12	0.04	0.06	0.09	0.06	0.06	0.11	0.05	0.07	0.12
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.33	1.23	7.39	1.26	1.12	7.34	1.21	1.18	7.38	1.27	1.24	7.33	1.33	1.31	7.28
	SD	0.03	0.04	0.08	0.02	0.04	0.11	0.03	0.03	0.07	0.07	0.05	0.12	0.03	0.05	0.09
3 (5)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.30	1.23	7.37	1.25	1.12	7.35	1.21	1.11	7.32	1.20	1.19	7.36	1.31	1.30	7.30
	SD	0.05	0.02	0.10	0.02	0.03	0.09	0.08	0.06	0.11	0.03	0.02	0.07	0.04	0.04	0.05

Data reported from Phase 1 repeat

Data obtained from non-fasted animals

Ca^{++} measured calcium; cCa++ corrected calcium

Table 6 Blood Gas Analysis: Phase 1: Group Mean Values: Females

Group / Dose Level (mg/kg)		Timepoint after Dosing: Predose			15 min			30 min			1 h			3 h		
		Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH	Ca^{++}	cCa++	pH
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.28	1.19	7.34	1.21	1.14	7.35	1.24	1.19	7.36	1.24	1.19	7.33	1.32	1.33	7.29
	SD	0.06	0.04	0.09	0.05	0.05	0.14	0.07	0.05	0.12	0.03	0.07	0.11	0.03	0.03	0.12
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.30	1.21	7.32	1.21	1.15	7.34	1.27	1.19	7.36	1.21	1.20	7.36	1.30	1.35	7.31
	SD	0.05	0.05	0.05	0.01	0.06	0.11	0.07	0.06	0.08	0.05	0.06	0.14	0.04	0.04	0.09
3 (5)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.25	1.17	7.37	1.24	1.14	7.31	1.23	1.12	7.25	1.19	1.18	7.38	1.34	1.35	7.34
	SD	0.03	0.04	0.12	0.05	0.05	0.10	0.05	0.03	0.10	0.04	0.03	0.06	0.03	0.05	0.10

Data reported from Phase 1 repeat

Data obtained from non-fasted animals

Ca^{++} measured calcium; cCa++ corrected calcium

[N000 study 203262 P97 - 100]

Calcium loading (im injection of 0.33 mmol/kg calcium) increased plasma calcitonin in fasted monkeys, but liraglutide had no effect on calcium induced increased plasma calcitonin (Figure 2)

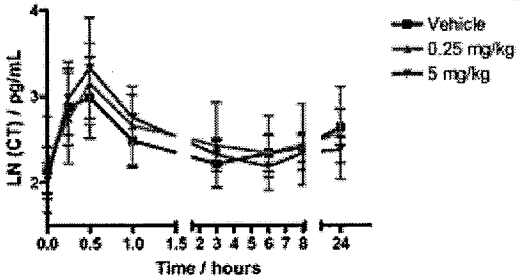
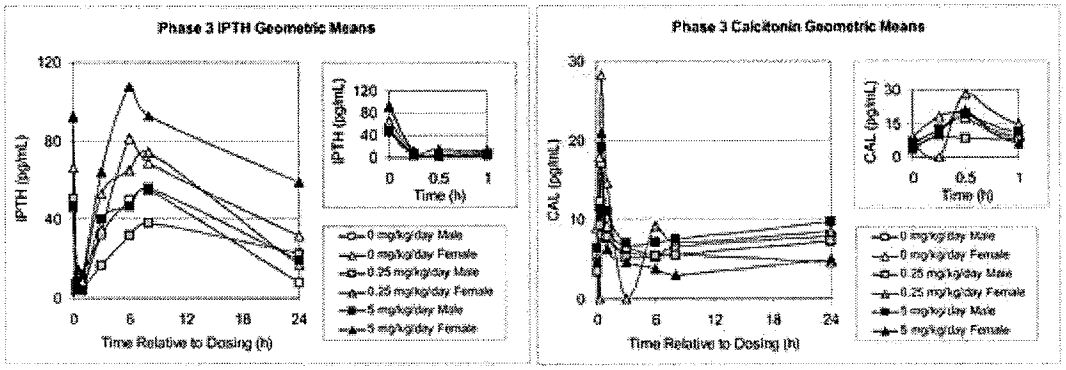


Figure 2 Plasma CT (natural logarithm transformed) values (mean and 95% CI) in fasted cynomolgus monkeys (5 male + 5 females / group) treated with liraglutide (5.0 and 0.25 mg/kg) or vehicle as a single dose and challenged with Ca^{++}.

[study 203262 P6]

10.5 IPTH & Calcitonin Results - Phase 3 (Calcium Loaded)



[study 203262 P634]

Table 9 Blood Gas Analysis: Phase 3: Group Mean Values: Males

Group/ Dose Level (mg/kg)		Predose			15 min			30 min			1 h			3 h		
		Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.35	1.32	7.33	1.45	1.40	7.33	1.43	1.35	7.35	1.35	1.37	7.32	1.36	1.32	7.34
	SD	0.03	0.02	0.11	0.12	0.07	0.08	0.04	0.07	0.08	0.04	0.04	0.04	0.02	0.06	0.06
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.37	1.34	7.35	1.39	1.33	1.29	1.33	1.28	7.39	1.35	1.33	7.33	1.35	1.31	7.38
	SD	0.03	0.03	0.08	0.10	0.03	0.08	0.10	0.11	0.12	0.07	0.07	0.12	0.01	0.05	0.06
3 (5)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.34	1.32	7.35	1.33	1.25	1.24	1.44	1.31	7.39	1.39	1.33	7.32	1.35	1.32	7.36
	SD	0.02	0.03	0.08	0.08	0.03	0.08	0.08	0.01	0.07	0.07	0.04	0.08	0.05	0.04	0.08

Data obtained from fasted animals with a calcium load dose of 0.33 mmol/kg body weight

Ca^{++} measured calcium; cCa^{++} corrected calcium

Table 10 Blood Gas Analysis: Phase 3: Group Mean Values: Females

Group/ Dose Level (mg/kg)		Predose			15 min			30 min			1 h			3 h		
		Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH	Ca^{++}	cCa^{++}	pH
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.35	1.36	7.31	1.45	1.41	1.31	1.34	1.35	1.34	1.36	1.33	1.35	1.36	1.33	1.35
	SD	0.03	0.06	0.05	0.12	0.13	0.12	0.11	0.10	0.08	0.06	0.07	0.12	0.01	0.05	0.10
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.34	1.39	7.30	1.43	1.33	1.33	1.43	1.34	1.38	1.36	1.33	1.35	1.34	1.31	7.30
	SD	0.03	0.08	0.05	0.08	0.07	0.09	0.06	0.07	0.08	0.06	0.05	0.06	0.03	0.05	0.07
3 (5)	Number	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5
	Mean	1.34	1.39	7.36	1.46	1.41	1.35	1.40	1.32	1.34	1.35	1.30	1.30	1.31	1.31	7.38
	SD	0.03	0.05	0.07	0.04	0.06	0.03	0.06	0.07	0.04	0.03	0.04	0.06	0.02	0.02	0.02

Data obtained from fasted animals with a calcium load dose of 0.33 mmol/kg body weight

Ca^{++} measured calcium; cCa^{++} corrected calcium

[N000 study 203262 P104 - 7]

Repeat Dose Phase

During the 85 week repeat dose phase, a control group male (monkey 3M) was sacrificed in week 63 because of aggressive behavior toward cage mates and technicians. Clinical signs of subcutaneous swelling and edema occurred at ≥ 0.25 mg/kg liraglutide in both males and females. Body weight, food consumption, ECG parameters, and organ weights were unaffected by treatment. Urinalysis changes were limited to the 5 mg/kg/day groups in week 11 with high urinary volume occurring in males and low urinary volume occurring in females 0 – 4 hours after dosing, but without any changes in the concentration of electrolytes, including calcium and magnesium. There were no treatment-related effects on plasma calcium, iPTH, or calcitonin during the repeat dose phase. Calcium loading on day 56 (im injection of 0.33 mmol/kg calcium) transiently increased plasma calcium, but calcium returned to predose levels within 3 hours after dosing. Peak plasma calcitonin levels elicited by calcium loading were similar in single and repeat dose phases suggesting repeat dosing with NNC 90-1170 had no effect on thyroid c-cell proliferation.

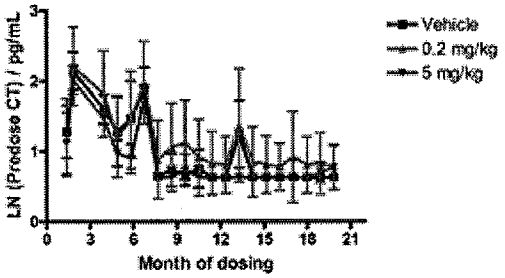
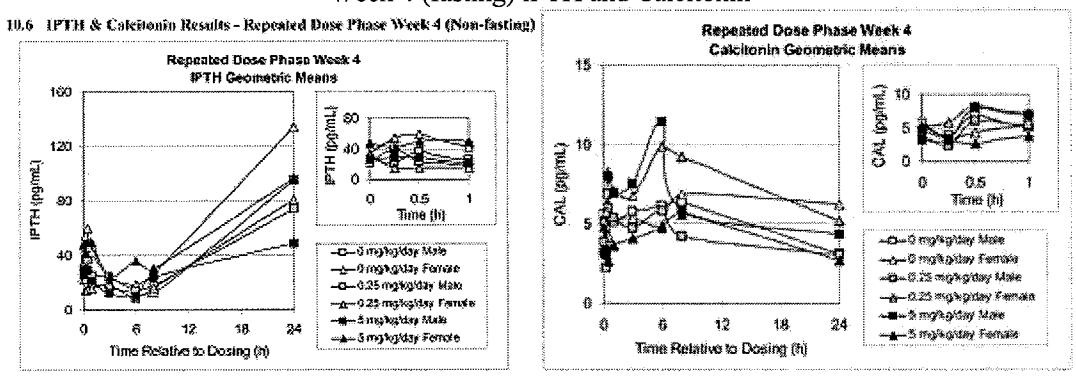


Figure 3 Plasma CT (natural logarithm transformed) values (mean and 95% CI) in cynomolgus monkeys (5 males and 5 female /group) treated with liraglutide (5.0 or 0.25 mg/kg) or vehicle.

[N000 study 203262 P7]

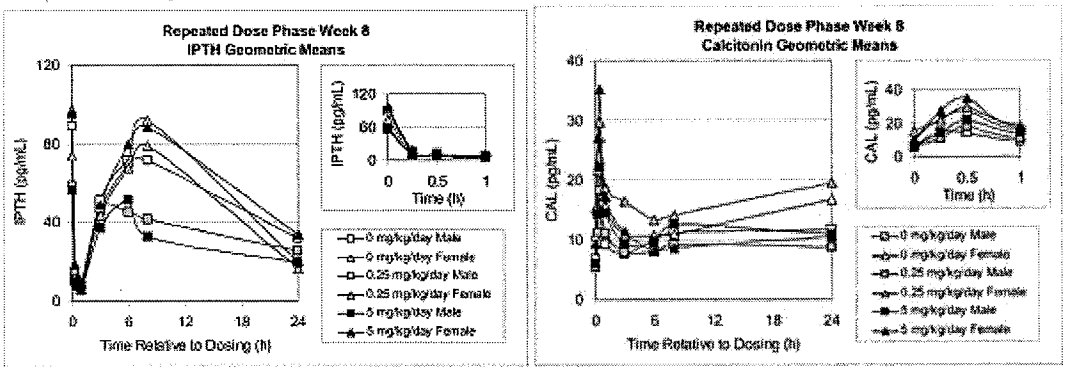
Week 4 (fasting) iPTH and Calcitonin



[N000 study 203262 P640]

Week 8 (fasting), Calcium, iPTH, and Calcitonin after Calcium Loading

10.8 IPTH & Calcitonin Results - Repeated Dose Phase Week 8 (Calcium Loaded)



[N000 study 203262 P646]

Table 37 Blood Gas Analysis: Week 57: Repeat Dose Phase: Group Mean Values: Males

Group/ Dose Level (mg/kg/day)		Timepoint after Dosing: Predose Ca++	Predose cCa++	Predose pH	30 min Ca++	30 min cCa++	30 min pH	3 h Ca++	3 h cCa++	3 h pH	6 h Ca++	6 h cCa++	6 h pH
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.14	1.13	7.38	1.11	1.11	7.40	1.12	1.13	7.41	1.14	1.15	7.42
	SD	0.02	0.02	0.06	0.03	0.02	0.05	0.04	0.04	0.03	0.05	0.05	0.09
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	4	4	4
	Mean	1.11	1.12	7.42	1.08	1.10	7.45	1.11	1.12	7.43	1.12	1.13	7.43
	SD	0.07	0.07	0.04	0.03	0.03	0.04	0.03	0.03	0.02	0.04	0.04	0.02
3 (5)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.16	1.17	7.42	1.11	1.13	7.46	1.14	1.16	7.43	1.15	1.16	7.43
	SD	0.05	0.03	0.04	0.04	0.03	0.07	0.03	0.05	0.05	0.05	0.05	0.05

Data obtained from fasted animals

Ca++ measured calcium; cCa++ corrected calcium

Table 38 Blood Gas Analysis: Week 57: Repeat Dose Phase: Group Mean Values: Females

Group/ Dose Level (mg/kg/day)		Timepoint after Dosing: Predose Ca++	Predose cCa++	Predose pH	30 min Ca++	30 min cCa++	30 min pH	3 h Ca++	3 h cCa++	3 h pH	6 h Ca++	6 h cCa++	6 h pH
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.15	1.13	7.36	1.10	1.10	7.40	1.19	1.16	7.34	1.18	1.16	7.39
	SD	0.04	0.04	0.06	0.09	0.09	0.03	0.06	0.05	0.03	0.08	0.06	0.09
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.15	1.13	7.38	1.12	1.13	7.44	1.13	1.13	7.38	1.14	1.15	7.43
	SD	0.05	0.05	0.07	0.04	0.01	0.00	0.02	0.04	0.08	0.04	0.03	0.05
3 (5)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.15	1.14	7.38	1.14	1.15	7.43	1.18	1.17	7.39	1.18	1.16	7.41
	SD	0.10	0.10	0.10	0.03	0.05	0.03	0.05	0.05	0.05	0.03	0.06	0.05

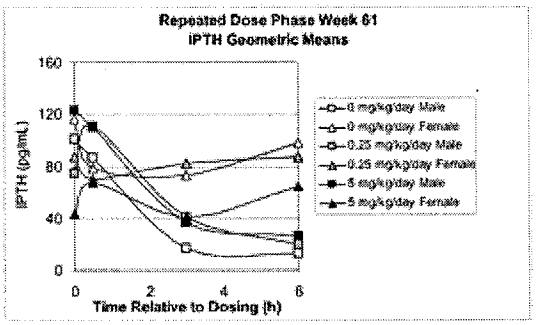
Data obtained from fasted animals

Ca++ measured calcium; cCa++ corrected calcium

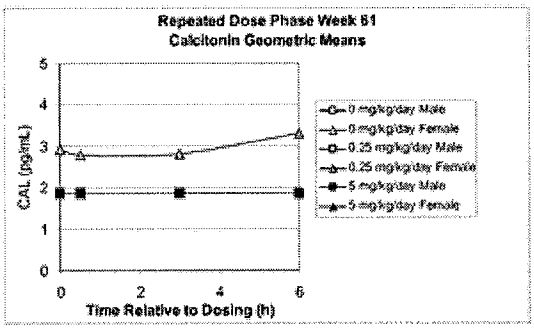
[N000 study 203262 P152 - 3]

Week 61 (fasted) iPTH and Calcitonin

10.25 IPTH Results - Repeated Dose Phase Week 61



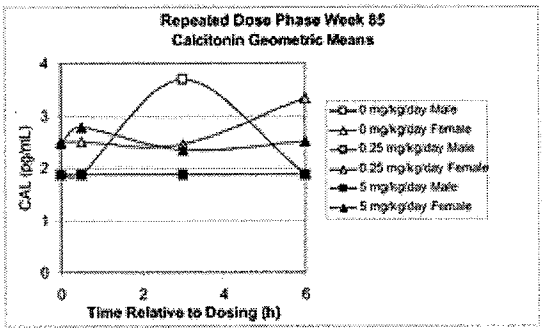
10.26 Calcitonin Results - Repeated Dose Phase Week 61



[N000 study 203262 P677 - 678]

Week 85 (fasted) Calcium and Calcitonin

10.32 Calcitonin Results - Repeated Dose Phase Week 85



[N000 study 203262 P684]

Table 51 Blood Gas Analysis: Week 85: Repeat Dose Phase: Group Mean Values: Males

Group/ Dose Level (mg/kg/day)		Timepoint after Dosing: Predose Ca++	Predose cCa++	Predose pH	30 min Ca++	30 min cCa++	30 min pH	3 h Ca++	3 h cCa++	3 h pH	8 h Ca++	8 h cCa++	8 h pH
1 (0)	Number	4	4	4	4	4	4	4	4	4	4	4	4
	Mean	1.39	1.14	7.43	1.09	1.11	7.44	1.33	1.14	7.41	1.18	1.15	7.40
	SD	0.01	0.02	0.04	0.03	0.03	0.02	0.04	0.02	0.05	0.04	0.02	0.04
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.34	1.15	7.41	1.08	1.11	7.46	1.30	1.14	7.48	1.12	1.12	7.42
	SD	0.08	0.03	0.08	0.04	0.03	0.04	0.04	0.03	0.03	0.04	0.04	0.03
3 (5)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.30	1.11	7.42	1.10	1.12	7.44	1.08	1.10	7.46	1.13	1.12	7.42
	SD	0.01	0.02	0.03	0.04	0.03	0.07	0.02	0.03	0.02	0.05	0.05	0.03

Data obtained from fasted animals

Ca++ measured calcium, cCa++ corrected calcium

Table 52 Blood Gas Analysis: Week 85: Repeat Dose Phase: Group Mean Values: Females

Group/ Dose Level (mg/kg/day)		Timepoint after Dosing											
		Predose			30 min			3 h			6 h		
		Ca++	cCa++	pH	Ca++	cCa++	pH	Ca++	cCa++	pH	Ca++	cCa++	pH
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.19	1.12	7.34	1.17	1.14	7.33	1.15	1.12	7.35	1.16	1.14	7.36
	SD	0.07	0.04	0.08	0.06	0.03	0.07	0.08	0.06	0.05	0.06	0.03	0.05
2 (0.25)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.18	1.16	7.36	1.18	1.17	7.39	1.12	1.13	7.43	1.12	1.12	7.40
	SD	0.06	0.06	0.04	0.04	0.04	0.04	0.04	0.05	0.03	0.09	0.08	0.07
3 (5)	Number	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	1.18	1.12	7.38	1.13	1.13	7.39	1.11	1.13	7.43	1.14	1.14	7.41
	SD	0.04	0.03	0.08	0.07	0.05	0.05	0.03	0.04	0.04	0.07	0.07	0.02

Data obtained from fasted animals

Ca^{2+} measured calcium; cCa^{2+} corrected calcium

[N000 study 203262 P166 - 7]

Treatment-related necropsy findings occurred at the injection site with the incidence of macroscopic thickened areas above control group levels at 5 mg/kg/day NNC 90-1170.

NECROPSY FINDINGS	GROUP DOSE	GROUP TOTALS					
		Males			Females		
		Grp 1 0 mg/kg	Grp 2 0.25 mg/kg	Grp 3 5 mg/kg	Grp 1 0 mg/kg	Grp 2 0.25 mg/kg	Grp 3 5 mg/kg
INJECTION/TREATMENT SITE							
Dark focus			1		2	1	1
Scab		1					
Thickened/Thickened areas		1	1	3		1	3
Reddened		1	1	2	3	2	
Enlarged		1	2	2	2	1	2
Reddened areas		1	1	1			
LUNG							
Pale				1			
Dark focus			1	1			
Spongy		1	3	3	1		1
Dark					1		
Adhesion(s)			1	1		1	

The absence of a numeral indicates that the lesion specified was not identified
Reference File Ref: [illegible]

[Study 203262 Compiled from Table 73]

There were no treatment-related histopathology findings in thyroid tissue sections stained with hematoxylin-eosin (Table 1) or stained for calcitonin immunoreactivity (Table 2).

Table 1 Histological findings of the thyroid and parathyroid gland(s) presented by group - evaluation of the HE stained sections

		Males			Females		
	Histological findings	Group 1 0 mg/kg	Group 2 0.25 mg/kg	Group 3 5 mg/kg	Group 1 0 mg/kg	Group 2 0.25 mg/kg	Group 3 5 mg/kg
Thyroid gland	Number of animals examined	5	5	5	5	5	5
	NAD	3		13, 14	18	23	
	Dilated ultimobranchial ducts	1, 2, 4, 5	6, 7, 8, 9	11, 12, 15	16, 17, 19, 20	21, 25	26, 28, 29, 30
	Mononuclear inflammatory cell infiltration, minimal	1, 2	6, 9			25	28, 29
	Mononuclear inflammatory cell infiltration, mild				20	24	27
	Ectopic thymus	4, 5	7, 8, 10	11, 15	16	24, 25	
	Developmental cyst(s)	2, 5				22	

NAD= No abnormalities detected; Numbers inserted for the findings are animal numbers

[Study 203262 P606]

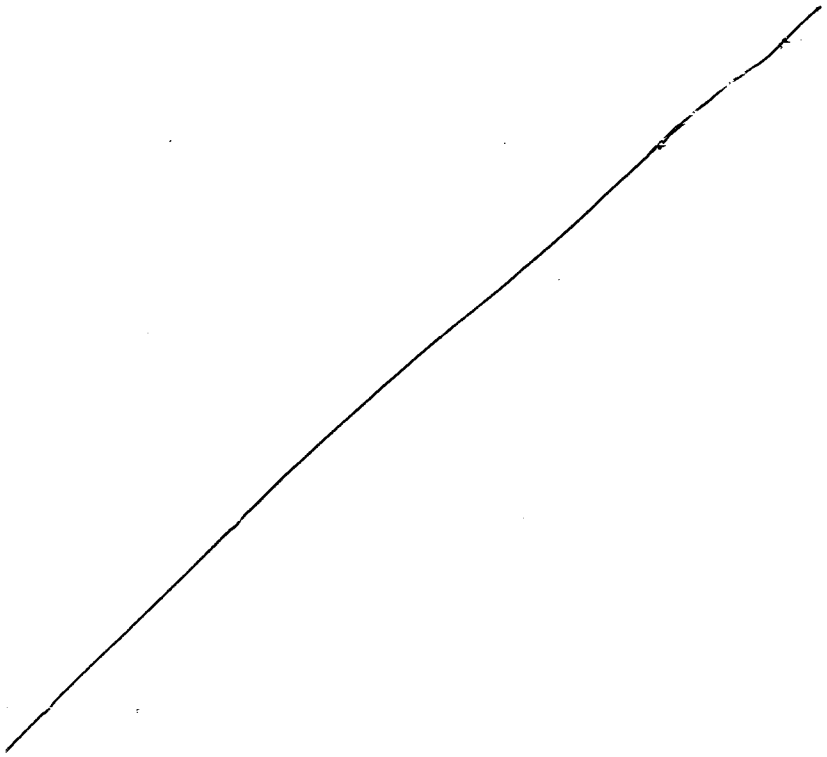
Table 2 Histological findings of the thyroid and parathyroid gland(s) presented by group - evaluation of the sections immunohistochemically stained for presence of calcitonin for specific identification of C-cells

		Males			Females		
	Histological Findings	Group 1 0 mg/kg	Group 2 0.25 mg/kg	Group 3 5 mg/kg	Group 1 0 mg/kg	Group 2 0.25 mg/kg	Group 3 5 mg/kg
Thyroid gland	Number of animals examined	5	5	5	5	5	5
	NAD in the C-cells	1, 2, 3, 4, 5	6, 7, 9, 10	11, 12, 13, 14, 15	16, 17, 18, 19, 20	21, 22, 23, 24, 25	26, 27, 28, 29, 30
	C-cells in ectopic thymus		8				

NAD= No abnormalities detected; Numbers inserted for the findings are animal numbers

[Study 203262 P607]

Week 87 toxicokinetics showed plasma NNC 90-1170 exposure increased with dose and pharmacologically relevant levels of drug (> 1 nM) persisted for up to 60 hours after subcutaneous dosing at $\geq$ 0.25 mg/kg/day.



b(4)

[Study 203262 P574-576]

Anti-NNC 90-1170 antibodies were detected using a RIA based on precipitation of radioactivity from monkey plasma after incubation with [^{125}I]liraglutide. Plasma samples were taken 3 days after dosing in weeks 59/60, 72/73, and 87/88. Five monkeys were antibody positive: one at 0.25 mg/kg (24F) and 3 at 5 mg/kg (13M, 27F, 30F) from weeks 59/60 onward and one male at 5 mg/kg (14M) from week 72 onward. Plasma levels of NNC 90-1170 may be high enough to interfere with detection of anti-NNC 90-1170 antibodies, particularly in the 5 mg/kg/day group, even 3 days after dosing. High plasma NNC 90-1170 prevented characterization of antibody neutralizing activity in confirmed positive samples.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Subcutaneously injected liraglutide was a non-genotoxic carcinogen in 2 year repeat dose studies of in rats and mice causing thyroid c-cell neoplasms in both species. To evaluate the human relevance of thyroid c-cell tumors, the sponsor performed mechanistic studies to show that liraglutide-induces C-cell tumors in rats and mice by causing GLP-1R mediated persistent calcitonin secretion from C-cells. The sponsor asserts proliferative C-cell lesions in rats and mice are not relevant to human safety assessment because GLP-1R-mediated calcitonin secretion is more robust in rodents compared to monkeys or humans, However, the weight of evidence does not support the proposed mode of action in rats or mice, therefore the relevance of rodent thyroid c-cell tumors to human risk assessment cannot be dismissed.

Thyroid C-cell Neoplasms

Thyroid parafollicular cells, or C-cells, synthesize calcitonin and secrete it in response to elevations in serum calcium. C-cells are unevenly distributed within the thyroid's follicular basal lamina,

and they are situated basally without directly contacting the follicular lumen. Unlike thyroid hormone synthesizing follicular cells which are derived from endoderm, C-cells arise from the ultimobranchial body composed of cells from the neural crest. Histologically, normal C-cells are difficult to discern by hematoxylin-eosin staining, but they are easily identified by immunochemical staining for calcitonin. There are some species differences in c-cell distribution within the thyroid gland. In humans, c-cells are concentrated at the junction of the upper and middle lobes, but in rats, they're more widely distributed with higher concentrations occurring in the central region. Normal c-cells and cells resulting from physiologic hyperplasia are uniformly and strongly stained by anti-calcitonin antibodies, but calcitonin staining of c-cells forming nodules can show variable and weaker staining patterns. Immunohistochemical staining for calcitonin is the most useful method for evaluating c-cell tumors, even though poorly differentiated tumors may be only weakly stained. Proliferative C-cell lesions can be assessed histologically and proliferative c-cell lesions occur along a continuum ranging from diffuse hyperplasia, focal hyperplasia, nodular hyperplasia / adenomas to carcinomas. Nodular aggregates showing displacement of the surrounding gland without invasion are considered adenomas. C-cell hyperplasia and adenomas are differentiated by size with a focus of c-cells > 5 average-sized contiguous follicles considered adenomas. However, whether or not these adenomas are autonomous neoplastic growths is unknown. C-cell carcinoma, also called thyroid medullary carcinoma, occurs when c-cell nodules or cords develop stromal or vascular invasion.,

Thyroid tumors in humans affect approximately 1% of the population with 95% originating from thyroid follicular cells and 5% from calcitonin-secreting C-cells (parafollicular cells). Thyroid carcinomas arising from C-cells are usually referred to as medullary thyroid carcinoma or MTC. The 5-year survival rate for MTC is approximately 50%. While approximately 75% of MTC occurs sporadically, 25% is familial due to inherited autosomal dominant gain of function point mutations in the REarranged during Transfection (RET) proto-oncogene. RET is a plasma membrane receptor tyrosine kinase that regulates the growth of cells derived from the neural crest, and point mutations causing constitutive receptor tyrosine kinase activity lead to MTC. Somatic RET mutations also occur in up to 50% of sporadic cases, therefore activating mutations in RET are the most common molecular pathology causing spontaneous and familial MTC in humans. While some RET mutations are only associated with familial MTC, others also cause pheochromocytomas, ganglioneuromas, and other endocrine tumors associated with multiple endocrine neoplasia type 2 (MEN2). Although other tumors occur during the clinical course of MEN2, MTC usually precedes them. In familial MTC, c-cell hyperplasia and hypercalcitoninemia precede carcinoma. Hypercalcitoninemia and C-cell hyperplasia are uncommon in humans, but they occur at an increased frequency in families prone to the development of MTC and MEN2. Physiologic c-cell hypertrophy and hyperplasia associated with hypercalcemia, hyperparathyroidism, or toxic goiter can be distinguished from hyperplasia associated with neoplastic growth. Physiologic c-cell hyperplasia is diffuse and identified by anti-calcitonin antibody staining and quantitative analysis whereas neoplastic hyperplasia is focal or nodular and can be detected by hematoxylin-eosin staining as mildly to moderately atypical c-cells confined to the basement membrane of thyroid follicles (Perry et al., Cancer (1996) 77(4): 750 – 756). Perry et al. proposed that only focal hyperplasia precedes both sporadic and familial c-cell neoplasms in humans. Hypercalcitoninemia can occur in both physiologic and neoplastic hyperplasia.

Thyroid C-cell hyperplasia and tumors were characterized in laboratory rats (DeLillis et al, Laboratory Investigation (1979) 40: 140 – 154) and mice (Van Zweiten et al, Am J Pathol, (1983) 110: 219 – 229) and there are species differences in the development of proliferative c-cell lesions. In rats, plasma calcitonin and the incidence of diffuse c-cell hyperplasia, focal c-cell hyperplasia, and c-cell adenomas increase with age. Although age-related diffuse and focal C-cell hyperplasia and adenomas are common in rats (incidence > 1%), c-cell carcinomas are rare in most common laboratory rat strains (incidence < 1%). In both familial MTC in humans and strain-dependent age-related c-cell tumors in rats, a prolonged period of diffuse and nodular c-cell hyperplasia and elevated serum calcitonin precedes the development of c-cell tumors. In mice, hypercalcitoninemia, c-cell hyperplasia, adenomas, and carcinomas are rare (incidence < 1%) and c-cell hyperplasia usually doesn't precede tumors. Because the course of MTC in rats and humans is similar, rats were considered a model for human MTC. However,

the most common molecular pathology of MTC in humans, activating RET mutations, is not common in rat thyroid c-cell tumors.

The incidence of C-cell lesions in rats is strain dependent and affected by diet. In rats, the ratio of c-cells to follicular cells and the number focal aggregates of c-cells increases with age. Several strains of rats with a high incidence of age-dependent spontaneous c-cell tumors have been proposed as models of inherited human MTC because the clinical course of the disease is similar with elevated serum calcitonin and age-related c-cell hyperplasia progressing to tumors, but the molecular pathology in at least 2 susceptible rat strains is not because they lacked activating mutations in RET. WAG/Rij rats, a substrain of Sprague Dawley rats, and Long-Evans rats develop c-cell tumors that are preceded by c-cell hyperplasia and hypercalcitoninemia. In Long-Evans rats, proliferative c-cell abnormalities progress from mild to severe diffuse hyperplasia, nodular hyperplasia (probably categorized as focal c-cell hyperplasia or adenomas), and finally carcinoma. Tumors first appear at about 1 year of age and increase in frequency thereafter. C-cells in young Long-Evans rats account for ~ 4% of thyroid substance morphometrically, increasing to 7% by 1 year of age and up to 20% in older rats with a change in thyroid gland distribution from predominantly central to more peripheral. The ratio of C-cells to follicular cells, focal aggregates of c-cells, and aggregates progressing to nodules increases with age. Up to 47% of 24 – 36 month old Long-Evans rats develop nodular hyperplasia (25%) or MTC (22%). Calcitonin is elevated in rats with severe diffuse c-cell hyperplasia, nodular hyperplasia, or carcinoma, but not in rats with mild diffuse hyperplasia. WAG/Rij rats also have a high incidence of spontaneous age-related proliferative C-cell lesions, but there were no differences in RET exon nucleotide sequences compared to Sprague Dawley rats (a non-MTC-susceptible strain) or no mutations known to activate human RET. A MEN-like phenotype spontaneously arising in a Sprague Dawley rat breeding colony characterized by bilateral juvenile cataracts, pheochromocytoma, thyroid c-cell hyperplasia, parathyroid hyperplasia, and pituitary adenoma was autosomal recessive without any difference in the RET nucleotide coding sequence between affected and unaffected rats.

In mice, c-cell tumors occur without any degree of c-cell hyperplasia. However, genetically engineered mice expressing a mutant RET under the control of a specific C-cell promoter (CGRP-Ret^{C634R}), harboring simultaneous heterologous deletions of Rb (retinoblastoma protein, a tumor suppressor) and p53 (protein 53, a tumor suppressing transcription factor) ($Rb^{+/-}$ x $p53^{+/-}$), expressing v-Ha-ras (Harvey rat sarcoma viral oncogene homolog) under the control of a c-cell specific promoter (CGRP-v-Ha-ras), or coexpressing a truncated form of the polyomavirus (Py) middle-T antigen and the full-length Py small-T antigen under control of a Py early promoter/enhancer all developed MTC. The C634R mutation in RET is a common mutation causing MEN 2A syndrome in humans, and nearly all mice with the mutation developed C-cell hyperplasia progressing to bilateral MTC and elevated calcitonin by the time they were 8 to 12 months old. Tumor penetrance of the CGRP-Ret^{C634R} mutation depends on the background strain with 0% of FVB/N mice developing tumors compared to 98% of CBA/ca mice. Overexpressing RET alone did not cause c-cell tumors. Mice (129 strain) harboring heterozygous deletions of both Rb and p53 developed c-cell hyperplasia progressing to MTC by 7 months of age. Spontaneous activating mutations in RET were detected in 4 of 8 MTC from $Rb^{+/-}$ x $p53^{+/-}$ mice. The importance of modifier genes in the development of MTC was also demonstrated by heterozygous deletion of only Rb ($Rb^{+/-}$ x $p53^{+/+}$) causing MTC in 56% of mice with a 129/Sv x C57BL/6 background, despite two active p53 alleles. The loss of Rb was detected in many types of human thyroid cancers, so it's deletion isn't specific to MTC. Transgenic mice (C57Bl/6 x SJL background strain) expressing v-Ha-ras under the control of the c-cell specific CGRP promoter develop c-cell hyperplasia progressing to calcitonin secreting MTC in 85 – 93% of mice within 6 – 12 months of age. Although the MTC tumor phenotype in mice resembles the human disease, the genotypes don't because ras mutations haven't been detected in human MTC. Comparison of thyroid tumor phenotypes in humans and genetically engineered mice are shown in Table 2 (below).

Table 2. Comparison of human thyroid tumors and genetically engineered mouse models of thyroid cancer with similar genetic mutations.

Genetic Alteration	Tumor Morphology*		Biologic Behavior		Hormonal Derangement	
	Human	Mouse	Human	Mouse	Human	Mouse
Ret/PTC1	Typical PTC	Typical PTC	Frequent local invasion, regional lymph node and less common distant metastases	Local invasion without any metastasis	None typical	Hypothyroidism
Ret/PTC3	Typical to solid PTC	Typical to solid PTC	Frequent local invasion, regional lymph node and less common distant metastases	Local invasion with rare lymph node metastases	None typical	NR†
$BRAF^{V600E}$	Tall cell variant or typical PTC with necrosis and anaplasia	Tall cell variant or typical PTC with foci of anaplasia	Very frequent local invasion and anaplasia; loss of differentiation markers	Local invasion and anaplasia; maintenance of thyroglobulin expression	None typical	High TSH euthyroid
TRK	Typical PTC	PTC with absence of characteristic nuclear abnormalities	Local invasion, regional lymph node and less common distant metastases	Invasion not reported; absence of metastases	None typical	None
ras	FTC or follicular variant PTC	Typical PTC	Local invasion, regional lymph node and/or hematogenous metastases	Local invasion with rare lung metastases	None typical	None
p53 inactivation/ loss	ATC	Increased anaplasia, invasion and metastases in Ret/ PTC models of PTC (p53 not examined alone)	Rapid local invasion and metastases with loss of differentiation markers	Local invasion with rare metastases and early death	None typical	Hypothyroidism (in combination with Ret/ PTC transgene expression)
Ret	MTC	MTC +/− PTC	C-cell hyperplasia progressing to MTC‡; distant spread common in advanced disease; tumors produce calcitonin	C-cell hyperplasia progressing to MTC; very rare metastases; tumors produce calcitonin	Hypercalcitonism	Hypercalcitonism

* PTC = papillary thyroid carcinoma; FTC = follicular thyroid carcinoma; ATC = anaplastic thyroid carcinoma; MTC = medullary thyroid carcinoma.
† NR = not reported.
‡ Hereditary cases (e.g., MEN 2A).

Table 2. Extended

[Knostman, KA et al, Vet Pathol. 2007 Jan;44(1):1-14]

Hyperprolactinemic male mice have elevated levels of calcitonin (Lu, CC et al, Metabolism (1999) 48: 221-6.), but MTC developed in 41% of 12 month old genetically engineered prolactin receptor null mice. These mice were hypercalcemic with increased parathyroid hormone levels, elevated calcitonin, and in isolated MTC tumors, the absence of common activating mutations in RET (mutations in codons 634 or 918).

Postulated Mode of Action

The sponsor's postulated mode of action is:

1. Circulating liraglutide binds to and activates GLP-1Rs on C-cells
2. GLP-1R activation on C-cells induces calcitonin release
3. Continued calcitonin release leads to increased calcitonin synthesis
4. Persistent stimulation of C-cells leads to C-cell hyperplasia in rodents
5. Long-term C-cell hyperplasia may lead to C-cell neoplasia in rodents.

Key events in the proposed mode of action are 1) liraglutide induced, GLP-1R-mediated calcitonin secretion leading to C-cell hyperplasia, 2) persistent C-cell hyperplasia due to increased calcitonin secretion (expected to be diffuse hyperplasia) leads to progressive, proliferative c-cell lesions. This mode of action precludes direct GLP-1R mediated transformation of c-cells and it depends on increased calcitonin secretion not being terminated by normal counter-regulatory responses. It is accepted that persistent hyperplasia may progress to adenomas then carcinomas. For liraglutide-induced calcitonin secretion to persist, liraglutide must increase the sensitivity of calcium-induced calcitonin secretion (an expected effect of increased intracellular cAMP) and/or abrogate the counter-regulatory responses that inhibit calcitonin secretion; primarily decreased blood calcium.

The sponsor asserts calcitonin secretion is a pharmacological effect of GLP-1R agonists, including liraglutide and exenatide. In rats, diffuse hyperplasia is considered a physiologic response to increased calcitonin demand, but when it persists, it progresses to focal hyperplasia, then adenomas, and

finally carcinomas. Since calcitonin is primarily secreted from thyroid C-cells, liraglutide induced secretion can be detected as increased plasma calcitonin. Plasma calcitonin is also increased with diffuse or focal hyperplasia and C-cell tumors. The sponsor asserts that persistent GLP-1R activation by liraglutide results in proliferative lesions, and less persistent activation by shorter acting GLP-1R agonist, like exenatide, does not, with the difference in effects on c-cell proliferation attributable to differences in pharmacokinetics, not pharmacodynamics.

Demonstrating calcitonin release is directly caused by liraglutide activating C-cell localized GLP-1Rs is relevant to human safety assessment because the sponsor proposes that in rodents, this is a robust mechanism for calcitonin release, but in humans it is not. Based on this species difference in GLP-1R-mediated calcitonin release, the sponsor asserts liraglutide-induced C-cell tumor findings in rodents are not relevant to human safety assessment.

Hypothetical Coupling of GLP-1 Receptor to Calcium-Induced Calcitonin Secretion

The GLP-1R is coupled to adenylyl cyclase via the stimulatory heterotrimeric G-protein, Gs. Agonist binding to the receptor activates Gs by inducing exchange of GTP for GDP in the Gsα subunit. The Gsα-GTP complex activates adenylyl cyclase increasing intracellular concentrations of cAMP. Increased intracellular cAMP activate protein kinase A (PKA) and cAMP-regulated exchange factor II cAMP-GEFII). Activated PKA and cAMP-GEFII increase hormone secretion by increasing intracellular calcium (activating L-type calcium channels and ryanodine receptor dependent and IP3 receptor dependent intracellular calcium release) and inhibiting efflux of intracellular potassium through KATP channels and Kv channels. In pancreatic beta cells, GLP-1R agonists increase glucose-dependent, but not glucose independent insulin release. GLP-1R agonists don't increase the sensitivity of beta cells to glucose, but increase the amount of insulin secreted by glucose stimulus. GLP-1Rs are linked to MAPK signaling, a pathway regulating mitosis, differentiation, and cell survival / apoptosis in beta cells.

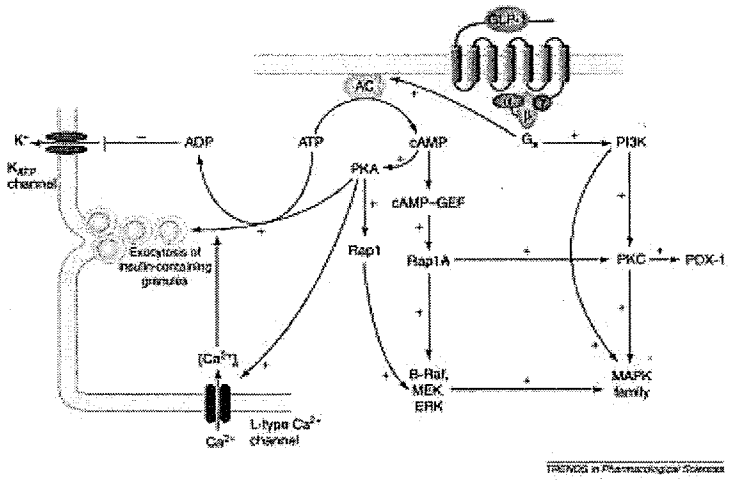


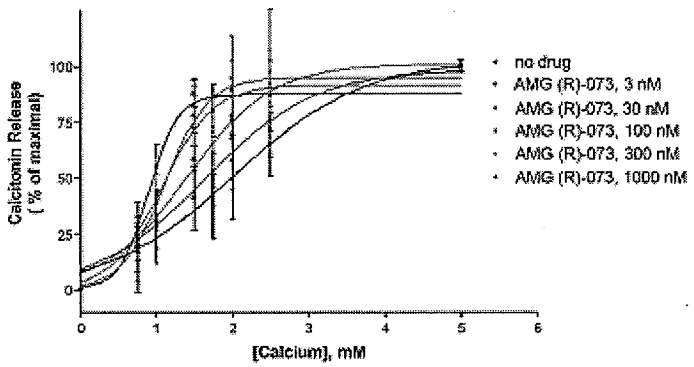
Figure 3 Signalling cascade for GLP-1 in the beta-cell (from Perry and Greig (53))

Calcium in the major physiologic stimulus for calcitonin secretion from thyroid c-cells, and it's effects are mediated by the G-protein coupled calcium sensing receptor (CaSR) on C-cells. Calcitonin secretion is calcium dependent with high extracellular calcium concentrations activating CaSR. The intracellular calcium ion concentration determines the rate of calcitonin secretion. Calcitonin release is enhanced by increased intracellular cAMP, which can by elicited by CGRP (a paracrine factor), norepinephrine (mediated by the β_2 adrenergic receptor), or glucagon (mediated by glucagon or GLP-1Rs) and inhibited by somatostatin (a paracrine effect). Since GLP-1R are coupled to Gs, any effect of

GLP-1 to directly modulate calcitonin release from C-cells is probably due to cAMP-dependent increased calcium-stimulated calcitonin release. In perfused rat thyroid, GLP-1(7-37) induced calcitonin secretion was calcium dependent; it occurred at high extracellular calcium (3 mM Ca), but not low calcium (1 mM). Hypothetically, GLP-1R activation elevates intracellular cAMP, elevated intracellular cAMP increases calcium-elicited calcitonin secretion. In rat c-cell lines (MTC 6-23 or CA77), GLP-1R agonists increase intracellular cAMP. Increased calcitonin decreases plasma calcium by deactivating osteoclasts and increasing urine calcium excretion, and as plasma calcium levels decrease, calcitonin secretion diminishes due to diminished CaSR activation. GLP-1R agonists causing sustained calcitonin secretion must either uncouple calcitonin secretion from extracellular calcium or desensitize the calcitonin-induced hypocalcemic response.

Type 2 CaSR agonists allosterically modulate the receptor to increase its calcium sensitivity and in thyroid c-cells, increase calcitonin secretion. Mice heterozygous for calcium sensing receptor gene deletion ($Casr^{+/-}$) had higher blood calcium levels, but lower calcitonin levels compared to wild type mice ($Casr^{+/+}$) indicating the CaSR calcium response curve was significantly blunted or rightward shifted in $Casr^{+/-}$ mice (Fudge NJ and Kovacs CS, BMC Physiol (2004) 20; 4-5). In calcitonin secreting MTC 6-23 cells, the CaSR agonist AMG-073 (cinacalcet, a marketed type II calcimimetic) causes a dose-dependent rightward shift in calcium-induced calcitonin secretion (Figure 2).

Figure 2. Effect of AMG (R)-073 on Calcium-Stimulated Calcitonin Release from MTC 6-23 Cells



MTC 6-23 cells were stimulated with increasing concentrations of extracellular calcium in the presence of a fixed concentration of AMG (R)-073. Each point represents the mean (+/- SD) of at least three experiments performed in triplicate or quadruplicate. The data were normalized to percent of maximal calcitonin release and curve fit to obtain EC_{50} values for calcium-stimulated calcitonin release. Amgen notebook number 36552, page 79.

[NDA 21-688 Study R2002079 P13]

In rats orally administered NPS R-568, a type II CaSR agonist, plasma calcitonin transiently increased within 10 – 90 minutes after dosing, but calcitonin levels return to baseline due to a counter-regulatory decrease in plasma calcium (Figure 4) (Fox J et al, JPET (1999) 290:480–486). Clamping blood calcium levels by iv infusion of calcium gluconate to maintain normocalcemia in NPS R-568 treated rats results in sustained hypercalcitoninemia (Figure 6).

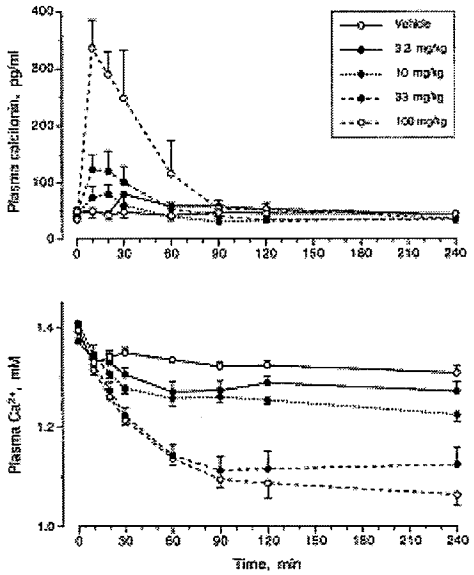


Fig. 4. Effects of oral administration of NPS R-568 on plasma levels of calcitonin and Ca^{2+} in normal rats. Values are means ± S.E.; n = 4 to 7 animals/dose. Some of these data were reported previously (Nemeth et al., 1996).

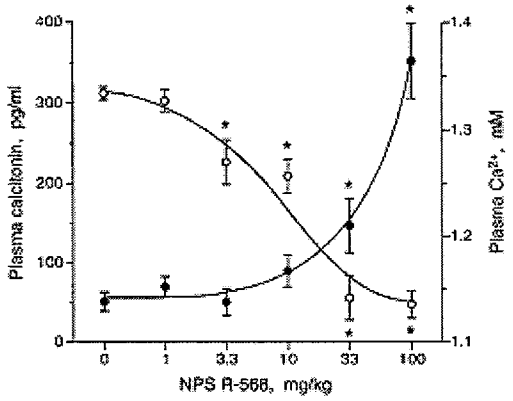


Fig. 5. Relationships between the dose of orally administered NPS R-568 and plasma calcitonin (●) and Ca^{2+} (○) levels in normal rats. Plasma calcitonin values are the maximum levels seen at either 10 or 20 min after dosing in each animal, and Ca^{2+} values are from 60 min (see Fig. 4). Values are means ± S.E.; n = 4 to 7/dose. *$p < .05$, significance of difference from vehicle-dosed rats.

[PMID 10411553 P483]

These results show calcitonin-induced hypocalcemia is a counter-regulatory response that decreases plasma calcitonin.

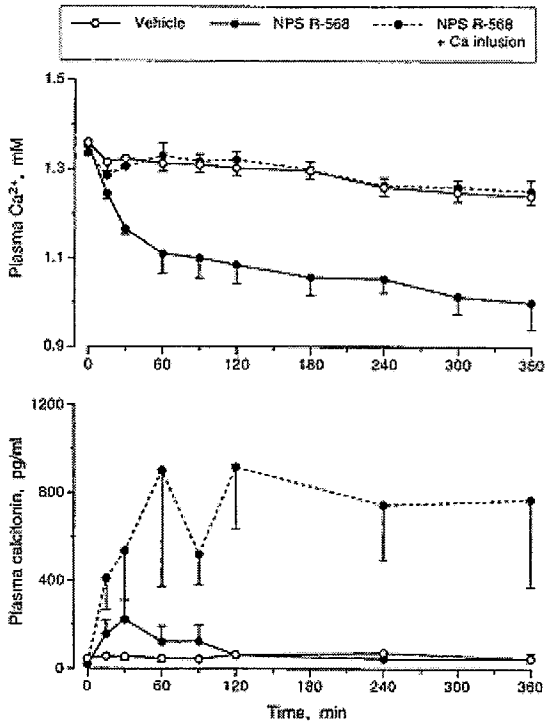


Fig. 6. Effect of prevention of induced hypocalcemia by i.v. infusion of calcium gluconate on plasma calcitonin response to orally administered NPS R-568 (100 mg/kg) in normal rats. Values are means ± S.E.; n = 4 to 6/group.

[PMID 10411553 P484]

CaSR agonist induced increased calcitonin causes hypocalcemia by inhibiting osteoclast mediated bone resorption. Orally administered NPS R-568 didn't affect plasma elimination of a pulsatile dose of intra-arterially injected $^{45}Ca^{+2}$, but it dose-dependently decreased unlabeled plasma calcium indicating the drop in calcium was due to diminished bone resorption, an expected physiologic effect of increased calcitonin.

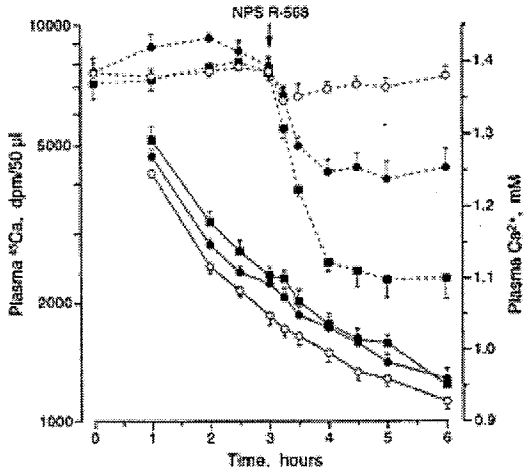


Fig. 7. Effects of oral administration of vehicle (○) or NPS R-568 (10 mg/kg, ●; 100 mg/kg, ■) on plasma ^{45}Ca activity (solid lines) and Ca^{2+} levels (dashed lines) in normal rats. ^{45}Ca (10 µCi i.v.) was injected at time 0, and the disappearance of plasma radioactivity followed before and after NPS R-568 administration at 3 h. Values are means ± S.E.; n = 5 or 6/dose.

[PMID 10411553 P484]

Cinacalcet transiently and dose-dependently increases calcitonin secretion at 1 or 30 mg/kg in normal rats (Figure 12 below).

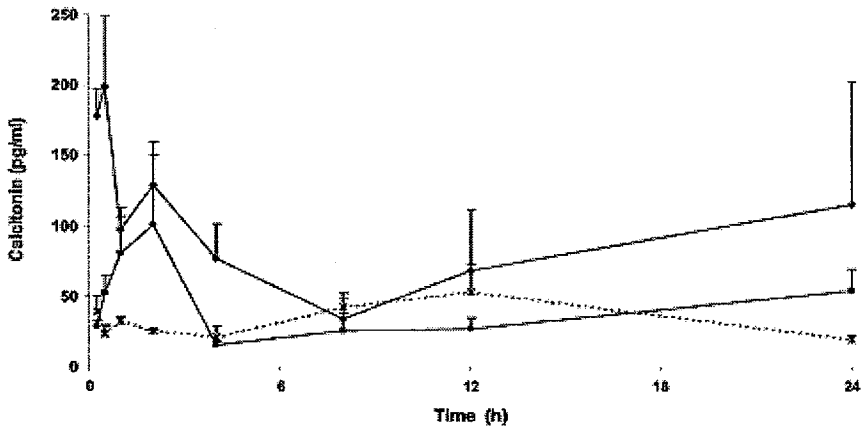


Fig. 12. Serum levels of calcitonin in normal rats administered orally with vehicle (x) or cinacalcet HCl at doses of 1 (●) or 30 (♦) mg/kg. Values represent mean ± S.E.M., n = 5–7/group. *, $p < 0.05$ versus vehicle (see text).

[Nemeth EF et al, JPET (2004) 308 ; 633]

Despite increasing calcitonin secretion, the CaSR agonist cinacalcet (AMG R-073) didn't cause thyroid tumors in 2 year carcinogenicity studies in mice or rats. In the rat carcinogen bioassay, cinacalcet actually dose-dependently decreased the incidence of c-cell adenomas in males and decreased the incidence of focal c-cell hyperplasia (a preneoplastic lesion) in low and high dose groups males and high dose group

females(see histopathology table below). The mechanism of CaSR agonist lowering proliferative C-cell lesions is possibly due to activating counter-regulatory mechanisms in response to transient hypercalcitoninemia (see Dr. Gemma Kuipers' nonclinical review of NDA 21-318) or by increased circulating calcitonin directly inhibiting c-cell growth. (Kakudo, K., et al. Acta Pathol Jpn. 1989 Sep;39(9):545-50)..

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS Males Cont 0 mg/kg /day	Grp 3 5 mg/kg /day	Grp 4 15 mg/kg /day	Grp 5 35 mg/kg /day	Females Cont 0 mg/kg /day	Grp 3 5 mg/kg /day	Grp 4 20 mg/kg /day	Grp 5 50/35 mg/kg /day
THYROID GLAND		(118)	(59)	(60)	(58)	(118)	(56)	(57)	(58)
No abnormality detected		76	42	37	47*	86	38	37	43
C-CELL CARCINOMA [M]		1	1	0	0	1	0	1	0
C-CELL ADENOMA [B]		25	6	2**	1***	10	5	2	3
Focal C-cell hyperplasia									
minimal		0	0	2	1	4	1	1	0
mild		8	0	1	1	4	3	5	1
moderate		5	1	4	0	5	4	1	1
marked		0	1	1	0	1	1	0	0
Total Incidence		13	2	8	2	14	9	7	2

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
[B] Benign tumour
[M] Malignant tumour
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified
Control Groups (1 and 2) are combined

[Excerpted from NDA 21-318]

Xenobiotic Effects on Thyroid C-cell Tumors in Rats

There is no established mechanism of thyroid C-cell tumor induction for 7 marketed drugs in 7 different pharmacologic classes that cause thyroid C-cell tumors in at least one sex in rat carcinogen bioassays (see summary table below). None of these marketed drugs caused thyroid C-cell tumors in mice. Six increased C-cell adenomas, two increase adenomas and carcinomas, and only 1, atenolol (a selective β_1 adrenergic receptor antagonist), increases C-cell carcinomas without increasing adenomas. Naratriptan, a 5-$HT_{1D/1B}$ receptor agonist, was the only drug that increased C-cell adenomas in both sexes of rats. Exenatide, a GLP-1receptor agonist, is suspected to increase GLP-1R mediated calcitonin secretion as a potential mechanism of inducing benign C-cell adenomas in female rats. In 2 year carcinogen bioassays in rats and mice, cinacalcet, a marketed CaSR agonist eliciting calcitonin release, didn't cause proliferative c-cell lesions in mice and reduced their incidence in rats.

Thyroid C-cell Tumors Occuring in Rodent Carcinogenicity Studies of Marketed Drugs and GLP-1 Receptor Agonists

Drug	Drug Class	Mice		Rats	
		M	F	M	F
Approved Drugs					
alendronate	bisphosphonate	-	-	adenoma (NOAEL 0.26X, LOAEL 1X)[2]	-
arformoterol	$_2$ receptor agonist	-	-	-	adenoma & carcinoma (NOAEL 55X, LOAEL 130X)[1]
atenolol	$_1$ receptor antagonist	-	-	carcinoma (NOAEL 150X, LOAEL 250X)[3]	-
colesevelam	bile acid sequestrant	-	-	-	adenoma (NOAEL 20X, LOAEL 40X)[3]
naratriptan	5-$HT_{1D/1B}$ receptor agonist	-	-	adenoma[B] (NOAEL 29X, LOAEL 180X)[1]	
palonosetron	5-HT_3 receptor antagonist	-	-	-	adenoma, combined adenoma & carcinoma (NOAEL 82X, LOAEL 182X)[1]
GLP-1 Receptor Agonists					
exenatide[E]	GLP-1 receptor agonist	-	-	-	adenoma[A] (NOAEL < 5X)[1]
exenatide LAR (extended release formulation)	GLP-1 receptor agonist	NT[C]		adenoma (≥ 0.3 mg/kg)[4]	adenoma, combined adenoma & carcinoma (> 0.3 mg/kg)[4]
liraglutide	GLP-1 receptor agonist	adenoma (NOAEL 0.6X, LOAEL 3.3)[1]	adenoma, combined adenoma & carcinoma (NOAEL 0.6X, LOAEL 3.3)[1]	adenoma, carcinoma (NOAEL 2.2X, LOAEL 7.6)[1], combined adenoma & carcinoma (NOAEL 0.5X, LOAEL 2.2)[1]	adenoma, combined adenoma & carcinoma (NOAEL < 0.5X)[1]
Approved Drug Cited By Sponsor					
teriparatide	PTH receptor agonist	NT[C]		adenomas, combined adenoma & carcinoma[D]	-

[1]Human exposure multiple calculated using plasma AUC comparison.

[2]Human exposure mutliple calculated using body surface area based dose comparison.

[3]Human exposure multiple calculated using weight based dose comparison.

[4]Body weight based subcutaneous dose administered every other week. Exposure multiples weren't calculated because exenatide plasma exposure during the rat carcinogenicity study or the human MRHD were not known.

[A]According to the drug label, incidences in female rats were 8% and 5% in the 2 control groups and 14%, 11%, and 23% in the low-, medium-, and high-dose groups, but increased tumor incidences in exenatide-treated groups were not statistically significant by trend analysis or control group pairwise comparison.

[B]According to the drug label "Two rat studies were conducted, 1 using a standard diet and the other a nitrite-supplemented diet (naratriptan can be nitrosated in vitro to form a mutagenic product that has been detected in the stomachs of rats fed a high nitrite diet)." Exposure multiples are based on results from the nitrite-diet supplemented study in which c-cell tumors occurred at lower exposures.

[C]NT = not tested

[D]Thyroid C-cell tumors are not listed as a drug-related finding in the approved drug label, but in male rats (n = 60/dose), there was a statistically significant dose-related trend for adenomas (incidence of 0, 2, 1, 3 at 0, 5, 30 , 75 mg/kg/day teriparatide) and combined adenomas and carcinomas (incidence of 1, 2, 1, 4 at 0, 5, 30 , 75 mg/kg/day teriparatide). Human exposure multiples for rat doses of 5, 30, and 75 mg/kg/day teriparatide calculated using plasma AUC comparison were 3, 20, and 60 times the human systemic exposure, respectively, for a 20 mcg/day dose. The incidence of C-cell adenomas or combined adenomas and carcinomas were not significantly different from controls for any dose group, therefore a NOAEL or LOAEL were not identified.

[E]Exenatide is the only marketed GLP-1 receptor agonist.

Norepinephrine, serotonin, and bisphosphonates affect bone metabolism. Secretory vesicles in MTC 6-23 cells, a rat thyroid c-cell line, costore serotonin and calcitonin (Tamir H et al, Synapse. 1992 Oct;12(2):155-68). In addition to secreting calcitonin, extracellular calcium also elicits CaSR-dependent secretion of serotonin from sheep thyroid c-cells in culture (McGehee DS et al., J Physiol. (1997) Jul 1;502 (Pt 1):31-44). Serotonin does not elicit calcitonin secretion from rat MTC 6-23 cells(Gilgenkrantz JL, Experientia. 1991 Oct 15;47(10):1067-9), but it does elicit calcitonin secretion from excised thyroid glands from young rats (M Zabel, Histochemistry. (1985);83(1):71-5). Rats treated with serotonin have increased bone mineral density and altered bone architecture (Gustafsson BI et al., J Cell Biochem 2006;97:1283–1291). Three serotonin receptors occur on osteoblasts; 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2B}.

Osteoblast-specific gene-inactivation studies show the 5-HT_{1B} receptor mediates serotonin-induced decreased osteoblast proliferation (Yadav VK et al, Cell. 2008 Nov 28;135(5):825-37). Non-specific inactivation of Htr2b gene in mice suggest a role for the 5-HT_{2B} receptor in mediating osteoblast recruitment and proliferation (Collet C, et al., FASEB J. (2008) 22(2):418-27), but these effects are apparently not mediated by the osteoblast localized receptor (Yadav VK et al, Cell. 2008 Nov 28;135(5):825-37). The specific role of 5-HT_{1D}, 5-HT_{1B}, or 5-HT_3 receptors in calcitonin secretion or c-cell proliferation isn't as well defined.

The nonselective $\beta_{1/2}$ receptor agonist, isoproterenol, stimulates calcitonin secretion from rat MTC 6-23 cells (Gilgenkrantz JL, Experientia. 1991 Oct 15;47(10):1067-9), but carcinogen bioassays of isoproterenol were not performed. β_2 adrenergic agonists increase serum calcium by directly activating osteoblast β_2 receptors that results in Rank ligand activation of osteoclasts. β receptor agonists may elicit calcitonin secretion either by directly activating c-cell β2 receptor or indirectly by elevating serum calcium. In either case, arformoterol induced c-cell tumors are not a pharmacologic class effect because they didn't occur in 2 year carcinogenicity studies of salmeterol and formoterol, 2 other long acting β_2 receptor selective agonists. The β_1 receptor selective antagonist atenolol increased c-cell carcinoma without increasing adenomas suggesting its effects are unrelated to changes in calcium or calcitonin.

Alendronate causes c-cell adenomas in male rats by an unknown mechanism. This is not a chemical class effect because 5 other marketed bisphosphonates including etidronate, risedronate, zoledronic acid, ibandronate, and pamidronate didn't cause c-cell tumors in rats.

The effects of the non-absorbed bile acid sequestrant colesevelam on calcitonin secretion is unknown and 2 year rat carcinogenicity studies of 2 non-absorbed bile acid sequestrants, colestipol and cholestyramine resin were not undertaken.

Drugs inhibiting thyroid hormone synthesis have different effects on C-cell proliferation. Thiamazole results in hyperplasia and adenomas of both follicular cells and C-cells (Capen, C.C. et al, Toxicologic Pathology (1989) 17: 266 – 293). Methimazole increases the number of TGF-1β secreting C-cells (Logan, A. et al, J Endocrinol. (1994) 141(1):45-57) and in a 2 year rat carcinogen assay, it increased the incidence of thyroid hyperplasia, adenoma, and carcinoma, but the proliferative thyroid lesion cell type(s) were not identified (drug label). Administration of propylthiouracil in drinking water for 21 days decreased C-cell density, C-cell calcitonin immunoreactivity, and plasma calcitonin levels in rats (Zbucki, RL et al., Folia Histochem Cytobiol. 2007;45(2):115-21).

C-cell hyperplasia in rats can be induced by ionizing radiation (5 – 10 μCi ^{131}I, Triggs and Williams, Acta Endocrinol. (1977) 85: 84-92). A diet high in calcium alone didn't further increase the incidence of C-cell tumors in irradiated rats, but a diet supplemented with high levels of vitamin D_3 did (Thruston and Williams, Acta Endocrinol. (1982) 100: 41 – 45). The effects of vitamin D and hypercalcemia on thyroid c-cell proliferation are not definitive because morphometric analysis of thyroids from rats treated for 3 months with high dose vitamin D3 showed the number of C-cells decreased, calcitonin mRNA levels decreased, and plasma calcitonin was unchanged despite persistent hypercalcemia.

Novelty of the Mode of Action

The sponsor is proposing that liraglutide increases the incidence of thyroid C-cell tumors in rats and mice by persistently stimulating GLP-1R mediated calcitonin secretion from C-cells resulting in increased calcitonin synthesis, c-cell hyperplasia, and progression of hyperplasia to adenomas with further progression to carcinomas. This is a novel mechanism based on the potential pharmacologic activity of GLP-1R agonists to elevate c-cell intracellular cAMP with a resulting increase in calcium-stimulated calcitonin secretion. Persistent stimulation of calcitonin secretion from thyroid c-cells results in c-cell hyperplasia. This mechanism depends on either uncoupling calcitonin secretion from extracellular calcium concentrations and/or desensitizing the hypocalcemic response to calcitonin. The key events in the proposed mode of action are persistent drug-related increased calcitonin secretion leading to C-cell hyperplasia and persistent hyperplasia leading to c-cell tumors.

In 2 year carcinogenicity studies of exenatide in rats and mice, no tumor findings occurred in mice or male rats. An increased incidence of focal thyroid C-cell hyperplasia, but not diffuse hyperplasia, occurred in exenatide-treated male (4/65, 5/65, 12/65, 11/65) and female rats (2/65, 6/65, 11/65, 8/65) at all doses in the 2 year rat carcinogenicity study (0, 0.018, 0.07, and 0.25 mg/kg/day, respectively), but it didn't occur in a 13 week repeat dose toxicity study at doses up to 0.25 mg/kg/day.

In the rat carcinogenicity study of exenatide, an increased incidence of C-cell adenomas above the sponsor's historical control group range occurred in females at all doses (8% and 5% in the two control groups and 14%, 11%, and 23% in the low-, medium-, and high-dose groups with systemic exposures of 5, 22, and 130 times, respectively, the human exposure resulting from the maximum recommended dose of 0.02 mg/day, based on plasma area under the curve (AUC), but the increased incidence was not statistically significant for either a dose related trend or pairwise comparison of treated groups to control.

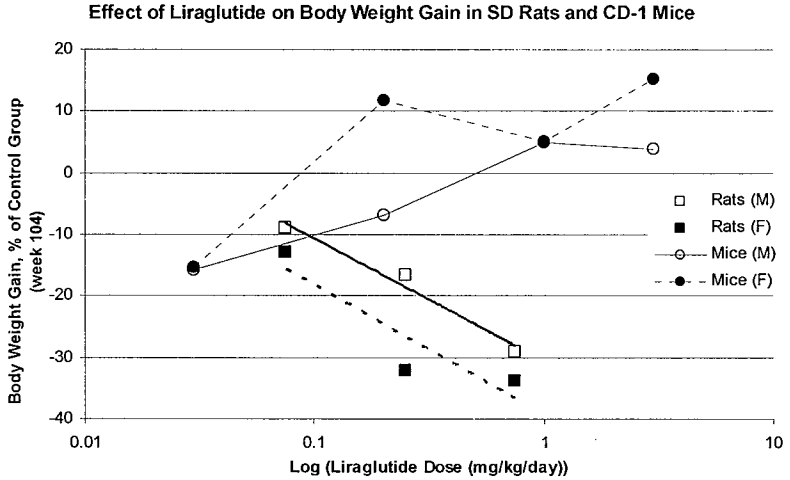
Other Potential Modes of Action

Other potential modes of action for liraglutide induced thyroid c-cell tumors in rats and mice include direct mitogenic activity of GLP-1R agonists on thyroid C-cells or activation of the RET proto-oncogene. Quantitative analysis or BrdU labeling studies of thyroid c-cells from liraglutide treated rats and mice and in vitro [^{3}H]thymidine incorporation assays using rat or human C-cell lines suggest liraglutide is not a C-cell mitogen. Liraglutide did not cross react with receptors linked to C-cell growth responses including human bombesin receptors, CCK2 receptors, or neuromedin receptors. Direct or indirect activation of RET has not been ruled out as a potential mode of action. Activation of RET kinase increases plasma calcitonin from wild type mice or mice implanted with human TT cell xenografts and based on the time course of RET kinase inhibitor activity, RET kinase induced increased plasma calcitonin is independent of it's effects as a C-cell mitogen. Liraglutide caused focal, but not diffuse, c-cell proliferation in both rats and mice suggesting it may be a c-cell tumor promoter, at least in vivo.

KEY EVENTS IN LIRAGLUTIDE INDUCED THYROID C-CELL TUMORS IN RODENTS

Liraglutide Pharmacodynamics in Rats and Mice

A pharmacodynamic effect of liraglutide, decreased body weight gain, was demonstrated in 2 year carcinogenicity studies in Sprague Dawley rats treated with 0.075, 0.25, or 0.75 mg/kg/day, but not in CD-1 mice treated with 0.03, 0.2, 1, or 3 mg/kg/day. Liraglutide dose-dependently decreased body weight gain in male and female rats at $\geq$ 0.075 mg/kg/day, but not in mice at any dose (see Figure below). GLP-1Rs may not regulate body weight or feeding behavior in mice because GLP-1R knockout mice were lean (Scrocchi et al., Nat Med (1996) 2:1254–1258) and inactivating the GLP-1R in obese ob/ob mice had no effect on food consumption or body weight (Scrocchi et al., Diabetes (2000) 49:1552–1560).



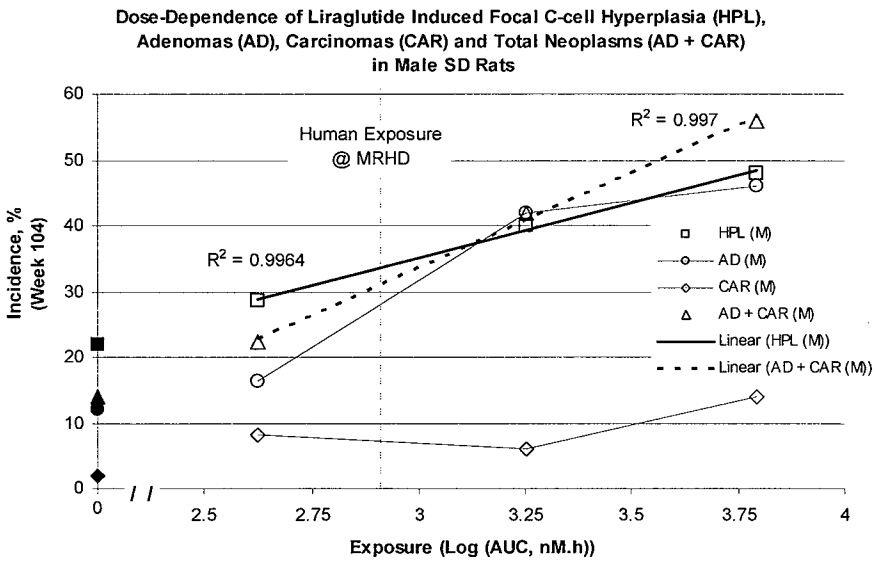
Liraglutide is pharmacologically active in mice because it dose-dependently increased glucose (10 mM) stimulated insulin release from perfused pancreatic islets from NMRI mice (ED_{50} 10nM), lowered blood glucose female diabetic ob/ob mice, and increased pancreas beta cell mass in ob/ob mice (C57BL/6J background strain). However, pharmacologic activity was not directly demonstrated in CD-1 mice.

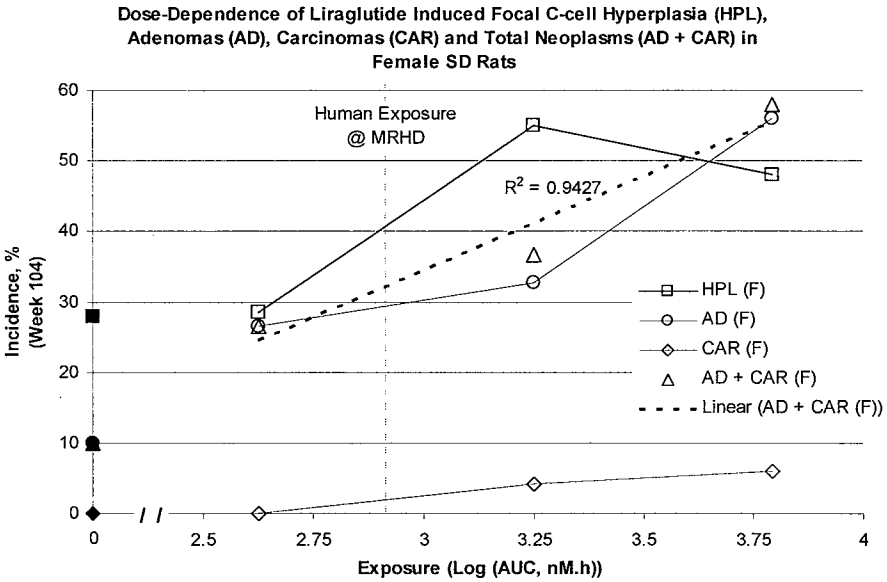
Liraglutide Dose and Duration Dependent Effects on Proliferative C-cell Lesions and Plasma Calcitonin

Rats

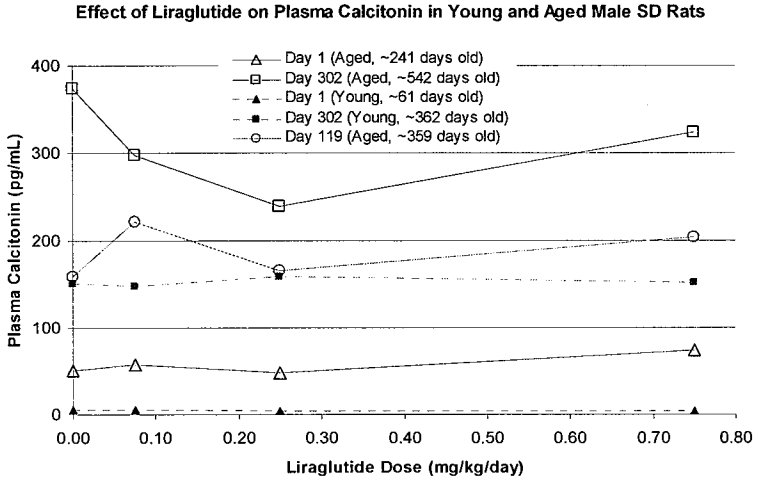
In rats, liraglutide dose-dependently increased proliferative thyroid c-cell lesions, including age-dependent focal hyperplasia, the progression of C-cell hyperplasia to adenomas, and the progression of C-cell adenomas to carcinomas. Liraglutide did not accelerate the onset of focal c-cell hyperplasia, but it did accelerate the onset of c-cell adenomas. Elevated plasma calcitonin was dependent on age and the incidence and severity of diffuse and focal C-cell hyperplasia, but not liraglutide dose or treatment duration. In rats, calcitonin was not a valid marker for liraglutide-induced C-cell tumors.

In a 2 year carcinogenicity study of 0.075, 0.25, or 0.75 mg/kg/day liraglutide in rats yielding exposures of AUC_{0-24} 423, 1785, or 6,225 nM.hr, respectively, liraglutide dose-dependently increased the incidence of focal C-cell hyperplasia (HPL), adenomas (AD), and combined adenomas and carcinomas (AD + CAR) in males at $\geq$ 0.25 mg/kg/day and in females at $\geq$ 0.075 mg/kg/day (Figures below, filled symbols on the y-axis show vehicle-treated control group incidences). Increased incidence of hyperplasia, adenomas, and combined adenomas and carcinomas was dose-related in males and females, and carcinomas were dose-dependently increased in males. The incidence of C-cell hyperplasia was similar to total C-cell tumors (adenomas and carcinomas combined), except at 0.25 mg/kg dose in females where the incidence of hyperplasia was higher.





Plasma calcitonin was not measured during the rat carcinogenicity study, but it was determined in mechanistic studies in male rats. Calcitonin measured in plasma of young male Sprague Dawley rats (2 months old at start of dosing) or aged male rats (8 months old at start of dosing) taken 3 hours after the first dose (day 1) of 0, 0.075, 0.25, or 0.75 mg/kg/day liraglutide and after dosing on day 302 showed increased plasma calcitonin was age dependent, but not liraglutide dose-dependent (see Figure below). Calcitonin levels in young rats treated with liraglutide for 302 days were similar to those in aged rats treated for 119 days, and these rats were the same chronological age at the time of sampling (~ 360 days old). Calcitonin levels were independent of liraglutide dose.

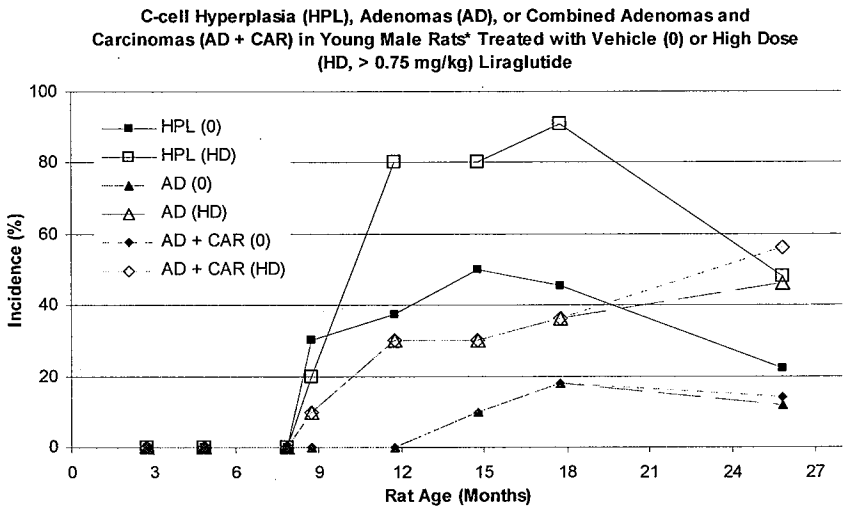


In single dose studies of subcutaneously injected liraglutide in normal and calcium loaded young male rats, liraglutide had little effect on calcitonin levels (data not shown, but included in review of studies 203281 / 203282), particularly compared to calcium loading. Liraglutide-treatment-related increased plasma PTH was possibly a counter-regulatory effect of decreased serum calcium secondary to any increase in plasma calcitonin, but the magnitude of any liraglutide-related decrease in serum calcium was small.

Liraglutide increased the incidence of age-dependent focal thyroid c-cell hyperplasia in rats, considered a pre-neoplastic lesion, but without increasing age-dependent diffuse c-cell hyperplasia. Liraglutide accelerated the progression of age-dependent focal c-cell hyperplasia to adenomas after at least 7 months of treatment and accelerated the progression of c-cell adenomas to carcinomas after at least 86 weeks of treatment, but without accelerating the onset of focal c-cell hyperplasia or without increasing the incidence of diffuse c-cell hyperplasia. (The first decedent with C-cell carcinoma in the high dose group occurred in week 86, but the first control group decedent with C-cell carcinoma died in week 93).

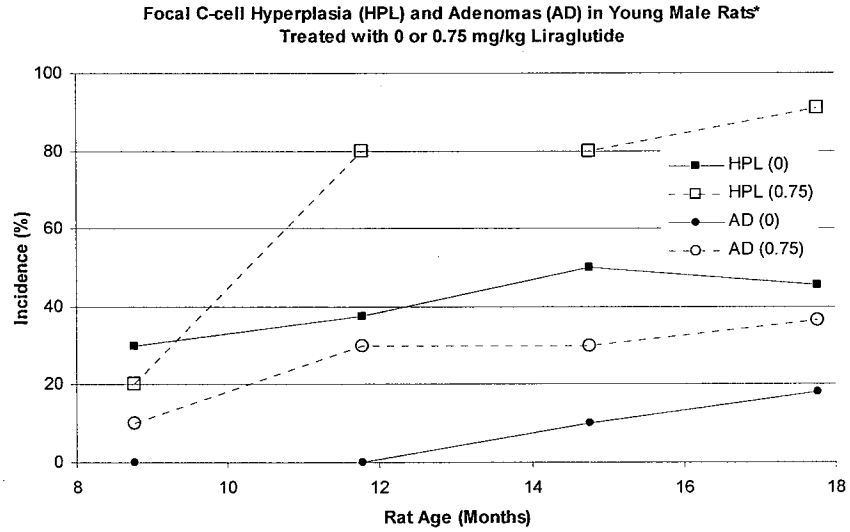
Nearly all liraglutide mechanistic studies in rats used only males, and this was acceptable because there were no qualitative sex differences in liraglutide-induced proliferative C-cell lesions. A time course for the development of focal c-cell hyperplasia, adenomas, and carcinomas in 2 month old male Sprague Dawley rats (approximate age when dosing was initiated) treated with vehicle or high dose liraglutide ($\geq$ 0.75 mg/kg) was constructed using data from 4 (0.75 mg/kg HD), 13 (1 mg/kg HD), and 26 week (1 mg/kg HD) repeat dose toxicity studies, mechanistic studies examining thyroid histopathology after treatment for 4, 30, 43, 56, and 69 weeks, and a 104 week carcinogenicity study (see Figure below). For rats treated for $\geq$ 30 weeks, the high dose of liraglutide was 0.75 mg/kg.

The incidence of C-cell adenomas, but not focal hyperplasia, increased in young males rats treated with 0.75 mg/kg/day liraglutide for 7 months (9 months old). This result indicated liraglutide increased progression to adenomas without affecting the incidence and without accelerating the onset of focal hyperplasia. After 10 months of treatment (12 month old rats), liraglutide increased the incidence of both focal hyperplasia and adenomas. After 24 months (26 month old rats), liraglutide increased the incidence of carcinomas.



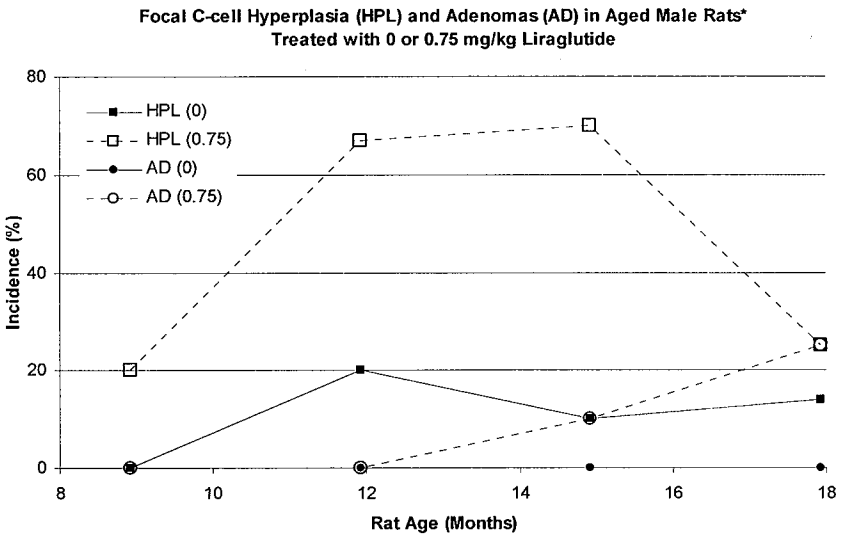
*Rats were ~ 2 months old when treatment started.

A mechanistic study to determine the effect of age on liraglutide–induced thyroid C-cell proliferative lesions treated 2 month old male rats ("young" rats) for 30 to 69 weeks (7 to 16 months) or 8 month old male rats ("aged" rats) for 4 to 43 weeks (1 to 10 months). In young male rats, focal c-cell hyperplasia didn't occur at up to 6 months of treatment with up to 1 mg/kg liraglutide (see Figure of incidence versus rat age, above). Compared to concurrent controls (0 mg/kg/day), liraglutide (0.75 mg/kg/day) increased the incidence of C-cell adenomas after 7 months of treatment, but without increasing the incidence of focal C-cell hyperplasia (see Figure below). At 12 months, liraglutide increased the incidence of both c-cell focal hyperplasia and adenomas, and this increase persisted up to the end of the study when rats were 18 months old.



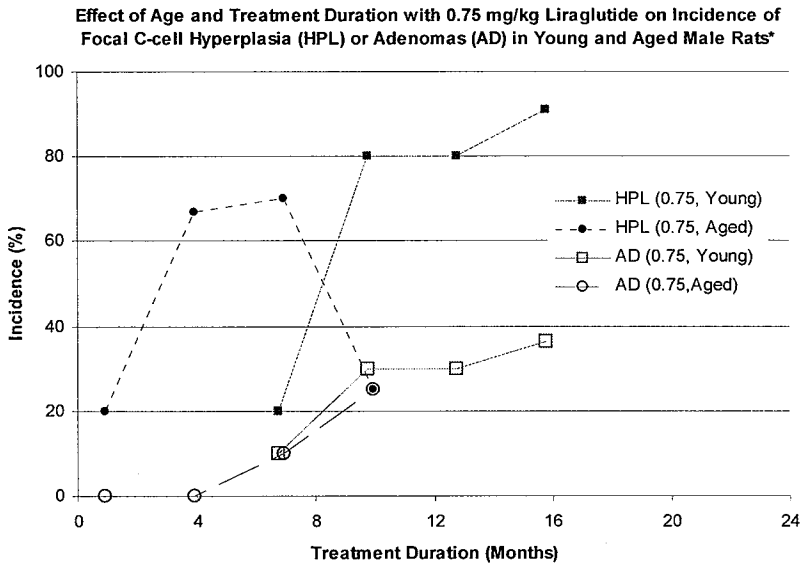
*Young male rats were 2 months old when treatment started.

In aged rats, 0.75 mg/kg/day liraglutide increased the incidence of focal c-cell hyperplasia after 1 month of treatment, but only increased the incidence of adenomas after 7 months (15 month old rats, see Figure below). The incidence of focal c-cell hyperplasia in aged rats decreased after 10 months of treatment (18 months old), and the decrease may be due to an increased incidence of adenomas, at least in part.



*Aged male rats were 8 months old at the start of treatment

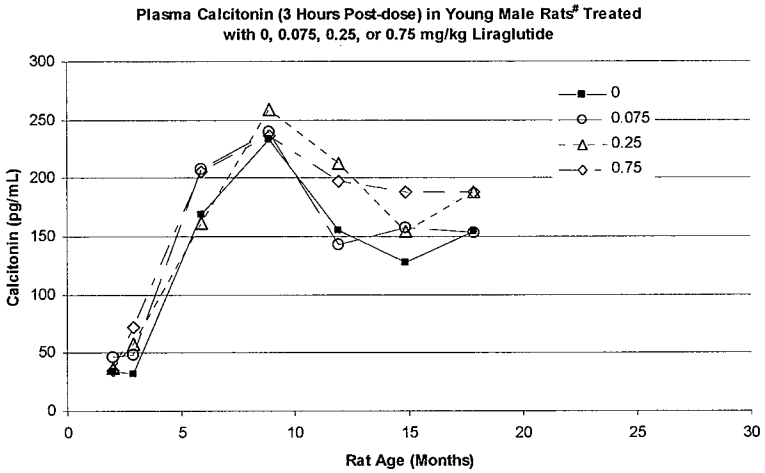
The figure below compares incidences of proliferative c-cell lesions based on liraglutide treatment-duration for young and aged male rats. This figure shows the incidence of C-cell adenomas increased with treatment duration. Liraglutide increases the incidence of age-related focal hyperplasia since liraglutide increased the incidence of focal hyperplasia only after at least 7 months of treatment in young rats and after only 1 month in aged rats, but without accelerating the onset of focal hyperplasia or without causing diffuse hyperplasia.



*When treatment started, young rats were 2 months old and aged rats were 8 month old.

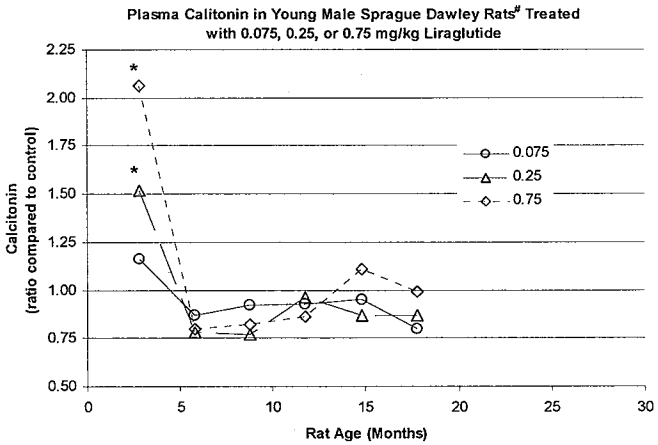
In male rats, liraglutide increased the incidence of age-related focal c-cell hyperplasia and increased the progression of focal hyperplasia to adenomas after $\geq$ 7 months of treatment, and increased the progression of adenomas to carcinomas after at least 20 months.

Plasma calcitonin levels increased with age, but there was a trend of higher calcitonin at 0.75 mg/kg liraglutide throughout the treatment period (see Figure below). Increased plasma calcitonin was not liraglutide dose-dependent in young male rats. Higher calcitonin levels in all groups occurring after 6 months of treatment can be attributed to increased diffuse and focal c-cell hyperplasia.



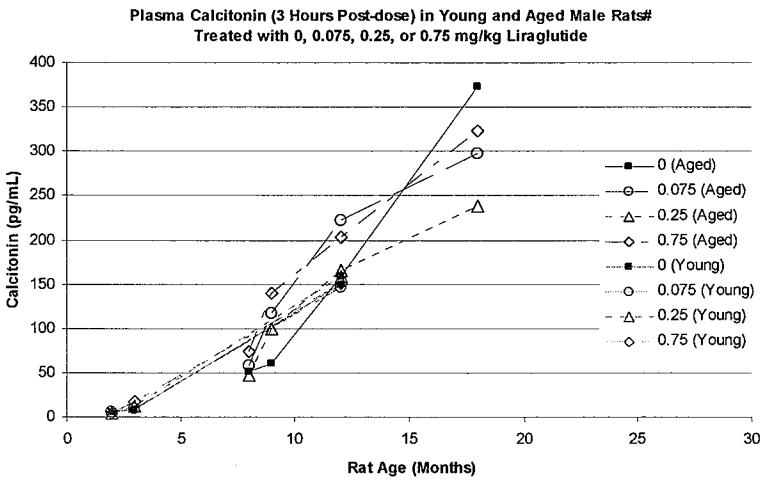
#Rats were 2 months old when treatment started.

The sponsor's analysis showed plasma calcitonin was significantly higher than concurrent controls after 1 month of dosing with ≥ 0.25 mg/kg liraglutide, but the effect didn't persist and there were no statistically significant increase compared to control after the first month (see Figure below).



#Rats were 2 months old when treatment started.
*Statistically significantly increased above concurrent control ($p < 0.05$).

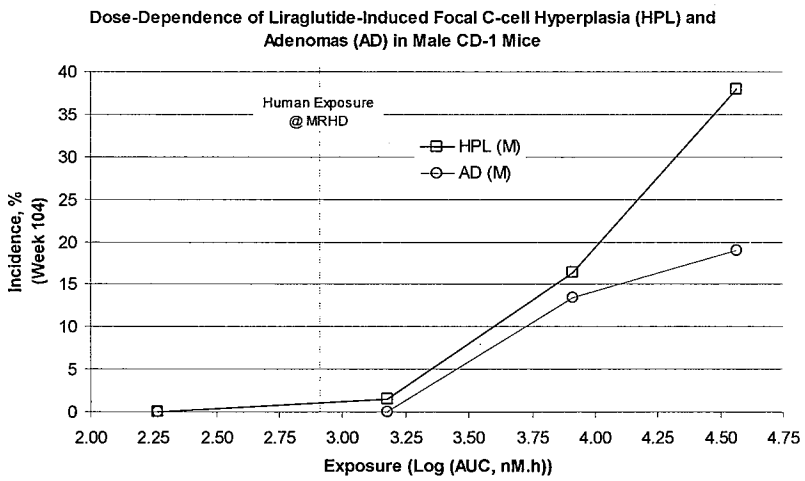
In both aged and young rats, plasma calcitonin increased with age, and increased plasma calcitonin correlated with increased c-cell hyperplasia, but not with liraglutide dose (see Figure below).

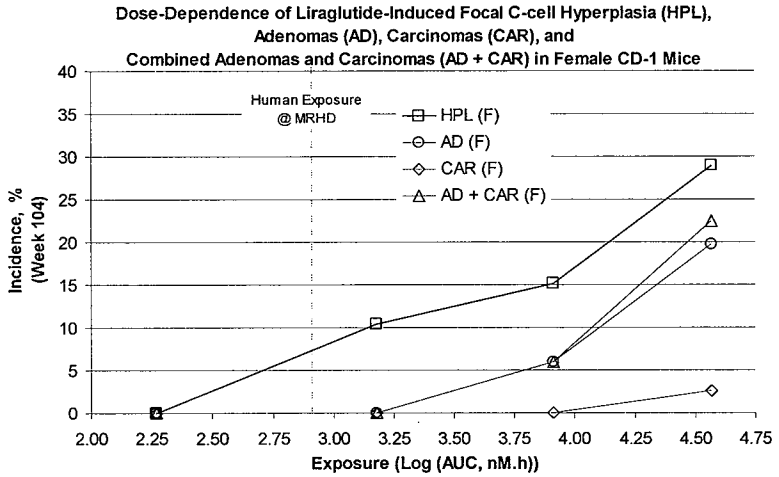


#When treatment started, young rats were 2 months old and aged rats were 8 month old.

Mice

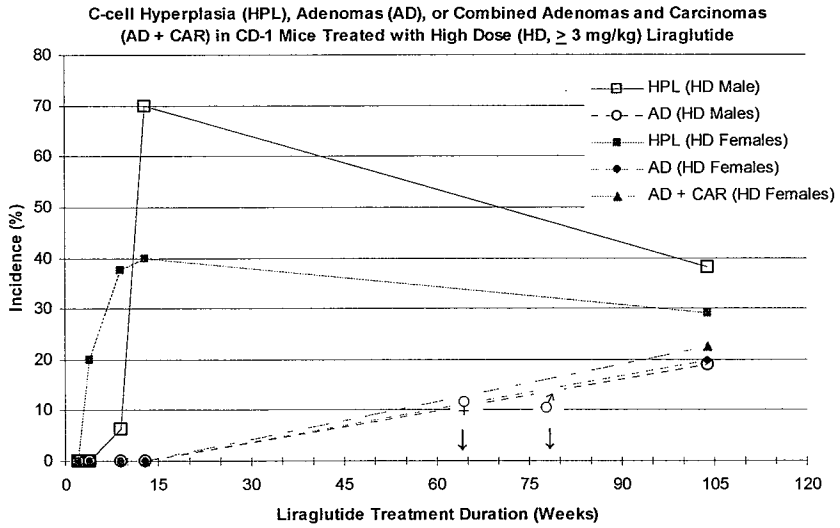
In a 2 year carcinogenicity study of 0.03, 0.2, 1, or 3 mg/kg/day liraglutide in mice yielding exposures of $AUC_{0\text{-}24}$ 185, 1,501, 8,153, or 36,830 nM.hr, respectively, liraglutide dose-dependently increased the incidence of focal C-cell hyperplasia (HPL) at $\geq$ 0.2 mg/kg/day in males and females, adenomas (AD) at $\geq$ 1 mg/kg/day in males and females, and combined adenomas and carcinomas (AD + CAR) in females at 3 mg/kg/day (Figures below, the incidence of hyperplasia, adenomas, or carcinomas in the control groups was 0% in both sexes). The incidence of C-cell hyperplasia was similar to or higher than total C-cell tumors (adenomas and carcinomas combined) at all doses. The higher incidence of hyperplasia at lower doses than those causing tumors and the higher incidence of hyperplasia compared to C-cell tumors at all doses is consistent with the hyperplasia preceding adenomas. In females, C-cell carcinoma only occurring at the highest dose was consistent with liraglutide accelerating the progression of adenomas to carcinomas.





A time course for the development of focal c-cell hyperplasia, adenomas, and carcinomas in high dose liraglutide-treated CD-1 mice was constructed using data from 4 week (5 mg/kg HD) and 13 week (5 mg/kg HD) repeat dose toxicity studies, mechanistic studies examining thyroid histopathology after treatment for 2 or 9 weeks (5 mg/kg HD), and a 104 week carcinogenicity study (3 mg/kg HD, see Figure below).

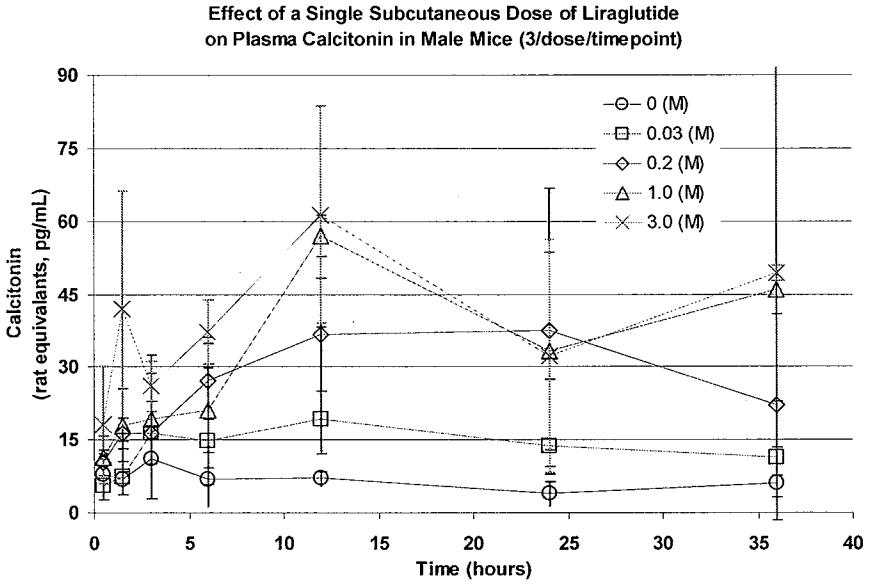
High dose liraglutide increased the incidence of focal c-cell hyperplasia after ≥ 4 weeks in females and after ≥ 9 weeks in males, but the incidence of diffuse hyperplasia was unaffected by liraglutide. C-cell hyperplasia preceded C-cell tumors. In the 104 week carcinogenicity study, c-cell tumors first occurred in high dose group decedents in week 64 in females (a C-cell carcinoma) and in week 78 in males (an adenoma), but focal hyperplasia occurred 17 weeks earlier in decedents from both sexes.

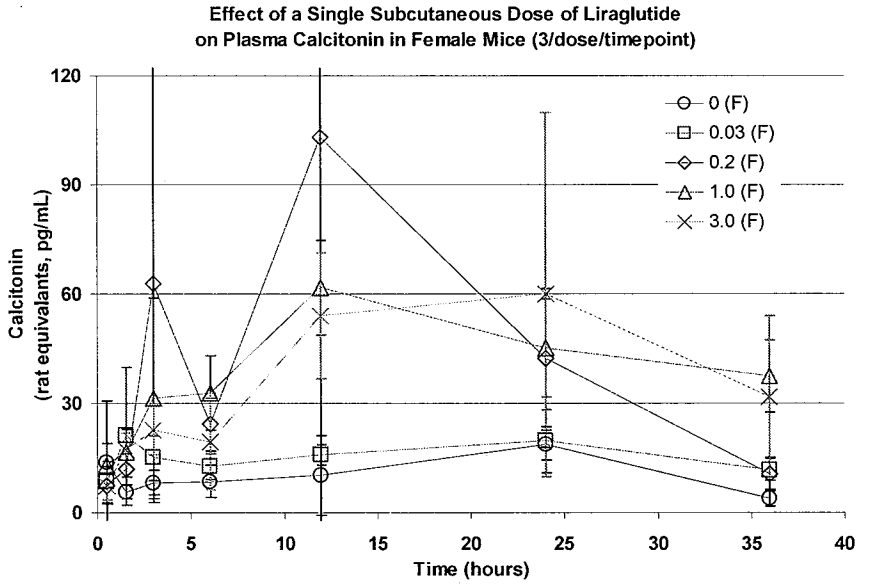


♀ and ♂ denote occurrence of first C-cell tumor in a decedent female and male, respectively.

After a single subcutaneous dose of 0, 0.03, 0.2, 1, or 3 mg/kg liraglutide, a trend of increased plasma calcitonin above control group levels occurred at ≥ 0.2 mg/kg in males and females, but with a high degree of variability. In males, the largest increase occurred ~12 hours after dosing with 3 mg/kg,

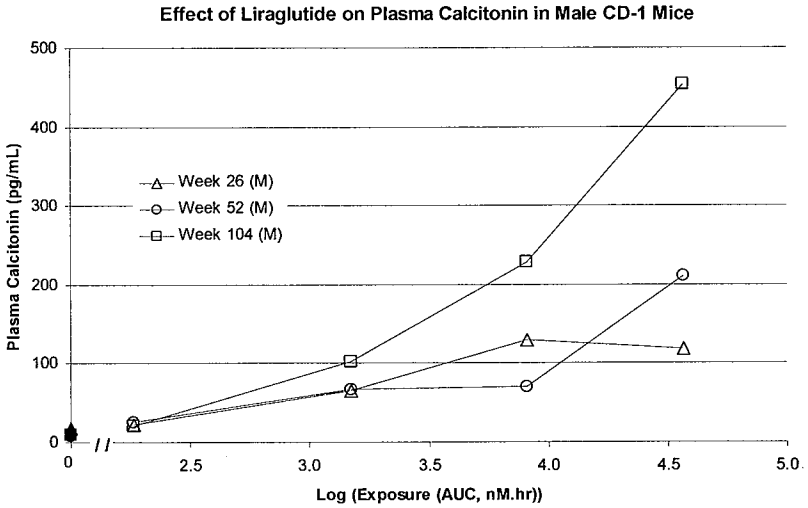
but in females, increased calcitonin was not related to dose. In females, the largest increase occurred in the 0.2 mg/kg group 12 hours after dosing.

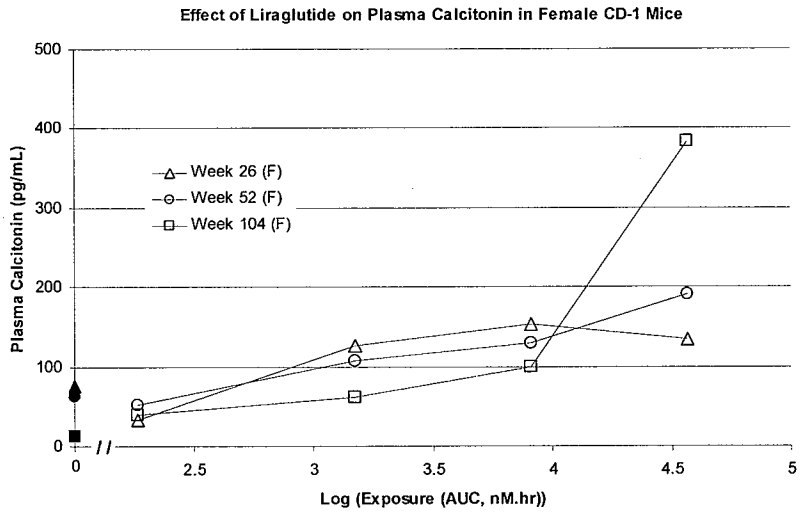




Plasma calcitonin was determined in the 2 year carcinogenicity study after dosing in weeks 26, 52, and 104 (see Figures below). Plasma calcitonin was significantly above control group levels (shown as filled symbols, corresponding to the respective sample week, on the y-axis) at $\geq$ 0.2 mg/kg/day in week 26 and at all doses in weeks 52 and 104. The increase was generally dose-dependent in both sexes. Dose-dependence was clearly established in week 104, probably due to an increased incidence of proliferative C-cell lesions in both males and females and decreased control group levels in females. A more robust response with increased treatment duration is consistent with an increased incidence of proliferative C-cell lesions, a pharmacologic effect of liraglutide to increase calcitonin secretion, or both. Although

plasma calcitonin was significantly increased above control group levels at ≥ 0.2 mg/kg/day liraglutide in week 26 and at all doses in weeks 52 and 104, plasma calcitonin increased with treatment duration at 3 mg/kg/day liraglutide in both sexes.





Exenatide is a marketed GLP-1R agonist with once daily subcutaneous injections causing c-cell adenomas in female rats, but not male rats or mice. Because liraglutide was more efficacious than exenatide at inducing proliferative c-cell lesions in both rats and mice, and because this effect was thought to be mediated by the GLP-1R, the sponsor considered differences in exenatide and liraglutide thyroid c-cell tumorogenicity may be due to pharmacokinetic differences. Since focal c-cell hyperplasia precedes c-cell adenomas in liraglutide-treated mice, but not in rats, and because focal c-cell hyperplasia in mice occurs within 13 weeks of treatment, the sponsor determined the effects of sustained plasma levels of exenatide on plasma calcitonin and thyroid c-cell histopathology in mice. Mice administered 0.25 mg/kg/day exenatide developed focal c-cell hyperplasia by week 12 that persisted to week 16 if exenatide was administered by constant subcutaneous infusion, but the incidence of hyperplasia was much lower for mice subcutaneously injected with the same bolus dose once a day. Repeat dosing of up to 1 mg/kg exenatide for up to 3 times a day for up to 13 weeks did not cause focal c-cell hyperplasia.

Pharmacokinetic / pharmacodynamic modeling of the effects of exenatide on plasma calcitonin and focal c-cell hyperplasia in mice indicate that sustained plasma levels of GLP-1R agonists, by daily subcutaneous injection of liraglutide or constant subcutaneous infusion of exenatide, results in persistent calcitonin secretion and C-cell focal hyperplasia, but not diffuse hyperplasia. These results suggest persistent GLP-1R activation induces increased plasma calcitonin and proliferative C-cell lesions (preneoplastic and neoplastic) in mice.

CONCLUSIONS

C-cell proliferative lesions in rats and mice appear to be a pharmacologic effect of long acting GLP-1R agonists including liraglutide and sustained release subcutaneous formulations of exenatide. A recently submitted safety report to ——— (serial 120 submitted 11/12/08) showed an extended release formulation of exenatide subcutaneously injected in rats once every other week increased the incidence of adenomas (males and females) and combined adenomas and carcinomas (females). Sustained release exenatide from subcutaneously implanted osmotic pumps in mice cause focal thyroid C-cell hyperplasia within 9 weeks of treatment. Liraglutide and sustained release formulations of exenatide are unique in causing proliferative c-cell hyperplasia in rats and mice. Persistent stimulation of thyroid c-cell calcitonin secretion leading to c-cell hyperplasia with progression to tumors has been proposed as a mode of action for treatment induced C-cell adenomas in rats for other drugs, but the mode of action was not thoroughly investigated. A search of approved drug labels identified 7 drugs positive for c-cell tumors in rat carcinogen bioassays, but none caused c-cell tumors in mice. A search of the NDA/IND review document database (DARRTS and DFS) did not identify any other approved or investigational drugs causing c-cell proliferative lesions in mice. The role of GLP-1Rs in mediating liraglutide-induced c-cell proliferative lesions is unknown, however rat carcinogenicity studies of exenatide which caused c-cell adenomas in female rats and an extended release formulation of exenatide (exenatide LAR) which cause c-cell adenomas in male rats and adenomas and combined adenomas and carcinomas in females, is consistent with persistent GLP-1R activation leading to c-cell neoplasms in rats.

b(4)

The table below summarizes findings in liraglutide mechanistic studies in each species.

Mode of Action Step	Rat	Mouse	Cynomolgus Monkeys	Humans
Presence of C-cell GLP-1 receptor[A]	Equivocal (GLP-1(7-37)amide autoradiographic binding in thyroid in 100% of rats, but cell type not identified)	Equivocal (GLP-1(7-37)amide autoradiographic binding in thyroid of 60% of mice, but cell type not identified)	Equivocal	Equivocal (GLP-1(7-37)amide autoradiographic binding in thyroid of 5.5% of humans, 28% of thyroid medullary thyroid tumors)
GLP-1 receptor activation linked to increased intracellular cAMP	Equivocal (+ in c-cell lines, but effects in c-cells unknown)	ND	ND	Equivocal (- in c-cell lines, but effects in c-cells unknown)
GLP-1 receptor activation linked to increased calcitonin release	In vitro: + in c-cell lines In vivo (plasma): Equivocal, increased calcitonin was age-dependent, not liraglutide-dose dependent	In vitro: ND In vivo: +	In vivo: - (single or repeat dosing up to 20 months)	In vitro: - (C-cell lines) In vivo: + (calcitonin dose-dependently increased in study weeks 26/28 at 0.6, 1.2, or 1.8 mg/day liraglutide)
Increased calcitonin synthesis	In vitro: + (c-cell lines) In vivo: Equivocal	In vitro: + (c-cell lines) In vivo: Equivocal	ND	In vitro: - (C-cell lines)
Characteristics of drug-related C-cell hyperplasia	Diffuse: - Focal: + (age-dependent, not liraglutide treatment-duration dependent)	Diffuse: - Focal: +	-	-
C-cell neoplasia	+ (adenomas (M & F), carcinomas (M), combined adenomas & carcinomas (M & F))	+ (adenomas (M & F), combined adenomas & carcinomas (F))	-	- (increased incidence of papillary thyroid carcinoma)

[A]Immunohistochemical localization studies of GLP-1 receptor in thyroid tissue sections from mice, rats, monkeys, and humans were indeterminant because the specificity of the anti-GLP-1 receptor antibody was not demonstrated. In situ hybrizidation studies localizing GLP-1 receptor mRNA in thyriod tissue sections were equivocal because receptor mRNA levels were very low.

The weight of evidence does not support the proposed mode of action for liraglutide-induced thyroid c-cell tumors in Sprague Dawley rats because:

1. Although published studies demonstrate GLP-1Rs in rat thyroid by autoradiographic tissue binding and GLP-1R agonist increased calcium-stimulated calcitonin release from perfused rat thyroid cells, the sponsor's immunohistochemical and in situ hybridization studies did not adequately demonstrate GLP-1Rs were localized to c-cells.
2. Although there are differences in GLP-1R expression, second messenger coupling, and calcitonin secretion between c-cell lines derived from rats or humans indicating c-cell GLP-1Rs are coupled to calcitonin secretion in rats but not humans, any differences occurring in c-cell lines may not reflect normal c-cell physiology in vivo.
3. In vivo, increased plasma calcitonin was age dependent, not liraglutide dose or treatment duration dependent.
4. A time course for progression of c-cell proliferative lesions in control and high-dose liraglutide-treated rats showed liraglutide increased the incidence of c-cell adenomas prior to increasing the incidence of focal c-cell hyperplasia. Liraglutide did not cause diffuse c-cell hyperplasia, considered a physiologic response to increased calcitonin secretion, and it didn't accelerate the onset of age-dependent focal c-cell hyperplasia.
5. Increased incidence of c-cell adenomas and carcinomas were treatment duration dependent, but the incidence of focal c-cell hyperplasia was age-dependent.

Liraglutide increased the incidence of c-cell carcinomas, which are otherwise rare in Sprague Dawley rats. These results suggest liraglutide accelerates the progression of age-related focal c-cell hyperplasia to adenomas in rats, but without causing diffuse hyperplasia or accelerating the onset of focal hyperplasia. Although plasma calcitonin levels markedly increased with age, the increase was not liraglutide dose-dependent, despite higher incidences of C-cell tumors in liraglutide-treated rats. These data suggest plasma calcitonin is not a biomarker for liraglutide-induced thyroid tumors in rats. The role of GLP-1R in mediating liraglutide-induced c-cell proliferative lesions is unknown, however rat carcinogenicity study results of exenatide which caused c-cell adenomas in female rats and an extended release formulation of exenatide (exenatide LAR) which cause c-cell adenomas in male rats and adenomas and combined adenomas and carcinomas in females, is consistent with persistent GLP-1R activation leading to c-cell neoplasms in rats.

The weight of evidence does not support the proposed mode of action in CD-1 mice because:

1. immunohistochemical localization and in situ hybridization studies of GLP-1R in thyroid did not adequately demonstrate GLP-1R protein or transcript localized to calcitonin immunoreactive c-cells. A published study showed that thyroid from 60% of mice (3/5) were positive for GLP-1Rs detected by autoradiographic ligand binding, but GLP-1 binding activity wasn't localized to a specific thyroid cell-type.

2. Although liraglutide (and sustained release exenatide) caused focal hyperplasia (a preneoplastic lesion), but without causing diffuse hyperplasia (a physiologic response to increased calcitonin demand).

3. In addition to thyroid c-cell tumors, fibrosarcomas on the dorsal surface were treatment related at 3 mg/kg in male mice. GLP-1Rs. GLP-1Rs coupled to MAPK/ERK, but not to adenylyl cyclase, occur in hair follicles and in cultured skin-derived cells expressing nestin (a marker of cells dedifferentiated by epithelial to mesenchymal transition) (List et al, Regulatory Peptides (2006) 134:149-157).

Liraglutide increases calcitonin secretion in mice, and with continue treatment, liraglutide increases thyroid c-cell proliferative lesions resulting in elevated basal calcitonin levels.

Based on repeat dose toxicity, carcinogenicity, and mechanistic studies of liraglutide in rats and mice and exenatide in mice, the weight of evidence does not support the proposed mode of action. In both rats and mice, there was insufficient evidence to conclude thyroid GLP-1Rs are localized on C-cells. Quantitative analysis of thyroid c-cells in rats and mice showed that liraglutide increased the incidence of focal hyperplasia (a preneoplastic lesion), but without increasing diffuse hyperplasia (a physiologic response). In rats, liraglutide increased the incidence of age-related focal c-cell hyperplasia, the progression of focal c-call hyperplasia to adenomas, and the progression of adenomas to carcinomas, but without accelerating the onset of focal c-cell hyperplasia or without causing a sustained dose-related increase in plasma calcitonin above the age-related increase that normally occurs in rats. In mice, liraglutide caused neoplasms on the dorsal skin surface and in thyroid c-cells. Liraglutide increased calcitonin secretion, initiated focal c-cell hyperplasia, and with continued treatment, induced c-cell tumors in males and females, but without increasing the incidence of diffuse c-cell hyperplasia.

RECOMMENDATIONS

Mechanistic studies of liraglutide-induced thyroid C-cell tumors in rats and mice do not support the sponsor's conclusion that these tumors are not relevant to human risk assessment. The sponsor should remove any statement indicating liraglutide induced thyroid c-cell tumors in rodents are not relevant to humans from any document communicating risk to clinical trial participants, including the investigator brochure or patient informed consent.

Question to the ECAC

The Division is seeking the ECAC's concurrence that mechanistic studies investigating the mode of action for liraglutide-induced thyroid c-cell proliferative lesions in rats and mice do not support the following statement in the sponsor's proposed drug product label:

> *"The mechanism responsible for induction of these tumors [thyroid c-cell tumors] in rodents has been elucidated, and they are caused by a non-genotoxic, specific GLP-1 receptor-mediated mechanism to which rodents are particularly sensitive and monkeys and humans are not."*

The reviewer proposes replacing the sponsor's statement with the following:

> *"The relevance of liraglutide-induced thyroid c-cell tumors in rats and mice to humans is unknown."*

Does the ECAC concur that the sponsor's proposed statement regarding the human relevance of thyroid C-cell tumors is not supported by mechanistic studies?

Yes, the ECAC agreed there was insufficient evidence to conclude liraglutide-induced thyroid c-cell tumors in rats and mice are not relevant to human risk assessment.

b(4)

Appendix D: Rat Embryofetal Historical Control Data from ———

Control group data from the definitive combined fertility and embryofetal toxicity study of liraglutide in rats (study 990284) is included as study 15.

NR = not reported (but 0 may be the correct value)

For the means and ranges, the values generally refer to absolute incidences, not percentages, because percentages are of limited values for low-incidence parameters. For the numbers of ribs, percentages are more applicable. Mean and range values are not provided for total numbers of abnormalities or foetuses examined, because the values are not considered helpful for data interpretation.

[035 P10]

Malformations

Major Foetal Abnormality	Incidence of Foetuses (Litters) in Study:										
	1	2	3	4	5	6	7	8	9	10	11
Start Date	Mar-97	Jun-97	Jul-97	Sep-97	Nov-97	Jan-98	Feb-98	Apr-98	Oct-98	Nov-98	Dec-98
Retinal split	0	0	0	0	0	0	0	0	0	0	0
Oropharynx misshapen and/or opening into larynx narrowed	0	0	0	0	0	0	0	0	0	0	0
Rib(s) kinked	0	0	0	0	1 (1)	1 (1)	0	0	1 (1)	2 (2)	2 (2)*
Interventricular septal defect	2 (1)	0	0	0	0	0	0	0	0	0	0
Diaphragmatic hernia	2 (2)	0	0	0	0	0	0	0	0	0	0
Umbilical hernia	0	0	0	0	0	0	0	0	0	0	0
Testes undescended	0	0	0	0	0	0	0	0	0	0	0
Vertebral column terminating in sacral region; tail thread-like	0	0	1 (1)	0	0	0	0	0	0	0	0
Number with major abnormality	4 (3)	0	2 (2)	0	3 (3)	3 (3)	0	0	1 (1)	2 (2)	6 (5)
Total number examined	292 (24)	300 (25)	303 (24)	220 (18)	279 (23)	254 (18)	227 (18)	330 (21)	307 (23)	345 (24)	350 (24)

Major Foetal Abnormality	Incidence of Foetuses (Litters) in Study:								Mean	Range
	12	13	14	15	16	17	18	19		
Start Date	Jan-99	Jun-99	Sep-99	Nov-99	Feb-00	Mar-00	Oct-00	Jan-01		
Retinal split	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Oropharynx misshapen and/or opening into larynx narrowed	0	0	0	0	0	0	0	0	0	0
Rib(s) kinked	3 (3)	0	0	0	1 (1)	0	4 (3)	0	0.8	0-4
Interventricular septal defect	0	0	2 (1)	0	0	0	0	3 (3)	0.4	0-3
Diaphragmatic hernia	0	0	0	0	0	0	0	0	0.1	0-2
Umbilical hernia	0	0	0	0	0	0	0	0	0	0
Testes undescended	0	0	1 (1)	0	0	0	0	0	0.1	0-1
Vertebral column terminating in sacral region; tail thread-like	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Number with major abnormality	5 (5)	0	5 (2)	2 (2)	2 (2)	2 (2)	5 (4)	6 (5)	-	-
Total number examined	391 (22)	245 (19)	241 (20)	355 (24)	364 (23)	338 (24)	284 (21)	294 (24)	-	-

[035 P11, 12]

Minor Skeletal Malformations / Variations

Minor Skeletal Abnormality/Variant	Incidence of Foetuses (Litters) in Study:										
	1	2	3	4	5	6	7	8	9	10	11
Start Date	Mar-97	Jun-97	Jul-97	Sep-97	Nov-97	Jan-98	Feb-98	Apr-98	Oct-98	Nov-98	Dec-98
Small additional ossified area within cranial suture or fontanelle	0	0	0	0	0	2 (2)	0	0	3 (1)	0	1 (1)
Cranial suture minimal deviation from normal course	0	0	0	0	0	0	0	0	0	0	0
Cervical rib(s)	0	0	0	0	0	1 (1)	0	0	3 (1)	1 (1)	0
Sternebra 6 ossified area elongated	1 (1)	0	0	2 (2)	0	0	0	0	0	1 (1)	0
Rib(s) minimally kinked	0	0	0	0	0	0	0	0	0	5 (3)	3 (3)*
Costal cartilage asymmetrically aligned	0	0	0	0	0	0	0	0	0	0	0
Pelvic girdle unilateral caudal displacement	0	0	0	0	0	0	0	0	0	0	0
Caudal vertebrae locally constricted	0	0	0	0	0	0	0	0	0	0	0
Number of Ribs											
13th reduced ribs	0	0	0	0	0	1 (1)	0	1 (1)	0	0	1 (1)
13 complete ribs	153 (24)	130 (25)	134 (24)	98 (18)	125 (21)	109 (19)	95 (18)	144 (21)	131 (23)	146 (24)	151 (24)
14th vestigial supernumerary rib(s)	13 (8)	20 (11)	18 (13)	16 (11)	16 (10)	16 (10)	18 (12)	10 (8)	23 (11)	25 (11)	15 (9)
14th reduced supernumerary ribs	0	0	0	0	0	0	0	0	0	0	0
Number with skeletal abnormality	1 (1)	0	0	2 (2)	0	NR	0	0	2 (2)	8 (8)	2 (2)
Total number examined skeletally	345 (24)	150 (25)	152 (24)	114 (18)	141 (22)	126 (19)	113 (18)	155 (21)	154 (23)	171 (24)	167 (24)

Minor Skeletal Abnormality/Variant	Incidence of Foetuses (Litters) in Study:								Mean	Range
	12	13	14	15	16	17	18	19		
Start Date	Jan-99	Jun-99	Sep-99	Nov-99	Feb-00	Mar-00	Oct-00	Jan-01		
Small additional ossified area within cranial suture or fontanelle	0	1 (1)	0	0	2 (2)	0	0	0	0.4	0-2
Cranial suture minimal deviation from normal course	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Cervical rib(s)	0	3 (3)	0	1 (1)	0	1 (1)	2 (2)	1 (1)	0.6	0-3
Sternebra 6 ossified area elongated	0	0	0	1 (1)	6 (2)	0	0	0	0.6	0-6
Rib(s) minimally kinked	2 (2)*	1 (1)	2 (2)	1 (1)	0	2 (2)	7 (4)	5 (4)	1.5	0-7
Costal cartilage asymmetrically aligned	0	0	0	1 (1)	0	0	1 (1)	0	0.1	0-1
Pelvic girdle unilateral caudal displacement	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Caudal vertebrae locally constricted	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Number of Ribs										
13th reduced ribs	0	0	1 (1)	2 (1)	2 (2)a	0	0	1 (1)	6%	0-13%
13 complete ribs	79 (15)	102 (19)	110 (20)	155 (24)	152 (23)	169 (24)	133 (21)	134 (24)	88%	84-94%
14th vestigial supernumerary rib(s)	35 (11)	19 (10)	10 (7)	20 (10)	26 (10)	9 (5)	13 (7)	15 (7)	12%	5-16%
14th reduced supernumerary rib(s)	0	1 (1)	0	0	0	0	0	0	0%	0-1%
Number with skeletal abnormality	0	5 (5)	2 (2)	5 (5)	10 (5)	3 (3)	10 (7)	9 (7)	-	-
Total number examined skeletally	94 (21)	122 (19)	121 (20)	177 (24)	180 (23)	170 (24)	144 (21)	150 (24)	-	-

a = includes one foetus with 13th vestigial rib(s)
b = one foetus with 12 complete ribs, but major skeletal abnormality

[035 P17, 18]

Minor Visceral Malformations / Variations

Minor Visceral Abnormality/Variant	Incidence of Foetuses (Litters) in Study:										
	1	2	3	4	5	6	7	8	9	10	11
Start Date	Mar-97	Jan-97	Jul-97	Sep-97	Nov-97	Jan-98	Feb-98	Apr-98	Oct-98	Nov-98	Dec-98
Subcutaneous haemorrhage:											
Head	3 (2)	0	5 (5)	0	7 (6)	6 (5)	0	11 (8)	8 (6)	12 (9)	8 (5)
Trunk (includes haemorrhages within fat pads and muscular tissue)	0	0	0	1 (1)	1 (1)	0	3 (3)	2 (1)	5 (4)	3 (3)	4 (3)
Limbs	0	0	1 (1)	1 (1)	0	0	0	1 (1)	2 (2)	0	1 (1)
Tail (annular)	0	0	0	0	1 (1)	0	0	0	0	0	0
Extradural haemorrhage	0	0	0	0	0	0	0	0	0	0	0
Subdural haemorrhage	0	0	1 (1)	0	0	0	0	0	0	0	0
Lateral brain ventricles minimally dilated	0	0	0	0	0	0	1 (1)	1 (1)	0	0	3 (3)
Olfactory brain ventricles minimally dilated	0	0	0	0	0	0	0	0	0	0	0
Pituitary gland small cavity	0	0	0	0	0	0	0	0	0	0	0
Minimal haemorrhage aqueous chamber of eye	0	0	0	0	0	0	0	0	0	1 (1)	0
Eye(s) enlarged	0	0	0	0	0	0	0	0	0	0	0
Eye(s) reduced (in size)	0	0	0	0	1 (1)	0	0	0	0	0	7 (7)
Eye(s) oval (in shape)	0	0	0	0	0	0	0	0	0	10 (2)	0
Thyroid reduced (in size)	0	0	1 (1)	0	1 (1)	0	0	1 (1)	0	0	1 (1)
Cervical remnant of thymus	NR	NR	NR	NR	NR	NR	NR	NR	NR	12 (8)	11 (7)
Innominate artery absent	0	0	0	1 (1)	0	0	0	0	0	0	0
Innominate artery lengthened	0	0	0	0	0	0	0	0	0	0	0
Origins of arteries arising from aortic arch displaced	0	0	0	0	0	0	0	0	0	0	0
Aortic arch lumen minimally misshapen	0	0	0	0	0	0	0	0	0	0	0
Ductus arteriosus narrowed	0	0	0	0	0	0	0	0	0	0	0
Azygos vein narrowed	0	0	0	0	0	0	0	0	0	0	0
Azygos vein displaced (includes right-sided)	0	0	0	0	0	0	0	0	0	0	0
Heart minimal abnormal rotation	0	0	0	0	0	0	0	0	0	0	0
Small interventricular septal defect	2 (2)	0	0	0	0	0	0	0	1 (1)	1 (1)	0
Lung lobes fused	0	0	0	0	0	0	0	0	0	0	0

Minor Visceral Abnormality/Variant	Incidence of Foetuses (Litters) in Study:								Mean	Range
	12	13	14	15	16	17	18	19		
Start Date	Jan-99	Jun-99	Sep-99	Nov-99	Feb-00	Mar-00	Oct-00	Jan-01		
Subcutaneous haemorrhage:										
Head	13 (6)	0	5 (2)	4 (3)	5 (4)	8 (5)	2 (2)	5 (3)	5.4	0-13
Trunk (includes haemorrhages within fat pads and muscular tissue)	5 (4)	0	4 (2)	4 (4)	0	2 (2)	3 (3)	1 (1)	2.0	0-5
Limbs	1 (1)	0	2 (2)	0	0	0	0	0	0.5	0-2
Tail (annular)	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Extradural haemorrhage	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Subdural haemorrhage	0	0	0	0	0	0	0	0	0.1	0-1
Lateral brain ventricles minimally dilated	0	0	1 (1)	1 (1)	0	0	0	3 (3)	0.5	0-3
Olfactory brain ventricles minimally dilated	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Pituitary gland small cavity	0	0	0	0	0	0	0	0	0	0
Minimal haemorrhage aqueous chamber of eye	1 (1)	0	0	0	0	1 (1)	0	2 (2)	0.3	0-2
Eye(s) enlarged	0	0	0	5 (3)	0	5 (5)	1 (1)	2 (2)	0.7	0-5
Eye(s) reduced (in size)	1 (1)	0	0	1 (1)	0	0	0	0	0.5	0-7
Eye(s) oval (in shape)	0	0	9 (2)	2 (2)	0	0	1 (1)	1 (1)	1.2	0-10
Thyroid reduced (in size)	1 (1)	1 (1)	0	1 (1)	1 (1)	0	1 (1)	2 (2)	0.6	0-2
Cervical remnant of thymus	20 (18)	3 (2)	11 (6)	12 (8)	12 (10)	13 (10)	14 (10)	15 (10)	12.3	3-20
Innominate artery absent	0	0	0	0	0	0	0	4 (3)	0.5	0-4
Innominate artery lengthened	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Origins of arteries arising from aortic arch displaced	0	0	0	2 (2)	0	0	0	0	0.1	0-2
Aortic arch lumen minimally misshapen	0	0	0	0	0	0	0	0	0	0
Ductus arteriosus narrowed	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Azygos vein narrowed	0	0	0	0	0	2 (1)	0	0	0.1	0-2
Azygos vein displaced (includes right-sided)	0	0	0	0	0	0	0	2 (2)	0.1	0-2
Heart minimal abnormal rotation	0	0	2 (1)	1 (1)	0	0	0	0	0.2	0-2
Small interventricular septal defect	0	0	2 (1)	1 (1)	0	0	0	0	0.4	0-2
Lung lobes fused	0	0	0	1 (1)	0	0	0	0	0.1	0-1

[035 P13, 15]

Minor Visceral Abnormality/Variant	Incidence of Foetuses (Litters) in Study:										
	1	2	3	4	5	6	7	8	9	10	11
Start Date	Mar-97	Jun-97	Jul-97	Sep-97	Nov-97	Jan-98	Feb-98	Apr-98	Oct-98	Nov-98	Dec-98
Minimal protrusion of median liver lobe with thinning diaphragm	a	a	a	a	a	a	a	a	a	6 (4)	a
Protrusion of median liver lobe with thinning diaphragm	4 (4)	9 (5)	7 (5)	7 (4)	7 (6)	8 (6)	9 (6)	34 (7)	39 (10)	5 (3)	12 (9)
Additional liver lobe within median cleft	0	0	0	0	0	0	0	0	1 (1)	4 (3)	1 (1)
Liver mottled	0	0	0	0	0	0	0	0	0	1 (1)	0
Hepatic haemorrhage	2 (2)	1 (1)	1 (1)	2 (2)	1 (1)	0	0	1 (1)	1 (1)	0	0
Intra-abdominal haemorrhage	2 (2)	1 (1)	0	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	3 (2)	0	3 (3)
Spleen misshapen	0	0	0	0	0	0	0	0	0	3 (1)	0
Kidney(s) displaced	0	0	0	0	0	0	0	0	0	0	0
Renal pelvises dilated	1 (1)	1 (1)	4 (3)	1 (1)	2 (1)	1 (1)	2 (2)	0	6 (4)	0	0
Ureter(s) dilated	1 (1)	1 (1)	8 (7)	4 (3)	3 (3)	2 (1)	11 (6)	2 (2)	9 (6)	5 (3)	4 (4)
Testisses displaced	2 (2)	1 (1)	5 (5)	0	2 (2)	6 (6)	4 (4)	5 (5)	5 (4)	6 (5)	6 (6)
Umbilical artery dilated	0	0	0	0	0	0	0	0	0	0	0
Umbilical artery left-sided	0	0	0	0	0	0	0	0	0	0	3 (3)
Small foetus	NR	NR	NR	NR	NR	NR	NR	NR	NR	1 (1)	NR
Visceral - probable fixation artefact											
Subdural space (brain/spinal cord) increased	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Subcutaneous space(s)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Number with minor visceral abnormality/variant	10 (5)	14 (8)	27 (19)	20 (10)	23 (11)	NR	28 (14)	56 (16)	48 (19)	58 (21)	62 (22)
Number examined by Wilson sectioning	146 (24)	330 (25)	151 (24)	116 (18)	138 (23)	113 (18)	114 (18)	155 (21)	153 (23)	174 (24)	169 (24)
Total number examined viscerally	202 (24)	300 (25)	303 (24)	250 (18)	279 (23)	229 (18)	227 (18)	330 (21)	307 (23)	345 (24)	338 (24)

a = The grading "minimal" was not used in these studies; it is not possible to state whether all finding were reported ungraded, or if the minimal findings were considered as being within normal limits

Minor Visceral Abnormality/Variant	Incidence of Foetuses (Litters) in Study:								Mean	Range
	12	13	14	15	16	17	18	19		
Start Date	Jan-99	Jun-99	Sep-99	Nov-99	Feb-00	Mar-00	Oct-00	Jan-01		
Minimal protrusion of median liver lobe with thinning diaphragm	4 (4)	3 (3)	2 (2)	14 (10)	9 (8)	8 (4)	11 (8)	6 (5)	7.0	2-14
Protrusion of median liver lobe with thinning diaphragm	4 (4)	5 (4)	10 (4)	13 (7)	7 (6)	8 (8)	8 (7)	3 (2)	8.4	3-19
Additional liver lobe within median cleft	1 (1)	12 (7)	20 (13)	27 (17)	21 (10)	20 (12)	24 (15)	34 (18)	8.7	0-34
Liver mottled	0	0	0	0	0	0	0	0	0.1	0-1
Hepatic haemorrhage	0	0	1 (1)	0	0	0	0	0	0.5	0-2
Intra-abdominal haemorrhage	3 (2)	1 (1)	2 (2)	2 (2)	0	0	1 (1)	0	1.2	0-3
Spleen misshapen	0	0	0	0	1 (1)	0	0	0	0.2	0-3
Kidney(s) displaced	0	0	0	0	0	1 (1)	4 (3)	2 (2)*	0.4	0-4
Renal pelvises dilated	3 (3)	2 (2)	0	4 (2)	5 (4)	1 (1)	1 (1)	0	1.8	0-6
Ureter(s) dilated	6 (6)	1 (1)	7 (3)	10 (6)	17 (8)	2 (2)	0	1 (1)	4.9	0-17
Testisses displaced	3 (3)	5 (5)	5 (5)	2 (2)	4 (4)	3 (3)	5 (5)	6 (6)	3.8	0-6
Umbilical artery dilated	0	0	0	2 (2)	0	0	0	0	0.1	0-2
Umbilical artery left-sided	0	3 (3)	3 (2)	5 (3)	7 (5)	3 (3)	3 (3)	7 (7)	1.7	0-7
Small foetus	4 (2)	2 (2)	17 (2)	1 (1)	3 (2)	2 (2)	0	2 (2)	3.6	0-17
Visceral - probable fixation artefact										
Subdural space (brain/spinal cord) increased	NR	NR	NR	3 (3)	NR	NR	NR	NR	-	-
Subcutaneous space(s)	NR	NR	NR	6 (4)	NR	NR	NR	NR	-	-
Number with minor visceral abnormality/variant	58 (21)	37 (15)	77 (17)	89 (23)	83 (23)	67 (23)	73 (21)	78 (23)	-	-
Number examined by Wilson sectioning	97 (22)	123 (19)	120 (20)	178 (24)	184 (23)	168 (24)	140 (21)	144 (24)	-	-
Total number examined viscerally	191 (22)	245 (19)	241 (20)	355 (24)	364 (23)	338 (24)	284 (21)	294 (24)	-	-

[035 P14, 16]

Appendix D: Rabbit Embryofetal Historical Control Data from ——— b(4)

Control group data from the definitive study of liraglutide embryofetal toxicity is included as study 6.

NR = not reported (but zero may be the correct value).

For the means and ranges, the values generally refer to absolute incidences, not percentages, because percentages are of limited values for low-incidence parameters. For the numbers of ribs, percentages are more applicable. Mean and range values are not provided for total numbers of abnormalities or foetuses examined, because the values are not considered helpful for data interpretation.

Malformations

Major Foetal Abnormality	Incidence of Foetuses (Litters) in Study:											Mean	Range
	1	2	3	4	5	6	7	8	9	10	11		
Start Date	Apr-95	May-95	Oct-95	Jun-96	Jan-97	Mar-99	Feb-00	Jun-00	Sep-00	Mar-01	Mar-01		
Hydrocephaly	0	0	0	0	0	0	0	0	0	0	0	0	0
Gross disorganisation of cranial/facial bones	0	0	0	0	0	0	1 (1)	0	0	1 (1)	0	0.2	0-1
Large unossified area in both parietals	0	0	0	0	1 (1)	0	1 (1)	0	0	0	0	0.2	0-1
Fused frontals	0	0	0	0	0	0	1 (1)	0	0	0	0	0.1	0-1
Connected parietals	0	0	0	1 (1)*	1 (1)*	0	1 (1)	0	0	0	1 (1)	0.4	0-1
Microphthalmia with/without retinal fold	0	0	0	0	0	0	0	0	0	0	0	0	0
Narrow ascending aorta	0	0	0	0	0	1 (1)	0	0	0	0	0	0.1	0-1
Retroesophageal right subclavian artery	3 (2)	2 (1)	0	0	0	2 (2)	0	0	0	0	0	0.6	0-3
Dilated ascending aorta, narrow/threadlike pulmonary trunk	0	0	2 (1)	0	0	1 (1)	0	0	0	0	1 (1)	0.4	0-2
Multiple vertebral irregularities with fused ribs	0	0	0	0	0	0	3 (3)	0	0	0	1 (1)	0.3	0-3
Dilated pulmonary trunk, incomplete aortic arch, malformed heart	0	0	0	0	0	0	0	0	0	0	0	0	0
Forelimb flexure	0	1 (1)	0	0	0	0	1 (1)	0	0	0	1 (1)	0.2	0-1
Curved scapula	0	0	0	0	0	0	0	0	0	0	0	0	0
Split sternum	0	0	1 (1)	0	0	0	0	0	0	0	0	0.1	0-1
Right kidney represented by small area of tissue with cyst attached	0	0	0	0	0	0	0	0	0	0	0	0	0
Hepatic duct diverticulum	0	0	0	0	0	0	0	0	0	0	0	0	0
Displaced umbilicus/umbilical hernia, gut of intestine adhered to umbilical vein	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachyury	0	0	0	0	0	0	0	0	0	0	0	0	0
Number with major abnormality	3 (2)	3 (2)	5 (2)	2 (2)	4 (4)	3 (3)	7 (4)	0	1 (1)	2 (2)	7 (5)	-	-
Total number examined	158 (16)	178 (19)	112 (14)	179 (20)	116 (15)	138 (17)	140 (18)	123 (15)	159 (18)	165 (20)	183 (23)	-	-

[035 P20]

Minor Visceral Malformations / Variations

Minor Visceral Abnormality/Variant	Incidence of Foetuses (Litters) in Study:											Mean	Range
	1	2	3	4	5	6	7	8	9	10	11		
Start Date	Apr-95	May-95	Oct-95	Jun-96	Jan-97	Mar-99	Feb-00	Jun-00	Sep-00	Mar-01	Mar-01		
Dilated lateral ventricles	0	0	0	0	0	1 (1)	0	1 (1)	0	0	0	0.2	0-1
Corneal opacity	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyst on right side of oesophagus	0	0	0	0	0	0	0	0	0	0	0	0	0
Variation in origin of minor arteries arising from aortic arch	3 (3)	5 (4)	1 (1)	0	0	6 (4)	25 (16)	3 (3)	12 (5)	11 (8)	14 (8)	7.1	0-23
Lungs unexpanded	0	0	0	0	0	1 (1)	0	0	0	0	0	0.1	0-1
Absent intermediate lung lobe	0	0	0	0	0	0	0	0	0	0	0	0	0
Additional lobe of liver within median cleft	0	0	0	0	0	0	0	0	0	0	0	0	0
Intestines and bladder distended	0	0	0	0	0	0	0	0	0	0	0	0	0
Bilobed or bifurcated gall bladder	0	0	5 (3)	0	0	0	4 (2)	0	2 (2)	1 (1)	0	1.1	0-5
Dilated ureter	0	0	0	0	0	0	0	0	0	0	0	0	0
Cystic ovary	0	0	0	0	0	0	0	0	1 (1)	1 (1)	0	0.2	0-1
Kinked tail	0	0	0	0	0	0	0	0	0	0	0	0	0
Number with visceral abnormality	3 (3)	5 (4)	6 (4)	0	0	8 (6)	25 (16)	3 (3)	12 (5)	11 (8)	14 (8)	-	-
Total number examined	158 (16)	178 (19)	112 (14)	179 (20)	116 (15)	138 (17)	140 (18)	123 (15)	159 (18)	165 (20)	183 (23)	-	-

[035 P21]

Minor Skeletal Malformations / Variations

Minor Skeletal Abnormality/Variant	Incidence of Fetuses (Litters) in Study:											Mean	Range
	1	2	3	4	5	6	7	8	9	10	11		
Study Date	Apr-95	May-95	Oct-95	Jan-96	Jan-97	Mar-99	Feb-00	Jun-00	Sep-00	Mar-01	Mar-01		
Sutural bone	2 (2)	1 (1)	4 (2)	6 (4)	1 (1)	2 (2)	5 (4)	0	1 (1)	7 (4)	1 (1)	2.7	0-7
Ossification irregularity/unossified area	2 (2)	0	2 (2)	1 (1)	1 (1)	1 (1)	9 (6)	5 (3)	3 (3)	7 (5)	9 (7)	3.6	0-9
Curve(s) of hyoid bent in/outward	7 (4)	6 (4)	8 (5)	1 (1)	6 (6)	4 (3)	10 (5)	4 (4)	5 (5)	13 (6)	7 (7)	6.5	1-13
Connected/fused jugal(s) to maxilla	6 (3)	2 (2)	2 (2)	6 (5)	4 (4)	5 (5)	6 (4)	5 (1)	3 (2)	2 (1)	2 (2)	3.5	2-6
Additional area of ossification ventral to 1st cervical centrum	0	0	1 (1)	0	0	0	0	0	0	0	0	0.1	0-1
Cervical rib	1 (1)	1 (1)	0	6 (4)	0	12 (6)	12 (7)	1 (1)	5 (3)	3 (3)	4 (3)	4.1	0-12
Superior angle of laminae) of axis incompletely ossified/unossified	0	1 (1)	0	0	0	0	0	0	0	0	0	0.1	0-1
Hemicentric vertebra	0	1 (1)	1 (1)	1 (1)	1 (1)	0	0	0	0	0	0	0.4	0-1
Interrupted rib	0	1 (1)	0	0	0	0	0	0	0	0	0	0.1	0-1
Fused/connected sternebra(e) with/without slight dorsal ventral dislocation of sternum	4 (4)	0	4 (3)	3 (3)	2 (2)	3 (2)	4 (3)	1 (1)	0	2 (2)	1 (1)	2.0	0-4
Asymmetric alignment/floating/additional costal cartilage element	0	0	1 (1)	1 (1)	0	3 (3)	2 (2)	0	0	1 (1)	5 (5)	1.2	0-5
Additional sternebral centre anterior to 1st	2 (2)	0	0	2 (2)	0	2 (2)	1 (1)	0	2 (1)	1 (1)	1 (1)	1.0	0-2
Vertebral arch defect(s)	0	0	0	0	0	1 (1)	0	1 (1)	0	0	0	0.2	0-1
Slight upward pelvic shift	0	0	NR	1 (1)	NR	15 (5)	NR	NR	NR	NR	NR	4.0	0-15
Slight downward pelvic shift	0	0	NR	1 (1)	NR	2 (2)	NR	NR	NR	NR	NR	0.8	0-2
Slight asymmetric alignment of pelvic bones	0	1 (1)	NR	0	NR	0	NR	NR	NR	NR	NR	0.3	0-1
One extra pre-sacral vertebra	0	0	0	1 (1)	0	0	8 (5)	10 (6)	25 (8)	13 (7)	25 (11)	7.3	0-25
Misaligned caudal vertebra	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of Ribs													
12 Complete ribs	96 (36)	121 (19)	27 (10)	103 (19)	35 (10)	86 (16)	71 (36)	59 (12)	76 (15)	97 (20)	101 (22)	33%	5-64%
Vestigial supernumerary rib(s) on 13th thoracic vertebra	19 (12)	10 (8)	8 (7)	20 (11)	13 (9)	31 (6)	18 (10)	19 (12)	15 (6)	24 (15)	22 (13)	18%	6-15%
Reduced supernumerary rib(s) on 13th thoracic vertebra	15 (7)	27 (12)	17 (8)	22 (11)	9 (6)	12 (8)	13 (8)	21 (10)	7 (7)	9 (6)	18 (13)	13%	5-13%
Complete supernumerary rib(s) on 13th thoracic vertebra	21 (9)	20 (8)	40 (12)	36 (12)	61 (12)	29 (11)	38 (13)	24 (9)	41 (8)	34 (12)	41 (19)	27%	11-54%
Number with skeletal abnormality	20 (9)	14 (9)	25 (11)	25 (14)	15 (11)	37 (13)	53 (15)	34 (13)	71 (16)	71 (18)	80 (20)	-	-
Total number examined	158 (16)	178 (19)	182 (14)	179 (20)	116 (15)	138 (17)	140 (18)	123 (15)	139 (18)	166 (20)	183 (23)	-	-

[035 P22]

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Anthony L Parola
7/10/2009 03:49:57 PM
PHARMACOLOGIST

Karen Davis-Bruno
7/10/2009 03:59:57 PM
PHARMACOLOGIST
see supervisor memo

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-341 (IND 61,040)
Sponsor and/or agent: Novo Nordisk Inc., 100 College Road West, Princeton, NJ 08540
Manufacturer for drug substance: Novo Nordisk A/S Hallas Allé, DK-4400 Kalundborg, Denmark

Reviewer name: Anthony L Parola, PhD
Division name: Metabolic and Endocrine Products
HFD #: 510
Review completion date: December 4, 2008 (draft), February 12, 2009 (revised)

Drug:

Trade name: Victoza®
Generic name: liraglutide
Code name: NNC 90-1170, NN2211
Chemical name: $Arg^{34}Lys^{26}$-(N-ε-(γ-Glu (N-α-hexadecanoyl)))-GLP-1[7-37]
CAS registry number: 204656-20-2
Molecular formula/molecular weight: $C_{172}H_{265}N_{43}O_{51}$: 3,751.20 Da
Structure: hygroscopic white powder highly soluble in water at pH > 7 (270 mg/mL) with decreased solubility at lower pH (0.05 mg/mL at pH 4 – 5).

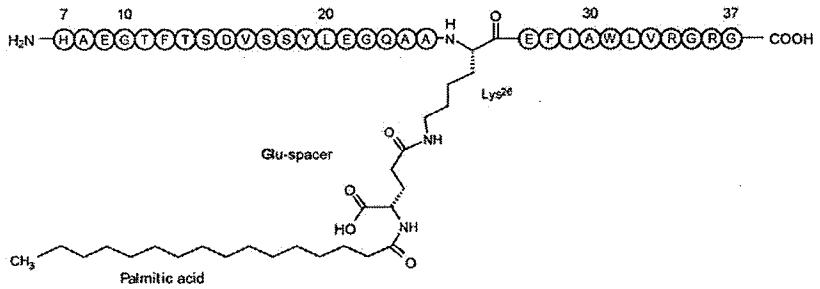


Figure 3–1 The Chemical Structure of Liraglutide.
[N000 IB P17]

Drug class: GLP-1 receptor agonist
Intended clinical population: Type 2 diabetics
Clinical formulation: solution for injection
Route of administration: subcutaneous
Sponsor's maximum recommended human dose: 1.8 mg/day (Cmax 36 nM, AUC_{0-24} 816 nM.h)

Background:

Liraglutide, a palmitoylated recombinant human GLP-1 analog with a prolonged elimination half-life due to increased peptidase resistance of the highly protein bound drug, is being developed to treat type 2 diabetes under IND 61,040. In 2 year repeat subcutaneous dose carcinogen bioassays in rats and mice, liraglutide was a non-genotoxic carcinogen increasing the incidence of thyroid c-cell tumors in rats and mice and fibrosarcomas on the dorsal surface, the body surface for drug administration, in male mice. The sponsor performed mechanistic studies to evaluate the human relevance of thyroid c-cell tumor findings. Mechanistic studies were reviewed and presented to the Executive Carcinogenicity Committee at a meeting on 9 December 2008 for concurrence with the Division's recommendation that the lack of clinical relevance of liraglutide-induced thyroid c-cell tumors in rodents was not supported by mechanistic studies.

Effect of Liraglutide on Thyroid Parameters in Humans

Because liraglutide caused proliferative lesions of thyroid c-cells in rats and mice, the sponsor monitored thyroid parameters in clinical studies. Plasma calcitonin was measured in several clinical trials and a calcium-stimulated calcitonin test was performed on a subgroup of subjects in 2 long term studies (1573 and 1574). Thyroid biochemistry parameters were monitored in 8 clinical studies and thyroid structure, determined by ultrasonography, was monitored in 4 studies. A search of adverse events related to calcitonin or thyroid was performed across 38 completed and 4 ongoing clinical studies. The sponsor evaluated GLP-1 receptor radioligand binding, second messenger coupling, and calcitonin secretion in a human thyroid C-cell line, TT cells.

Thyroid

In completed clinical trials, the rate of total, serious, and non-serious thyroid adverse events (Table 2-16, number of events divided by subject years of exposure multiplied by 1000) was higher in liraglutide treated subjects (event rates of 35.7, 4.5, and 31.2 for total, serious, and non-serious AEs, respectively) compared to non-liraglutide-treated subjects (placebo or comparator, event rates of 22.0, 0.9, and 21.1 for total, serious, and non-serious AEs, respectively).

Table 2–16 Summary of Treatment Emergent Thyroid AEs - All Completed Trials - Safety Analysis Set

	Liraglutide	Non-liraglutide
Safety Analysis Set	4211	2272
Total Exposure (yrs)	2241.4	1138.6
Number of Subjects with Serious Thyroid Adverse Events (events)	7 (10)	1 (1)
Number of Subjects with Non-serious Thyroid Adverse Events (events)	57 (70)	(24) 24
Total Number of Subjects with Thyroid Adverse Events (events)	61 (80)	24 (25)
Rate of Thyroid Serious Adverse Events (R)	4.5	0.9
Rate of Thyroid Non-serious Adverse Events (R)	31.2	21.1
Rate of All Thyroid Adverse Events (R)	35.7	22.0

R: Number of events divided by subject years of exposure multiplied by 1000

[N000 Module 2.5 P107]

Table 2-20 shows thyroid adverse events that occurred after at least 1 dose in clinical studies. Thyroid adverse events that increased in liraglutide-treated clinical trial subjects were goiter, hyperthyroidism, thyroid cyst, and thyroid disorder. The incidence of treatment-emergent benign and malignant thyroid neoplasms was higher in liraglutide-treated compared to non-liraglutide treated subjects. The incidence of papillary thyroid tumors were notably higher in liraglutide-treated subjects with the earliest onset occurring within 50 days of initiating treatment

with 1.8 mg liraglutide + metformin + rosiglitazone (Table 2-23). Despite dose-related elevated plasma calcitonin levels in liraglutide-treated subjects, thyroid c-cell tumors were not noted in liraglutide-treated subjects.

Table 2–20 Treatment Emergent AEs - Thyroid (AE Onset Date after First Drug Date) - by SOC and Preferred Term - All Completed Trials - Safety Analysis Set

	Liraglutide				Non-Liraglutide			
	N	(%)	E	R	N	(%)	E	R
Safety Analysis Set	4211				2272			
Total Exposure (yrs)	2241.4				1138.6			
All Adverse Events related to Thyroid	46	(1.1)	62	27.7	19	(0.8)	19	16.7
Endocrine Disorders	19	(0.5)	24	10.7	7	(0.3)	7	6.1
Goitre	13	(0.3)	14	6.2	1	(0.0)	1	0.9
Hypothyroidism	3	(0.1)	3	1.3	4	(0.2)	4	3.5
Hyperthyroidism	2	(0.0)	2	0.9	0	(0.0)	0	0.0
Thyroid Cyst	2	(0.0)	2	0.9	0	(0.0)	0	0.0
Thyroid Disorder	2	(0.0)	2	0.9	0	(0.0)	0	0.0
Autoimmune Thyroiditis	1	(0.0)	1	0.4	2	(0.1)	2	1.8
Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)	19	(0.5)	22	9.8	4	(0.2)	4	3.5
Thyroid Neoplasm	15	(0.4)	16	7.1	4	(0.2)	4	3.5
Papillary Thyroid Cancer	4	(0.1)	4	1.8	0	(0.0)	0	0.0
Benign Neoplasm Of Thyroid Gland	1	(0.0)	1	0.4	0	(0.0)	0	0.0
Parathyroid Tumour Benign	1	(0.0)	1	0.4	0	(0.0)	0	0.0
Investigations	14	(0.3)	16	7.1	8	(0.4)	8	7.0
Blood Calcitonin Increased	10	(0.2)	11	4.9	6	(0.3)	6	5.3
Blood Thyroid Stimulating Hormone Incr	2	(0.0)	3	1.3	1	(0.0)	1	0.9
Blood Calcitonin Abnormal	1	(0.0)	1	0.4	0	(0.0)	0	0.0
Thyroxine Decreased	1	(0.0)	1	0.4	0	(0.0)	0	0.0
Blood Thyroid Stimulating Hormone Decr	0	(0.0)	0	0.0	1	(0.0)	1	0.9

N: Number of Subjects with adverse events
%: Proportion of subjects in analysis set having adverse events
E: Number of adverse events
R: Number of events divided by Subject years of exposure multiplied by 1000

[N000 Module 2.5 P108]

Table 2–23 Treatment Emergent Adverse Events of Papillary Thyroid Cancer – All Completed Trials and Ongoing Trials

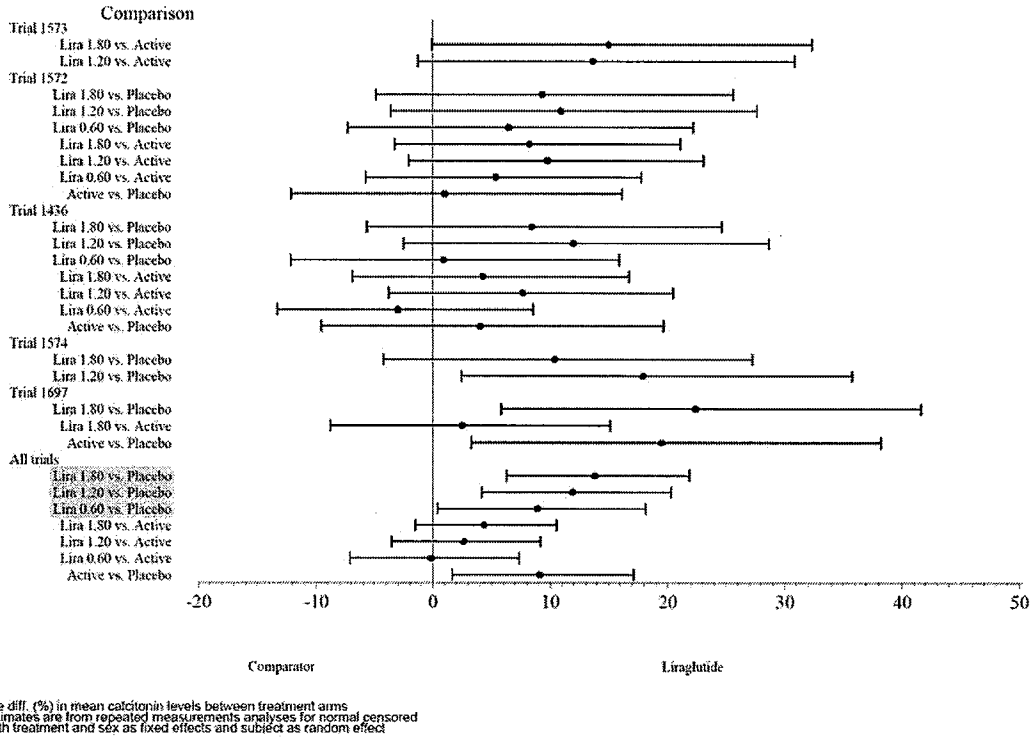
Trial	Subject ID	Age Yrs/Gender	Treatment	Preferred Term [MedDRA] (Relationship) (Outcome) (severity)	Duration of Therapy at Onset	Duration of Event
1334	16004	70/F	Liraglutide 0.6 mg	Papillary thyroid cancer (U) (R*) (mild)	99 days	NA
1573	261006	62/F	Liraglutide 1.2 mg	Thyroid disorder (P) (R) (moderate) Papillary thyroid cancer (P) (R) (moderate) Benign neoplasm of the thyroid gland (P) (R)	356 days	113 days
1436	506001	59/M	Liraglutide 1.8 mg+glimepiride	Papillary thyroid cancer Possible (liraglutide) Unlikely (glimepiride) (R) (moderate)	175 days	149 days
1574	326016	53/F	Liraglutide 1.8 mg+metformin+ rosiglitazone	Goitre (U) (R) (mild) Papillary thyroid cancer (U) (R) (moderate)	22 days 50 days	30 days 63 days
	326008	59/M	Metformin+rosiglitazone	Papillary thyroid cancer (U) (R) (moderate)	1 day	91 days

*Updated based on information received after end of trial (Appendix 7.4, Listing 5)
Gender: M=male and F=female
Relationship: P=evaluated as possibly or probably related by investigator, U=unlikely related
Outcome: R=recovered, NR=not recovered

[N000 Module 2.5 P115]

Calcitonin

A Forest plot of individual clinical trials and pooled results from week 26/28 of long term clinical trials showed significant, dose-dependent increased plasma calcitonin compared to placebo at all liraglutide doses, but no significant difference between liraglutide and active comparator at any dose.



Cross-reference: Appendix 7.3, Figure 84

Figure 3–9 Forest plot of Calcitonin Continous Analysis – Week 26/28 – All Long-term Trials - Safety analysis set

[N000 Module 2.5 P192]

At week 52, calcitonin was significantly higher than placebo at both 1.2 or 1.8 mg/day liraglutide, and calcitonin was significantly higher than active comparator at 1.8 mg/day (Figure 85 in Appendix 7.3 not found, but the report containing the appendix wasn't hyperlinked. Data from clinical trial 1573.)

A calcium stimulated calcitonin test was performed in a subset of subjects from long-term clinical studies 1573 (90 subjects) and 1574 (54 subjects). There were no significant differences in calcium-stimulated calcitonin secretion between comparator or liraglutide (1.2 or 1.8 mg/day) groups prior to initiating treatment or after 52 weeks of treatment.

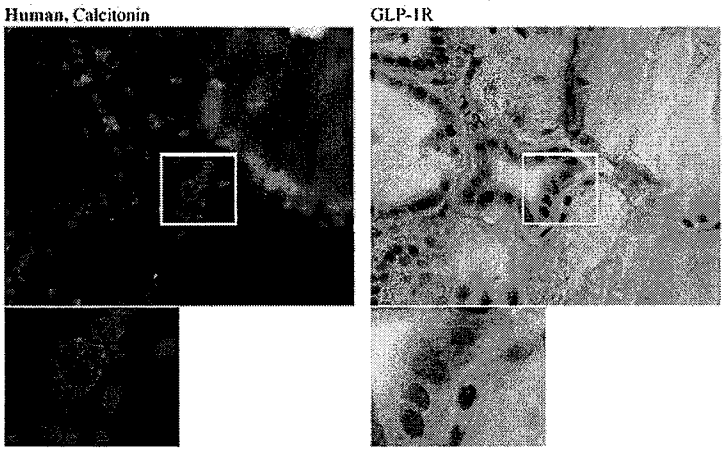
GLP-1 Receptors in Human Thyroid C-cells

Autoradiography using ^{125}I-GLP-1(7–36)amide in thyroid tissue slices showed GLP-1 receptors occurred in thyroid of 1 of 18 humans, compared to 60% of thyroids from mice and 100% of thyroids from rats (Table 3) (Körner M et al., . J Nucl Med(2007) 48: 736–743). Although specific thyroid cell types binding ^{125}I-GLP-1(7–36)amide were not identified, the

TABLE 1
GLP-1 Receptor Incidence and Density in Human Tumors

[Körner M et al., . J Nucl Med 48: 736–743, 2007]

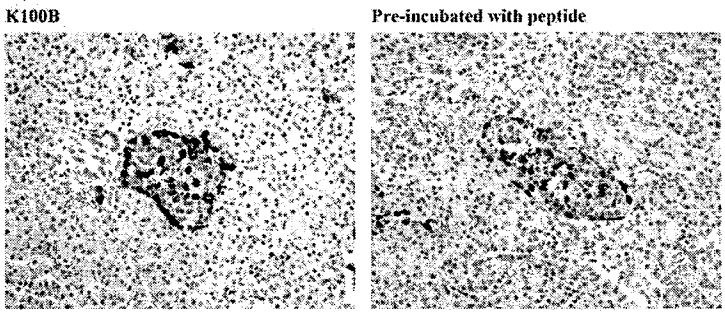
In study report 204370, colocalization of GLP-1 receptors and calcitonin immunoreactivity in human thyroid tissue were equivocal for GLP-1 receptor because the specificity of the rabbit anti-human GLP-1 receptor antibody, K100B, was not adequately demonstrated (staining only partially blocked by preabsorption with the antigenic peptide, antibody stains pancreatic islets in GLP-1 receptor knockout mice) and staining was weak and not always colocalized with calcitonin (see Figure below).



[N000 4.2.3.7.3 P20]

K100B strongly stained islets from pancreatic tissue, but preabsorption of K100B with the antigenic peptide only partially blocked staining (Figure 12, below).

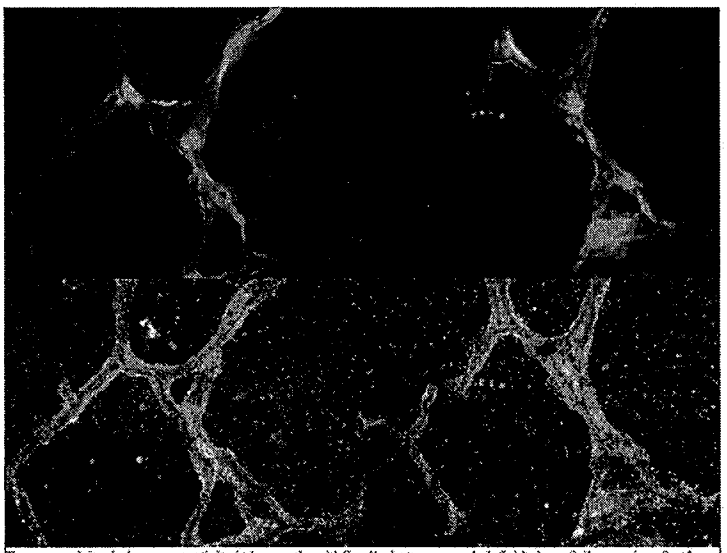
Figure 12 Pancreas serial sectioned, human



[N000 4.2.3.7.3 P30]

In situ hybridization of species specific ^{35}S-labeled riboprobes to GLP-1 receptor mRNA was evaluated in paraffin-embedded thyroid tissue sections from humans (study 20040515PR4). Thyroid c-cells were identified by indirect fluorescent microscopy after staining with an Alexa488-coupled anti-calcitonin antibody. In situ hybridization to pancreatic islets served as a positive control for GLP-1 receptor probes and hybridization of a ^{35}S-labeled riboprobes to calcitonin served as a control for mRNA quality in thyroid tissue. A ^{35}S-labeled probe to cyclophilin, a low to medium abundance transcript, served as a addition control for mRNA quality in samples of thyroid and pancreas.

GLP-1 receptor mRNA was not detectable in thyroid c-cells identified by anti-calcitonin antibody staining and Figure 6 shows colocalization of GLP-1 receptor mRNA and calcitonin was not compelling (Figure 6). Anti-sense human GLP-1 receptor probes labeled human pancreatic islet cells and an antisense calcitonin probe labeled calcitonin immunoreactive cells in human thyroid tissue sections (data not shown)



Top row: calcitonin immunoreactivity in human thyroid C-cells; bottom row: dark field view of silver grains after 5 weeks exposure of emulsion. Left column: in-situ hybridisation with antisense riboprobe to hGLP-1R mRNA; right column: in-situ hybridisation with sense riboprobe to hGLP-1R mRNA.

Figure 6 Human thyroid GLP-1R mRNA co-localisation with C-cell calcitonin

[N000 4.2.3.7.3 P20]

GLP-1 Receptors in Human Thyroid C-cell Lines

The human thyroid C-cell line, TT, was devoid of functional GLP-1 receptors coupled to calcitonin secretion, but cAMP-coupled receptor pathways may be perturbed in this cell line. In TT cells, forskolin, a direct activator of cAMP, increased calcitonin secretion, but pentagastrin, a potent human and rat calcitonin secretagogue, did not. Furthermore, known TT cell mitogens pentagastrin and epidermal growth factor did not stimulate TT cell mitosis. More detailed reviews of studies characterizing GLP-1 receptors in human TT cells are included in reviews of studies characterizing GLP-1 receptor signaling in rat c-cell lines in Section 2.6.6.8 because rat and human cell lines experiments were included in the same reports.

Western blotting of TT cell proteins after separation by SDS-PAGE using the anti-GLP-1 receptor polyclonal antibody K102B did not identify the GLP-1 receptor protein, but the results are not valid because the specificity of the K102B antibody was not adequately demonstrated (study report 205218). TT cell GLP-1 receptor transcript levels were very low to undetectable by real-time PCR with estimated transcript levels of 1 GLP-1 receptor transcript / 1000 beta actin transcripts (report 204415).

Although the sponsor claims specific binding of radiolabeled, but not fluorescent GLP-1(7-37) was demonstrated in human TT cells, specific ligand binding wasn't demonstrated in either study (study report 1425-006 and 205088 using [^{125}I]GLP-1(7-37) or GLP-1(7-36)-Lys(6-FAM), respectively).

GLP-1 receptor agonists liraglutide or exenatide didn't stimulate intracellular cAMP accumulation or calcitonin secretion from TT cells, but the positive control forskolin did. In vivo in humans, pentagastrin stimulates CCK_2 receptor mediated calcitonin release, but pentagastrin had no effect on cAMP accumulation or calcitonin secretion from TT cells (study report 13737-025). Micromolar concentrations of forskolin did not elicit calcitonin secretion from 3 other human thyroid c-cell lines: SINJ, SHER-1 or MTC-SK cells (report 14725-062).

Liraglutide, GLP-1(7-37) or exenatide were not mitogenic in a [^{3}H]thymidine DNA incorporation assay using human TT cells, but these cells didn't respond to the positive control mitogens gastrin or epidermal growth factor (study report 205295).

Liraglutide doesn't bind to human calcitonin receptors. In a scintillation proximity format assay, up to 5 μM liraglutide or GLP-1(7-37) did not inhibit binding of 53 pM [^{125}I]calcitonin (salmon) to BHK cell expressing a recombinant human calcitonin receptor (report 14718-007).

In conclusion, specific GLP-1(1-37)amide binding occur in normal thyroid in at least a subgroup of people, but GLP-1 receptors have not been localize to specific cells in thyroid. When it occurs, the density of receptor binding sites in thyroid was similar to pancreas. GLP-1 receptors are more commonly found in human MTCs than in normal thyroid. In clinical studies, liraglutide dose-dependently increased plasma calcitonin compared to placebo at 0.6, 1.2, and 1.8 mg/day at 26 or 28 months of treatment. In clinical studies, the incidence of treatment-emergent benign and malignant thyroid neoplasms was higher in liraglutide-treated compared to non-liraglutide treated subjects.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:
Special Toxicity

Mechanistic Studies (Rodent Thyroid C-cell tumors)

- c-cell / Rodent C-cell findings: Assessment of human relevance
- 204370 / An immunohistochemical investigation of the GLP-1R in tissue from mice, rats, cynomolgus monkeys, and humans
- 20040515PR4 / Investigation of GLP-1 receptor mRNA expression in mouse, rat cynomolgus monkey, and human thyroid C-cells and in pancreatic islets studied by in situ hybridization
- 14725-006 / GLP-1 receptor expression in rat and human cell lines
- 205088 / Quantitative analysis of GLP-1 receptor levels on 2 rat (rMTC 6-23 and CA-77) and one human (TT) C-cell line
- 205218 / Western blot analysis of GLP-1R expression in rats and human C-cell lines
- 204415 / Real-time (TaqMan) RT-PCR quantification of glucagon-like peptide 1 receptor in C-cell lines
- 13737-025 / Thyroid C-cell line GLP-1 receptor functional data: cAMP accumulation and calcitonin release
- 14725-062 / Further human C-cell lines
- 205295 / Investigation of the mitogenic potential of liraglutide in rats and human C-cell lines
- 14718-007 / Calcitonin receptor binding studies
- 13736-092 / Liraglutide binding to rat gastrin (CCK2R) and bombesin (BB2R) receptors in AR42J cells
- 040301 / Assessment of beta and non-beta cell mass in pancreatic islets of cynomolgus monkeys treated with liraglutide for 52 weeks: [redacted] study 577863, NN study 200241 b(4)
- 205106 / NNC 90-1170 single dose study in mice with subcutaneous administration
- 204268 / A 9 week exploratory study with reversibility in mice – Combined evaluation of the in life phase, hormone analysis, molecular analysis, pathology, and statistical analysis
- 204289 / 13 week toxicity study in mice with subcutaneous administration. Calcitonin determinations in mouse plasma
- 203281 / Effects on calcium homeostasis after a single subcutaneous administration to male rats in a fasted condition – Combined evaluation of the in life phase, hormone analysis, statistical analysis, and molecular analysis

- 203258 / The effects on calcium homeostasis after a single subcutaneous administration to male rats in a nonfasted condition – Combined evaluation of the in life phase, hormone analysis, and statistical analysis
- 203282 / Study on acute effects on calcium homeostasis related hormones after single dose subcutaneous administration in fasted and calcium treated rats
- 203317 / Effects on calcium homeostasis related parameters and thyroid volume fractions after up to six weeks daily subcutaneous administration followed by a 2 week reversibility period in male rats – Combined evaluation of in life phase, hormone analysis, and statistical analysis
- 204163 / Effects on plasma calcitonin, thyroid C-cell mass, and formation of antibodies after up to 483 days daily subcutaneous administration in young aged male rats – Combined evaluation of the in life phase including antibody analysis, calcitonin
- 204021 / Quantification of thyroid C-cells by digital image analysis on histological sections prepared from specimens from ——— studies 577863 (cynomolgus monkeys) and 455476 (Crl: CD rats) b(4)
- 203262 / Effects on calcium homeostasis related parameters after up to 87 weeks daily subcutaneous administration in male and female cynomolgus monkeys – combined evaluation of in life phase including thyroid histopathological evaluation
- 204402 / Study on the acute effects on calcitonin and toxicokinetics after single dose subcutaneous administration in fasted mice
- 205074 / In vivo study with administration of NNC 0113-0000-0000 by subcutaneous administration as bolus injections (once, twice, three times daily) or continuous infusion in female mice
- 205050 / NNC 0113-0000-0000 and liraglutide. Study on calcitonin and toxicokinetics after 3–days of subcutaneous administration in fasted male mice
- 205025 / Preliminary investigative study by subcutaneous administration (3 times a day) to CD-1 mice for 2 or 13 weeks – Combined evaluation of the in life phase including hormone analysis and C-cell pathology of the thyroid gland and molecular analysis
- 205205 / Investigatory toxicity study by osmotic minipump subcutaneous administration to CD-1 mice for 12 or 16 weeks
- 2005 001 / Modeling of exendin-4 concentration and effect on plasma calcitonin in mice
- 2005 005 / Modeling of pharmacokinetics and effect on plasma calcitonin after once daily dose administration of liraglutide
- 205121 / Characterization of the distribution of C-cells in thyroids from cynomolgus monkeys

TABLE OF CONTENTS

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Carcinogenicity:

Subcutaneously injected liraglutide was a non-genotoxic carcinogen in 2 year repeat dose studies causing thyroid c-cell tumors in male and female rats and mice and fibrosarcomas on the dorsal surface (the body surface used for drug administration) in male mice. In Sprague Dawley rats treated with 0.075, 0.25, or 0.75 mg/kg/day liraglutide for 104 weeks, liraglutide plasma AUC_{0-24} values were approximately 0.5, 2.2, or 7.6 times the mean human plasma drug exposure from a single 1.8 mg/day subcutaneous dose, respectively. Treatment-related thyroid c-cell adenomas occurred at $\geq$ 0.25 mg/kg in males and at $\geq$ 0.075 mg/kg in females, c-cell carcinomas occurred at 0.75 mg/kg in males, and combined c-cell adenomas or carcinomas occurred at $\geq$ 0.25 mg/kg in males and at $\geq$ 0.075 mg/kg in females. In CD-1 mice treated with 0.03, 0.2, 1, or 3 mg/kg liraglutide for 104 weeks, liraglutide plasma AUC_{0-24} values were approximately 0.2, 1.8, 10, or 45 times the mean human plasma drug exposure from a single 1.8 mg/day subcutaneous dose, respectively. Treatment-related thyroid c-cell adenomas occurred at $\geq$ 1 mg/kg in male and female mice, combined c-cell adenomas or carcinomas occurred at $\geq$ 1 mg/kg in females, and fibrosarcomas on the dorsal skin and subcutis, the body surface for drug administration, occurred at 3 mg/kg in males.

Special toxicology:

Mechanistic Studies of Liraglutide-Induced Proliferative Thyroid C-cell Lesions in Rats and Mice

To evaluate the human relevance of liraglutide-induced thyroid c-cell tumors, the sponsor performed mechanistic studies to support their proposed mode of action that:

1. Circulating liraglutide binds to and activates GLP-1 receptors on thyroid C-cells.
2. GLP-1 receptor activation on C-cells induces calcitonin release.
3. Continued calcitonin release leads to increased calcitonin synthesis.
4. Persistent stimulation of calcitonin secretion and synthesis in C-cells leads to C-cell hyperplasia in rodents.
5. Long-term C-cell hyperplasia may lead to C-cell neoplasia in rodents.

A schematic of the sponsor's hypothetical mode of action is shown below.

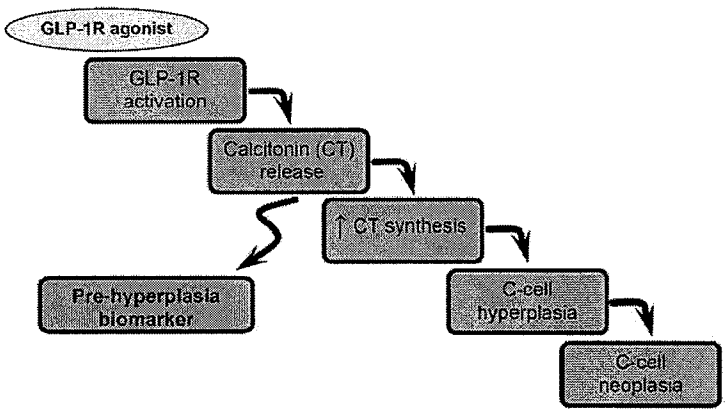


Figure 2 Key events in the process leading to rodent C-cell proliferation after long-term treatment with GLP-1 receptor (GLP-1R) agonists

[N000 4.2.3.7.3 Assessment Document P15]

The key events are 1) persistent liraglutide-induced GLP-1 receptor-mediated calcitonin release from thyroid C-cells results in c-cell hyperplasia and 2) persistent hyperplasia progresses to adenomas, then carcinomas. The sponsor proposed that GLP-1 receptor agonist-induced calcitonin secretion from c-cells is more robust in rodents compared to primates, therefore this mode of action is relevant to liraglutide induced C-cell tumors in rats and mice, but not humans.

Rats and mice have different susceptibilities to naturally occurring and xenobiotic-induced thyroid c-cell tumors. In rats, plasma calcitonin, diffuse c-cell hyperplasia (considered a physiologic response), focal c-cell hyperplasia (considered a preneoplastic lesion), and c-cell adenomas increase with age. In Sprague Dawley rats, thyroid c-cell adenomas are common in control groups of 2 year studies (incidence > 1%), but c-cell carcinomas are not (incidence < 1%). In mice, focal c-cell hyperplasia, adenomas, and carcinomas are rare in control groups of 2 year studies (incidence < 1%). In rats, proliferative c-cell lesions progress from diffuse hyperplasia to focal hyperplasia to adenomas, but in mice, when adenomas occur, they are rarely preceded by focal c-cell hyperplasia. Seven marketed drugs with rat thyroid c-cell tumor findings in their label were identified (including exenatide), but none of them caused c-cell tumors in mice and a mechanism for drug-induced c-cell tumors wasn't established for any of them (see Overall Conclusions and Recommendations section).

The sponsor used rat and human C-cell lines to characterize species differences in GLP-1 receptor agonist binding, signal transduction, coupling to calcitonin secretion, GLP-1 receptor agonist-induced regulation of calcitonin and GLP-1 receptor transcription, or ligand-induced mitogenesis. However, the behavior of the human TT cells, a thyroid C-cell line, did not agree with previously published studies with respect to known mitogens or known calcitonin secretagogues, therefore any differences in GLP-1 receptor agonist effects in rat and human cell lines are not proof of species differences occurring in vivo.

Since rats and mice differ with respect to their susceptibility to drug-induced thyroid c-cell tumors and the incidence and course of development of spontaneous c-cell tumors, mechanistic studies addressing the mode of action of liraglutide induced proliferative C-cell lesions were considered separately.

Rats

Thyroid c-cell GLP-1 receptor in rats

There is no direct evidence of rat thyroid c-cell GLP-1 receptors coupled to calcitonin secretion. Rat c-cell GLP-1 receptors are inferred from autoradiography of rat thyroid tissue using radiolabeled GLP-1, in vitro pharmacology studies of GLP-1 receptor agonist binding and

adenylyl cyclase activation in rat c-cell lines, GLP-1 receptor mRNA in rat c-cell lines, and GLP-1 induced calcitonin secretion from perfused rat thyroid and rat c-cell lines.

Published studies suggest GLP-1 receptors occur on rat thyroid c-cells, and studies with perfused rat thyroid and rat c-cell lines suggest the receptor mediates calcium-dependent calcitonin secretion. Autoradiography of thyroid tissue slices labeled with ^{125}I-GLP-1(7–36)amide showed detectable GLP-1 binding sites, but binding wasn't attributed to a specific cell type (Korner et al. J Nucl Med 48: 736–743, 2007). GLP-1 receptors were demonstrated in rat c-cell lines CA77 (Lamari et al, FEBS Lett. 393(2-3): 248 – 52, Crespel et al, Endocrin 137: 3674 – 80) and MTC 6-23 (Vertongen et al, Endocrin 135: 1537 – 42). In CA77 cells, GLP-1 receptor mRNA was detected by RT-PCR amplification using transcript specific primers and by Northern blot. GLP-1 receptors in CA77 cells were coupled to adenylyl cyclase activation via Gs, calcitonin secretion (up to 52% increase over baseline), and increased calcitonin mRNA (2.9 fold). GLP-1 receptor expression in MTC 6-23 cells were demonstrated by radioligand binding, the presence of the receptor transcript by PCR amplification using receptor specific probes, and GLP-1 (7-36)amide activation of adenylyl cyclase.

An immunohistochemical study of GLP-1 receptor in rat thyroid tissue sections stained with anti-calcitonin antibodies to identify c-cells did not confirm because the specificity of the rabbit polyclonal anti-human GLP-1 receptor antibody, K102B, wasn't demonstrated (study 204370). GLP-1 receptor specificity of K102B was not adequately demonstrated because; 1) K102B staining wasn't blocked in the presence of the peptide antigen used to generate the antibody and 2) Western blot analysis of protein from c-cell lines did not demonstrate GLP-1 receptor specific staining (study 205218). Furthermore, results from Western blots of SDS-PAGE electrophoresed proteins from rat c-cell lines CA77 and MTC 6-23 and the human TT c-cell using K102B were equivocal because stained proteins were unlikely to be GLP-1 receptors (study 205218).

An in situ hybridization study of GLP-1 receptor mRNA in tissue sections from rats showed GLP-1 receptor transcript levels were low to undetectable in thyroid, but much higher in pancreas, a positive control (study 20040515PR4).

GLP-1 receptors were demonstrated in rat thyroid c-cell lines CA77 and MTC 6-23 by ^{125}I-GLP1(7-37) radioligand binding (study 14725-006), GLP-1(7-36)-Lys(6-FAM) fluorescent ligand binding (study205088), PCR amplification of the receptor transcript, and GLP-1 receptor agonist induced cAMP accumulation (study 13737-025). GLP-1(7-37) was 48 fold more potent than liraglutide at stimulating cAMP accumulation in MTC 6-23 cells. The presence of GLP-1 receptors in rat c-cell lines doesn't confirm the presence of the receptor in thyroid c-cells in vivo.

C-cell GLP-1 receptor activation linked to calcitonin release

There is no direct evidence that rat thyroid calcitonin secretion is mediated by a c-cell GLP-1 receptor. In subchronic and chronic repeat dose studies of liraglutide in male Sprague Dawley rats, the magnitude of any effect was small, typically < 2 fold, and transient because it didn't persist after a few months of treatment. Although GLP-1 receptor agonist appear to increase it, plasma calcitonin levels probably remain within a normal physiologic range and elicit a counter-regulatory hypocalcemic response.

The best evidence for GLP-1 mediated calcitonin release in rats comes from a published study by Crespel (Endocrinol 137(9): 3674 – 3680). Perfusion of rat thyroid glands with 1 or 10 nM GLP-1 in the presence of low calcium (1 mM) or high calcium (3 mM) showed GLP-1 induced calcitonin secretion was calcium dependent (Figure 5). However, it should be noted that 1 mM calcium is a subphysiologic concentration (4 mg/dL) whereas 3 mM is within a normal physiologic range (12 mg/dL). Persistent calcitonin secretion in the presence of GLP-1 probably doesn't reflect normal physiology because the major counter-regulatory response, decreased serum calcium (due to inhibition of osteoclast-mediated resorption), can't occur.

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FIG. 5. Effect of glucagon (■) or tGLP-1 (●) on calcitonin secretion from perifused rat thyroid in the presence of 1 mM calcium (A) and 3 mM calcium (B and C). A stabilization period of 120 min at 0.5 mM calcium preceded the stimulation phase of 90 min at either 1 or 3 mM calcium and was followed by a 30-min period at 0.5 mM calcium. Perifusion terminated with of a 15-min period at 5 mM calcium for viability control of each preparation. Results are the mean ± SEM of four to eight experiments and are expressed as a percentage of the maximal secretion obtained at 5 mM calcium for each perifusion.

[Crespel et al. Endocrinol 137(9): 3674 – 3680)]

This study also demonstrated GLP-1 elicited calcitonin release from CA-77 cells, a rat c-cell line, but calcitonin secretion from MTC 6-23 cells was calcium independent (Scherub et al Horm Met Res [Suppl] 21: 18 – 21). Differences in calcium dependence of GLP-1 elicited calcitonin release from perfused thyroid and MTC 6-23 cells indicates cell lines may not accurately reflect regulation of calcitonin secretion from c-cells in vivo.

GLP-1 receptor agonists induce calcitonin secretion from cultured MTC 6-23 cells with the rank order potency expected for GLP-1 receptor mediation: exenatide (EC_{50} 55 pM) > GLP-1 (1-37) (EC_{50} 80 pM)>> liraglutide (EC_{50} 5,300 pM). Calcium dose-dependently stimulated calcitonin release from MTC 6-23 cells and liraglutide enhanced calcium-stimulated calcitonin secretion. Pentagastrin, a potent calcitonin secretagogue in humans and rats, had no effect on calcitonin secretion from rat MTC 6-23 cells suggesting that receptor-coupled calcitonin secretion in the cell line was different from thyroid c-cells in vivo.

In young rats (~ 2 months old at the start of treatment), single and repeat dosing with liraglutide for up to 6 weeks increased plasma calcitonin, but the effects didn't persist in chronically treated rats. Liraglutide-induced increased calcitonin provokes a counter-regulatory response of decreased plasma calcium and subsequently, increased PTH. The effect of

subcutaneously administered liraglutide on plasma calcitonin in rats was determined after single doses and repeat dosing up to 69 weeks.

A single dose study of subcutaneously injected 0 (vehicle) or 0.75 mg/kg liraglutide in male Sprague Dawley rats with monitoring plasma calcium parameters for up to 6 hours after dosing showed plasma calcitonin was modestly, transiently, but significantly increased compared to concurrent controls 0.5 and 1 hour after dosing and PTH levels increased 6 hours post-dose (study 203281). The transient increase in calcitonin was countered by decreased blood calcium, increased PTH, and increased excretion of calcium in urine (not monitored in this study). In calcium loaded rats (intraperitoneal injection of 1 mM/kg calcium) treated with 0 or 0.75 mg/kg liraglutide, calcitonin levels peaked within 15 minutes of dosing with higher levels in the liraglutide group (study 203282). The magnitude of increased plasma calcitonin in response to calcium loading was > 10 fold greater than any increase due to liraglutide. Plasma calcitonin levels were similar or below control group levels from 0.5 – 6 hours after dosing. Within 24 hours of a single s.c. injection of 0 or 0.75 mg/kg liraglutide to male Sprague Dawley rats, liraglutide increased urine volume and calcium excretion, but without significantly increasing plasma calcitonin. Decreased plasma calcium was considered an effect of increased calcium excretion, and increased PTH was a counter-regulatory response to decreased plasma calcium.

A 6 week study of 0 or 0.75 mg/kg liraglutide injected s.c. once a day to male Sprague Dawley rats included a 4 week interim sacrifice group, a 2 week recovery group. On day 45, fasting treated rats were calcium loaded to determine its effect on any liraglutide-induced changes in plasma PTH and calcitonin. Calcium loading vehicle or liraglutide treated rats on day 45 markedly reduced plasma PTH and increased calcitonin with return to baseline levels within 24 hours after calcium loading. Calcitonin levels in the liraglutide-treated group trended higher than concurrent controls in non-fasted rats sampled in week 4 and in fasted rats sampled in week 5. After a 2 week recovery period, plasma calcitonin levels in rats treated with liraglutide for 6 weeks trended lower than concurrent controls.

In a chronic repeat dose study of liraglutide in young and old male Sprague Dawley rats, any liraglutide-related increase in plasma calcitonin was transient and occurred early in treatment. In a 69 week repeat dose study of 0 (vehicle), 0.025, 0.25, or 0.75 mg/kg/day liraglutide in young male Sprague Dawley rats (2 months old) treated for 7, 10, 13, or 16 months or aged rats (8 months old) treated for 1, 4, 7, or 10 months, calcitonin levels were > 1.3 fold higher than concurrent controls prior to and after dosing at 0.25 and 0.75 mg/kg/day on day 28 in young rats, and at 0.75 mg/kg/day in aged rats. Calcitonin levels > 1.3 fold higher than concurrent controls occurred sporadically at all doses in aged rats, but the increase was small (at or near 1.3) and these increases were considered incidental because they weren't related to dose, duration of therapy, or time of drug administration. Calcium levels were unaffected by liraglutide treatment.

GLP-1 receptor agonist-induced calcitonin release increases calcitonin synthesis

In normal rats, a single dose of liraglutide decreased thyroid calcitonin peptide and mRNA levels, but in calcium loaded rats, it increased both. Repeat dosing up to 4 weeks did not significantly increase thyroid calcitonin transcript levels.

A single subcutaneous injection of 0.75 mg/kg liraglutide decreased thyroid calcitonin and calcitonin transcript levels in fasted rats, but in calcium loaded rats, liraglutide increased thyroid calcitonin and calcitonin transcript levels. Thyroid calcitonin and calcitonin transcript levels were determined 6 hours after a single subcutaneous injection of 0 (vehicle) or 0.75 mg/kg liraglutide to male Sprague Dawley rats (study 203281) or calcium loaded rats (single intraperitoneal injection of 1 mM/kg calcium). Calcium loading reduced thyroid calcitonin up to 2.2 fold up to 6 hours in vehicle treated controls, but in the liraglutide group, calcitonin levels were up to 4.7 fold higher than concurrent controls 6 hours after calcium loading. In fasted rats (without calcium loading), liraglutide decreased thyroid calcitonin up to 2.7 fold up to 6 hours compared to controls. Calcium loading liraglutide treated rats resulted in increased thyroid

calcitonin up to 3.8 fold up to 6 hours after dosing indicating concurrent liraglutide treatment and calcium loading increases calcitonin synthesis, whereas liraglutide treatment alone or calcium loading alone do not. In fasted rats, liraglutide decreased thyroid calcitonin mRNA up to 3.5 fold compared to controls, for up to 6 hours after dosing whereas calcium loading increased calcitonin mRNA levels in liraglutide treated rats.

After 4 weeks of dosing male Sprague Dawley rats with 0 or 0.75 mg/kg/day liraglutide (s.c. injections once a day), there were no treatment-related differences in relative thyroid calcitonin mRNA levels (study 203317).

Persistent c-cell stimulation (persistent elevated plasma calcitonin) leads to c-cell hyperplasia

There was no compelling evidence of liraglutide-induced diffuse thyroid c-cell hyperplasia, an expected physiologic response to increased calcitonin demand, but liraglutide increased the incidence of age-dependent focal c-cell hyperplasia, a preneoplastic lesion. There was no evidence of diffuse c-cell hyperplasia preceding focal hyperplasia in liraglutide-treated rats. Liraglutide appears to be a tumor promoter in rats because liraglutide-induced focal c-cell hyperplasia was age-dependent while liraglutide-induced c-cell tumors were treatment-duration dependent.

Treatment with up to 1 mg/kg/day liraglutide subcutaneously injected once a day in male and female Sprague Dawley rats for up to 26 weeks, did not increase the incidence of focal thyroid c-cell hyperplasia or c-cell adenoma. In a 2 year repeat dose carcinogenicity study of 0, 0.025, 0.25, or 0.75 mg/kg/day liraglutide, an increased incidence and severity of focal thyroid c-cell hyperplasia was dose-related at $\geq$ 0.25 mg/kg/day in males and females. Retrospective quantitative analysis of thyroid c-cells in rats from the 26 week chronic rat toxicity and the 104 week carcinogenicity study did not find any evidence of liraglutide-induced diffuse c-cell proliferation or any effect on the ratio of thyroid c-cells to follicular cells in the high dose groups (1 mg/kg/day liraglutide in the 26 week study and 0.75 mg/kg/day in the 104 week study).

In a repeat dose study of 0 or 0.75 mg/kg/day liraglutide administered to male Sprague Dawley rats for up to 6 weeks with BrdU administered within 48 hours of the terminal sacrifice to label proliferating cells, group mean absolute and relative thyroid weight in the liraglutide treated group was significantly lower than concurrent controls. However, quantitative analysis of c-cells (immunoreactive with anti-calcitonin antibody) and BrdU labeled c-cells showed despite differences in thyroid weight, there were no treatment-related differences in follicular cell volume, c-cell volume, or volume of proliferating c-cells. Elevated plasma calcium increases calcitonin secretion from thyroid c-cells in rats and mice, but elevated calcium doesn't necessarily result in c-cell hyperplasia. Hypercalcemia induced by hypervitaminosis D3 in rats (25,000 IU/day D3 concurrently administered with or without $CaCl_2$) did not cause c-cell hyperplasia (Fernández-Santos et al, Histol Histopathol. (2001) 16(2):407-14).

To determine the time course and characteristics of liraglutide-induced c-cell hyperplasia, the sponsor carried out single and repeat subcutaneous dose studies of up to 69 weeks with monitoring of calcium parameters including plasma calcium, calcitonin, and PTH, and quantitative and qualitative thyroid microscopic pathology. In a repeat dose study of 0, 0.075, 0.25, or 0.75 mg/kg/day liraglutide injected once a day in young (2 months old) or old (8 months old) male Sprague Dawley rats for up to 69 weeks with sacrifices occurring after 30, 43, 56, and 69 weeks for young rats and 4, 17, 30, or 43 week for aged rats (study 204163), focal thyroid c-cell hyperplasia first occurred in the 0.75 mg/kg/day group after 30 weeks of dosing in young rats and after just 4 weeks of dosing in aged rats. The age of onset, 9 months, was the same in both young and aged rats. C-cell adenomas first occurred in the 0.75 mg/kg/day group after 30 weeks of dosing in young or aged rats (9 months at age of onset in young rats, 15 month age of onset in aged rats), so the duration of treatment was the same, 30 weeks. In young rats, both focal c-cell hyperplasia and adenoma occurred after 30 weeks of treatment. Therefore, liraglutide-induced focal c-cell hyperplasia appears to age-dependent, but liraglutide-induced c-cell adenomas are

treatment-duration dependent. Although c-cell carcinomas occurred in a 104 week rat carcinogen bioassay, c-cell carcinomas didn't occur in "young rats" treated for up to 69 weeks or "aged rats" treated for up to 43 weeks in repeat dose mechanistic studies using the same doses.

Persistent liraglutide-induced c-cell hyperplasia progresses to c-cell neoplasms

Progression of liraglutide-induced focal thyroid c-cell hyperplasia to adenoma was treatment-duration dependent, but it occurred in the absence of any evidence of persistent elevated plasma calcitonin over and above the age-related increase that normally occurs in rats. Repeat dose mechanistic studies of subcutaneously administered liraglutide up to 69 weeks in young male Sprague Dawley rats and up to 43 weeks in aged rats showed liraglutide-induced focal thyroid c-cell hyperplasia was age dependent. In a 2 year repeat subcutaneous dose carcinogenicity study of 0.03, 0.2, 1, or 3 mg/kg/day liraglutide in Sprague Dawley rats, a strain not susceptible to thyroid c-cell tumors, the NOAEL for focal c-cell hyperplasia was < 0.075 mg/kg/day liraglutide in males and 0.075 mg/kg/day in females with minimal to marked c-cell hyperplasia occurring at the lowest observed effect level (LOEL) in both sexes (0.075 mg/kg/day in males and 0.25 mg/kg/day in females). C-cell adenomas occurred at $\geq$ 0.25 mg/kg/day in males and at $\geq$ 0.075 mg/kg/day in females. The LOEL for c-cell adenomas in females was lower than the LOEL for hyperplasia. The incidence of c-cell carcinomas exceeded the concurrent and historical control range occurred at $\geq$ 0.075 mg/kg/day in males and at $\geq$ 0.25 mg/kg/day in females. In the 2 year carcinogenicity study, the incidence of combined c-cell tumors (adenoma / carcinoma) exceeded the incidence of focal hyperplasia at $\geq$ 0.25 mg/kg/day in males and at 0.75 mg/kg/day in females. Although a prolonged period of diffuse and nodular c-cell hyperplasia and elevated serum calcitonin typically precedes the development of c-cell tumors in both humans and rats, that didn't occur in the mechanistic studies of liraglutide induced c-cell tumors in rats.

Mice

Thyroid c-cell GLP-1 receptor in mice

Immunohistochemical and in situ hybridization studies of GLP-1 receptors in mouse thyroid did not provide sufficient evidence of GLP-1 receptors on c-cells.

A published autoradiographic ligand binding study of [^{125}I]GLP-1(7-36) in thyroid tissue sections from mice showed mice are heterogeneous with specific tissue binding occurring in thyroid from 3/6 mice (Korner M et al, J Nucl Med(2007) 48: 736-743). Mouse thyroid cell type(s) labeled by [^{125}I]GLP-1(7-36) were not identified.

An immunohistochemical colocalization study using mouse thyroid tissue slices was equivocal for colocalization of GLP-1 receptor and calcitonin immunoreactivities on the same cells because GLP-1 receptor immunoreactivity was weak and the specificity of the anti-GLP-1 receptor antibody was not demonstrated. The specificity of K100B, a polyclonal rabbit anti-human GLP-1 receptor antibody, was not adequately demonstrated because; 1) the antibody stained pancreas from GLP-1 receptor knockout mice and 2) immunohistochemical staining in the presence of the peptide antigen used to generate the antibody did not block staining.

An in situ hybridization study of GLP-1 receptor mRNA in tissue sections from mice was equivocal with low to undetectable levels of GLP-1 receptor transcript in thyroid, but much higher levels in pancreas, a positive control.

C-cell GLP-1 receptor activation linked to calcitonin release

There were no in vitro studies in mouse thyroid c-cells or mouse c-cell lines linking GLP-1 receptor activation to calcitonin release. There is no direct evidence of liraglutide induced, thyroid c-cell GLP-1 receptor mediated calcitonin release in mice, but GLP-1 receptor agonists liraglutide and exenatide increase plasma calcitonin and thyroid calcitonin mRNA in mice prior to inducing focal c-cell hyperplasia. The magnitude of any GLP-1 receptor agonist elicited increase in plasma calcitonin was substantially smaller than that of intraperitoneally injected

calcium. There was a trend of increased plasma calcitonin after the first liraglutide dose, and increased plasma calcitonin was sustained for up to 2 years of continuous treatment in a mouse carcinogenicity study. Focal c-cell hyperplasia develop after 4 - 9 weeks of liraglutide treatment and neoplasms develop after 64 weeks. Proliferative c-cell lesions account for increased basal and GLP-1 receptor agonist stimulated calcitonin release in liraglutide-treated mice.

A methodological issue confounded results from studies of GLP-1 receptor agonist effects on plasma calcitonin in mice. Mouse plasma calcitonin was quantified using a rat calcitonin immunoradiometric assay (IRMA), but reports of the sensitivity, specificity, and validity of the assay for mouse calcitonin weren't submitted to the NDA (reports 205089 & 205189). Although peptide sequences of rat and mouse calcitonin differ by only a single amino acid, cross-reactivity of the rat IRMA with human calcitonin is only 12%, despite human and rat sequences differing by only 2 amino acids.

A recently published study characterizing bone and mineral homeostasis in GLP-1 receptor deficient mice supports the sponsor's hypothesis that the GLP-1 receptor showed receptor signaling is linked to bone resorption by a calcitonin dependent pathway (C. Yamada et al., Endocrinology (2008) 149(2):574–579). GLP-1 receptor knockout mice had increased osteoclasts, increased bone resorption, and decreased thyroid calcitonin mRNA, but plasma levels of ionized calcium and intact PTH were unaffected. Administering 10 IU/kg eel calcitonin suppressed elevated urinary excretion of deoxypyridinoline, a biomarker of increased bone resorption, in GLP-1 receptor knockout mice. Furthermore, GLP-1 receptor agonists don't directly affect osteoclast or osteoblast activity.

The effect of subcutaneously administered liraglutide and exenatide on plasma calcitonin in mice was determined after single and repeat dosing. Plasma calcitonin levels were measured for up to 16 weeks of exenatide treatment and up to 2 years of liraglutide treatment.

Single bolus subcutaneous doses of 0.2, 1, or 3 mg/kg liraglutide increased calcitonin in CD-1 mice (male and females combined)1.8, 2.4, or 2.4 fold compared to concurrent controls, respectively, within 1.5 to 36 hours after dosing. In nearly all dose groups at all time points, some mice were considered liraglutide non-responsive because plasma calcitonin levels were within the range of values for the control group.

In a 3 day repeat subcutaneous dose study of 0.06 or 25 mg/kg/day liraglutide in male CD-1 mice, day 3 pre-dose group mean plasma calcitonin dose-dependently increased in both liraglutide groups. However, there was evidence that some high dose group mice didn't respond to liraglutide treatment (plasma calcitonin < 50 pg/mL).

Liraglutide increased plasma calcitonin within 2 weeks of daily subcutaneous dosing in CD-1 mice, the increase was sustained with continued treatment for up to 9 weeks, and it was reversed within 6 weeks after treatment was stopped. In a 9 week study of 0.2 or 5 mg/kg/day liraglutide in CD-1 mice, the time course of liraglutide effects on plasma calcitonin were determined prior to dosing and 0.5 and 3 hours after on day 14 and at a single time point after dosing on days 14 and 63 (at the end of 2 and 9 weeks of treatment). Liraglutide increased plasma calcitonin in males 0.5 and 3 hours after dosing on day 14 and in females, calcitonin was above concurrent control levels 3 hours after dosing at 0.2 mg/kg/day and at all time points in females treated with 5 mg/kg/day. In males, calcitonin was increased only at 5 mg/kg/day and only at the end of the of the 9 week treatment period, but in 5 mg/kg/day liraglutide females, calcitonin was elevated after 2 and 9 weeks. Calcitonin levels in both males and females in the 5 mg/kg/day group returned to control group levels by the end of a 6 week recovery period.

In a 13 week repeat subcutaneous dose toxicity study of 0.2, 1, or 5 mg/kg/day liraglutide in CD-1 mice, plasma calcitonin levels increased at all liraglutide doses within 24 hours post-dose after the first dose and in week 13. In a 2 year carcinogenicity study of 0.03, 0.2, 1, or 3 mg/kg/day liraglutide subcutaneously injected once a day, plasma calcitonin was measured in weeks 26, 52, and 104. Group mean calcitonin in males was significantly higher the concurrent control group at $\geq$ 0.2 mg/kg/day in week 26 (male and female), then at all doses in weeks 52

and 104. In females, calcitonin increased at ≥ 0.2 mg/kg/day liraglutide in weeks 26 and 52, and at all doses in week 104. Between weeks 26 and 104, group mean plasma calcitonin increased more than 2 fold at 3 mg/kg/day in males and females, but not at lower doses. Proliferative C-cell lesions in liraglutide treated mice accounts for increased calcitonin at 3 mg/kg/day at the end of the carcinogenicity study.

To support their hypothesis that liraglutide-associated increased plasma calcitonin is GLP-1 receptor mediated, the sponsor evaluated the effects of a second agonist, exenatide, on plasma calcitonin and proliferative c-cell lesions in mice. Pharmacokinetic / pharmacodynamic modeling of liraglutide effects on plasma calcitonin in mice indicated that more frequent or continuous dosing with exenatide would be necessary to achieve comparable effects on plasma calcitonin due the shorter elimination half life of exenatide compared to liraglutide.

In a single subcutaneous bolus dose study of 0.25, 1, or 5 mg/kg exenatide in CD-1 mice, exenatide had little or no discernable affect on plasma calcitonin for up to 24 hours after dosing, particularly compared to the robust response elicited by intraperitoneal infusion of calcium.

Because of its short half life, exenatide was administered more frequently, up to 3 times daily, by subcutaneous bolus dosing or by continuous subcutaneous infusion using implanted ALZet osmotic minipumps.

Subcutaneous bolus injections of 0.25 mg/kg/day exenatide once a day or in divided doses 2 or 3 times a day (0.125 or 0.083 mg/kg/injection, respectively) in female CD-1 mice didn't affect plasma calcitonin levels after 2 days of treatment, but continuous subcutaneous infusion of 0.25 mg/kg/day liraglutide (ALZet osmotic minipump) significantly increased plasma calcitonin above control group levels on study day 2.

In a 3 day repeat subcutaneous bolus injection study of 0.06 or 0.25 mg/kg/day exenatide administered once a day or 0.03 or 0.125 mg/kg/injection administered twice a day (0.06 or 0.25 mg/kg/day) to male CD-1 mice, day 3 predose plasma calcitonin levels were higher in mice dosed twice a day, but there was no significant difference between 0.03 and 0.125 mg/kg/injection doses. Within 6 hours after dosing, plasma calcitonin levels were similar to controls. The effect of once a day exenatide dosing on plasma calcitonin was minimal.

In a repeat subcutaneous dose study of 0.083, 0.33, or 1.67 mg/kg/injection exenatide administered 3 times daily, (0.25, 1, or 5 mg/kg/day total dose) for 2 weeks, group mean calcitonin was significantly higher in all exenatide treated groups; up to 6.2 fold higher than concurrent controls in males and up to 8.1 fold higher in females. In a 13 week study of 0.33 mg/kg/injection exenatide administered 3 times daily for 8 days (1 mg/kg/day total dose) followed by 1 mg/kg/injection administered 3 times daily for 12 additional weeks (3 mg/kg/day total dose), calcitonin levels were significantly increased in exenatide treated males, but not in females, at the end of the 13 week period. At the end of the 13 week treatment with multiple daily subcutaneous injections of exenatide, increased plasma calcitonin and increased thyroid calcitonin mRNA lacked correlative focal c-cell hyperplasia in males.

Plasma calcitonin levels were determined in a 16 week repeat dose study of 0.25 or 1 mg/kg/day exenatide administered by continuous subcutaneous infusion or 0.25 mg/kg injected once a day in CD-1 mice. Compared to concurrent controls, daily subcutaneous injections of 0.25 mg/kg/day did not significantly increase plasma calcitonin levels after 12 or 16 weeks of treatment. In mice treated by continuous infusion, calcitonin levels were higher than concurrent controls in weeks 4, 8, 12 and 16. In weeks 12 and 16, calcitonin levels in exenatide groups treated by continuous infusion were at least 4 fold lower than in weeks 4 and 8, probably because treatment was stopped 24 hours prior to sampling in weeks 12 and 16, but in weeks 4 and 8, samples were taken while treatment was ongoing.

Pharmacokinetic / pharmacodynamic modeling of exenatide effects on plasma calcitonin in mice, using an EC_{90} of 270 pM exenatide to increase plasma calcitonin, estimated continuous infusion of 0.25 mg/kg/day would be sufficient to cause sustained elevated blood levels of calcitonin while subcutaneous bolus injections of ≤ 1.67 mg/kg/injection administered 3 times

daily would not. This modeling result is consistent with the absence of thyroid c-cell proliferative lesions in a carcinogenicity study of 0, 0.018, 0.08, or 0.25 mg/kg/day exenatide subcutaneously injected once a day in CD-1 mice for up to 98 weeks in males and up to 96 weeks in females and the presence of c-cell hyperplasia in mice after 12 or 16 weeks of treatment with a constant subcutaneous infusion of 0.25 or 1 mg/kg/day exenatide for 12 or 16 weeks.

GLP-1 receptor agonist-induced calcitonin release increases calcitonin synthesis

There is no direct evidence of liraglutide induced, thyroid c-cell GLP-1 receptor mediated calcitonin release in mice, but GLP-1 receptor agonists liraglutide and exenatide increase plasma calcitonin and thyroid calcitonin mRNA prior to inducing focal c-cell hyperplasia. A recently published study showed GLP-1 receptor knockout mice (Glp-1r$^{-/-}$) mice had cortical osteopenia, bone fragility, increased numbers of osteoclasts, increased bone resorption, higher levels of urinary deoxypyridinoline (a marker of bone resorption), and reduced levels of thyroid calcitonin mRNA (Yamada et al. Endocrin (2008), 149(2):574–579). GLP-1 had no direct effect on osteoclasts and osteoblasts, so in mice, GLP-1 receptors control bone resorption through a calcitonin-dependent pathway. Subcutaneous injection of 24 nmol/kg exenatide (90 mcg/kg) increased calcitonin transcript levels in thyroid of wild-type mice, and calcitonin transcript levels were significantly reduced in GLP-1 receptor knockout mice (see Figure 4 below from Yamada et al., Endocrinology (2008) 149(2):574–579) without affecting blood levels of ionized calcium or iPTH (data not shown) .

FIG. 4. Calcitonin deficiency resulted in increased bone resorption in Glp-1r$^{-/-}$ mice. **C**, Relative expression levels of calcitonin mRNA in thyroid from WT mice injected ip with PBS or 24 nmol/kg exendin-4 (Ex-4) 6 h before RNA isolation. Values are expressed as means +/- SE; n = 5 mice per group. *, P < 0.01, PBS vs. exendin-4 treatment. **D**, Relative expression levels of calcitonin mRNA in thyroid from WT and Glp-1r$^{-/-}$ mice determined by quantitative real-time PCR. Values are expressed as means +/- SE; n = 4 mice per group. *, P < 0.05; **, P < 0.01, WT vs. Glp-1r$^{-/-}$ mice.
[Excerpted from Yamada et al. Endocrin (2008), 149(2):574–579]

In a 9 week study of 0, 0.2, or 5 mg/kg/day liraglutide in CD-1 mice, thyroid calcitonin mRNA levels in the 5 mg/kg/day group significantly increased 3.9 fold over concurrent controls.

After 2 weeks of repeat subcutaneous dosing with 0, 0.083, 0.33, or 1.67 mg/kg/injection exenatide administered 3 times daily, (0.25, 1, or 5 mg/kg/day total dose), calcitonin mRNA levels in thyroid were significantly, dose-dependently increased 2.3 - 4.8 fold at 0.25, 1, and 5 mg/kg/day exenatide, and GLP-1 receptor mRNA was unaffected.

Persistent c-cell stimulation (persistent elevated plasma calcitonin) leads to c-cell hyperplasia

Evaluation of GLP-1 receptor agonist-induced c-cell hyperplasia in mice was confounded by inconsistent definitions of c-cell hyperplasia across studies. A Pathology Peer Review and Pathology Working Group Review to peer review thyroid c-cell histopathology findings in 4, 9, and 13 week studies in mice, chaired by Peter C. Mann, DVM, reached a consensus diagnosis for c-cell findings in these studies.

To determine the time course of liraglutide-induced thyroid c-cell hyperplasia in CD-1 mice, c-cells in thyroid tissues sections were identified by calcitonin immunoreactivity and

examined microscopically from mice treated with subcutaneously injected liraglutide for 2 weeks (study 204338), 4 weeks (study 203261), 9 weeks (study 204338), or 13 weeks (study 203261) and for 2 years. In the 9 week study, the time course of reversal of c-cell hyperplasia was determined after 6 and 15 week recovery periods.

In a 9 week study of 0, 0.2 or 5 mg/kg/day liraglutide with an interim sacrifice in week 2 and recovery periods lasting 6 or 15 weeks, there were no qualitative or quantitative microscopic changes in thyroid c-cells in week 2. After 9 weeks of treatment, a low incidence of minimal c-cell hyperplasia occurred in males at $\geq$ 0.2 mg/kg/day (1/16 at 0.2 or 5 mg/kg/day), and a dose-related increased incidence and severity of up to mild c-cell hyperplasia occurred in females at $\geq$ 0.2 mg/kg/day (1/16 at 0.2 mg/kg/day, 6/16 at 5 mg/kg/day). C-cell hyperplasia was fully reversed in males and partially reversed in females at the end of a 6 week recovery period, and after a 15 week recovery period, minimal hyperplasia only occurred in 1/16 females at 5 mg/kg/day. In a 4 week repeat subcutaneous dose toxicity study of 0, 0.1, 0.5, 1, or 5 mg/kg/day liraglutide in CD-1 mice, minimal to moderated c-cell hyperplasia occurred in 2/10 females in the 5 mg/kg/day group, but review of the finding by the Pathology Working Group dismissed the finding as "developmental disturbances associated with incomplete fusion of the ultimobranchial duct with the thyroid lobe resulting in only partial delivery of c-cells in the thyroid, and were not considered related to treatment."

In a 13 week repeat dose study of 0, 0.2, 1, or 5 mg/kg/day liraglutide in CD-1 mice, dose-related increased incidence and severity of minimal to mild c-cell hyperplasia occurred at $\geq$ 0.2 mg/kg/day in males and females. The Pathology Working Group agreed with the study pathologists diagnosis of c-cell hyperplasia, but disagreed with the characterization as focal.

Persistent calcitonin release resulting in C-cell hyperplasia would expected for treatments that induce hypercalcemia in mice and rats. However, hypercalcemia induced by implanting canine CAC8 adenocarcinomas in nude mice (Okada et al., Vet Path (1994) 341: 339-347) or hypervitaminosis D3 in rats (25,000 IU/day D3 concurrently administered with or without $CaCl_2$) (Fernández-Santos et al, Histol Histopathol. (2001) 16(2):407-14) did not cause c-cell hyperplasia. These results suggest that hypercalcemia itself may not be sufficient to induce c-cell hyperplasia in rats or mice.

Persistent liraglutide-induced c-cell hyperplasia progresses to c-cell neoplasms

Repeat dose studies of subcutaneously administered liraglutide up to 13 weeks in CD-1 mice showed focal thyroid c-cell hyperplasia occurred after $\geq$ 9 weeks of treatment, and liraglutide-induced hyperplasia was largely reversible in males and females. Diffuse hyperplasia, an expected physiologic response to increased calcitonin demand, was not liraglutide treatment related. In a 2 year repeat subcutaneous dose carcinogenicity study of 0.03, 0.2, 1, or 3 mg/kg/day liraglutide, the NOAEL for proliferative c-cell lesions was 0.03 mg/kg/day with minimal to marked focal c-cell hyperplasia occurring at $\geq$ 0.2 mg/kg/day in males and females, c-cell adenomas occurring at $\geq$ 1 mg/kg/day in males and females, and c-cell carcinomas occurring at 3 mg/kg/day in females. In the 2 year study, focal c-cell hyperplasia was considered a preneoplastic lesion because:

1. the incidence and severity of focal hyperplasia increased with dose in both males and females.
2. focal hyperplasia occurs at lower doses than c-cell tumors
3. the incidence of focal c-cell hyperplasia in mice with adenomas in the 3 mg/kg/day group was 56% in males and 33% in females.
4. in decedents, a finding of c-cell hyperplasia preceded c-cell tumors by 17 weeks in both males and females.

Cynomolgus Monkeys

Subcutaneously administered liraglutide had no effect on plasma calcitonin, thyroid c-cell proliferation, or calcium homeostasis parameters including plasma calcium and iPTH in studies up to 87 weeks long. Four mechanistic studies were performed: 1) immunohistochemical colocalization of calcitonin and GLP-1 receptor immunoreactivity in thyroid and pancreas tissue sections, 2) in situ hybridization determining GLP-1 receptor transcript levels in c-cells of thyroid tissue sections and pancreatic tissue, 3) quantifying c-cells in thyroid tissue sections from control and high dose monkeys from a pivotal 52 week repeat dose toxicity study, and 4) determining calcium homeostasis parameters (plasma calcium, iPTH, and calcitonin) and thyroid histopathology in monkeys treated with 0, 0.25, or 5 mg/kg/day liraglutide for up to 87 weeks.

In a dedicated study characterizing thyroid c-cells in male and female cynomolgus monkeys (study 205121), the sponsor determined calcitonin immunoreactive c-cells were primarily located in the middle third of each thyroid lobe in clusters of 2 to 10 cells attached to thyroid follicular epithelium or in parafollicular positions (cell clusters between follicles). type(s) labeled by [^{125}I]GLP-1(7-36) were not identified.

GLP-1 receptors were not localized on thyroid c-cells in monkeys. An immunohistochemical colocalization study using monkey thyroid tissue slices was equivocal for colocalization of GLP-1 receptor and calcitonin immunoreactivities on the same cells because GLP-1 receptor immunoreactivity was weak and the specificity of the anti-GLP-1 receptor antibody, K100B, was not demonstrated (study 204370). The specificity of K100B, a polyclonal rabbit anti-human GLP-1 receptor antibody, was not adequately demonstrated because; 1) the antibody stained pancreas from GLP-1 receptor knockout mice and 2) immunohistochemical staining in the presence of the peptide antigen used to generate the antibody did not block staining. An in situ hybridization study of GLP-1 receptor mRNA in tissue sections from monkeys was equivocal with undetectable levels of GLP-1 receptor transcript in thyroid, but much higher levels in pancreas, a positive control (study 20040515PR4).

Repeat subcutaneous dosing of up to 5 mg/kg/day liraglutide for up to 87 weeks in cynomolgus monkeys had no effect on plasma calcitonin or thyroid c-cells. In a definitive 52 week chronic toxicity study in monkeys, there were no thyroid c-cell proliferative lesions or plasma calcium changes. PCNA immunohistochemical staining thyroid tissue from control group and 5 mg/kg/day high dose monkeys in the 52 week study showed liraglutide had no effect on c-cell proliferation. In an 87 week mechanistic study identifying calcitonin immunoreactive c-cells in thyroid tissue sections of monkeys treated with 0, 0.25, or 5 mg/kg/day liraglutide, high plasma liraglutide levels interfered with the anti-liraglutide antibody screening and neutralization assays, and in the absence of any pharmacodynamic effect, the inability to characterize the anti-liraglutide antibody response confounds interpretation of the study. In the 87 week study, single or repeat doses of 0.25 or 5 mg/kg liraglutide had no effect on plasma calcium, plasma calcitonin, plasma iPTH, or calcium-induced secretion of calcitonin or iPTH. At the end of 87 weeks, liraglutide had no effect on macroscopic or microscopic pathology of calcitonin immunoreactive thyroid c-cells.

2.6.6.8 Special Toxicology Studies: Mechanistic studies of liraglutide-induced thyroid c-cell proliferative lesions

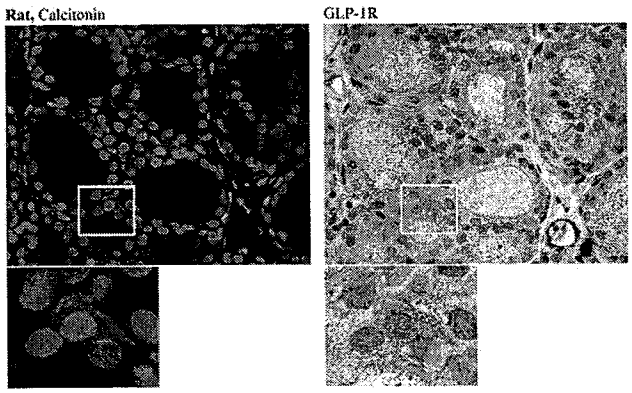
GLP-1 Receptor Localization and Signaling

- 204370 / An immunohistochemical investigation of the GLP-1R in tissue from mice, rats, cynomolgus monkeys, and humans

Immunohistochemical studies of thyroid tissue sections from mice, rats, cynomolgus monkeys and humans using anti-calcitonin antibody to identify c-cells and an anti-GLP-1 receptor antibody to determine if c-cells express GLP-1 receptors were inconclusive. A

fluorescent Alexa488-labeled anti-human calcitonin polyclonal antibody and a primary rabbit anti-human GLP-1 receptor polyclonal antibody (recognizing the receptor's amino terminus) labeled with chromogenic secondary goat anti-rabbit secondary antibody coupled to HRP (biotinylated antibody, avidin coupled HRP) were used for colocalization. Three different rabbit anti-human GLP-1 receptor antibodies were produced. Due to species differences in GLP-1 receptor immunoreactivity, antibody K100B was used to stain human, mouse, and monkey tissues and K102B was used for rat tissues. Pancreas tissue slices were used for GLP-1 receptor positive controls in all 4 species. Results using human tissues were already presented. Specificity of both anti-GLP-1R polyclonal antibodies (K100B and K102B) was not demonstrated because in both cases, staining was weak and staining wasn't blocked or it was only partially blocked by preincubation with the antigenic peptide. Furthermore, K100B stained cells in thyroid and pancreas from GLP-1R knockout mice, and Western blot of GLP-1R containing cell lines using K102B didn't demonstrate receptor specific labeling.

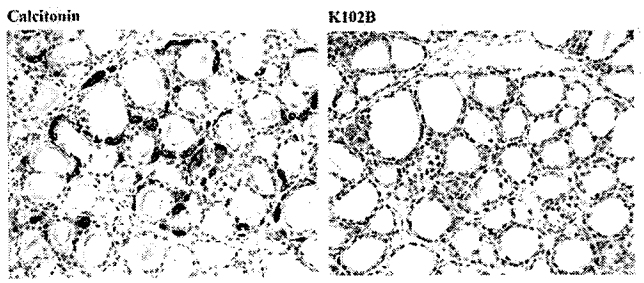
The figures below show specific calcitonin staining (left) and only weak GLP-1R staining in rat thyroid.



[N000 4.2.3.7.3 P19]

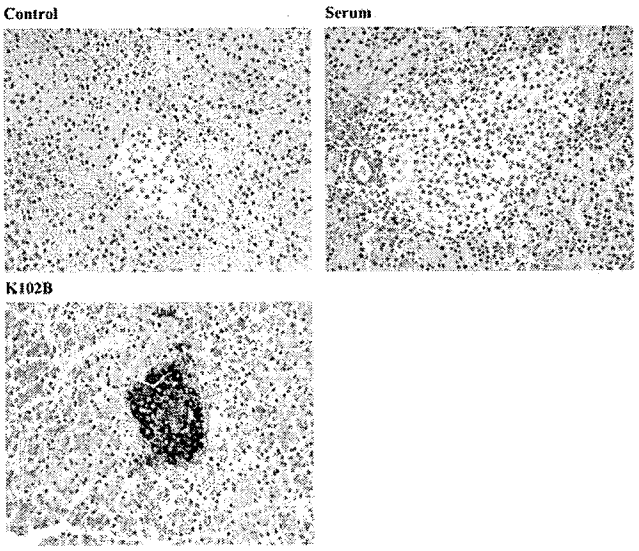
Figure 7 shows calcitonin immunoreactivity in rat thyroid was strongly labeled, but GLP-1 receptor labeling with K102B was weak with questionable specificity because staining wasn't blocked by preabsorption of the antibody with the GLP-1 receptor peptide antigen (data not shown). Figure 8 shows K102B stained specific cells in rat pancreas, a GLP-1R positive control, but stained cell types were not determined.

Figure 7 Thyroid gland serial sectioned, rat



[N000 4.2.3.7.3 P25]

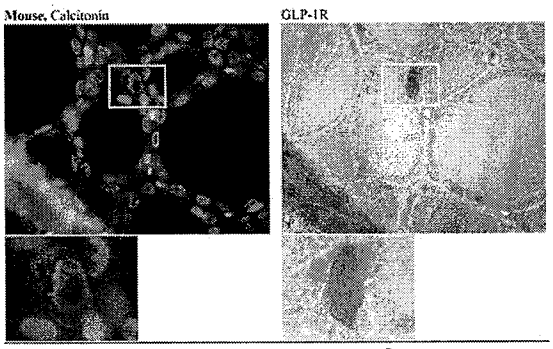
Figure 8 Pancreas serial sectioned, rat



[N000 4.2.3.7.3 P26]

In mouse thyroid tissue sections stained with Alexa488-labeled anti-human calcitonin polyclonal antibody or K100B, a polyclonal anti-GLP-1R antibody, calcitonin staining had high background levels(Figure 1, left). GLP-1R staining was evident in one calcitonin positive cell (Figure 1, right, enclosed in a square), but other cells not labeled by anti-calcitonin antibody were also stained by K100B.

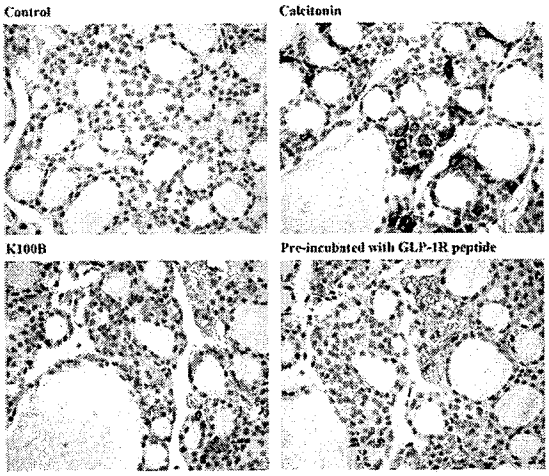
Figure 1 Mouse and rat thyroid gland, double staining



[N000 4.2.3.7.3 P19]

Figure 3 shows calcitonin immunoreactive cells in mouse thyroid, but GLP-1 receptor immunoreactivity with K100B was weak and specificity wasn't clearly demonstrated because preabsorption of the antibody with the GLP-1R peptide antigen had little effect on staining.

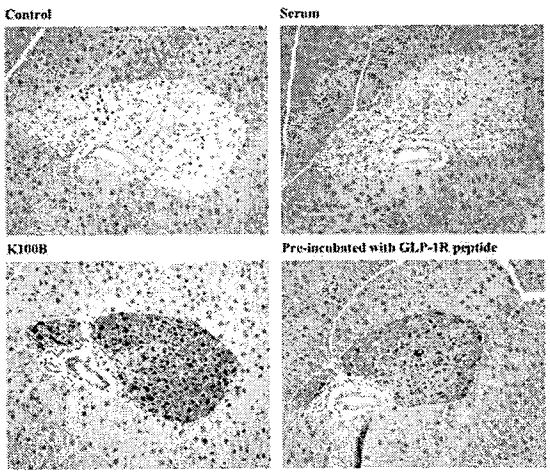
Figure 3 Thyroid gland serial sectioned, mouse



[N000 4.2.3.7.3 P21]

Figure 4 shows specific cell staining by K100B in mouse pancreas, a GLP-1R positive control. However, the specificity of K102B was not clearly demonstrated because staining was only partially blocked by preincubating the antibody with the antigenic peptide.

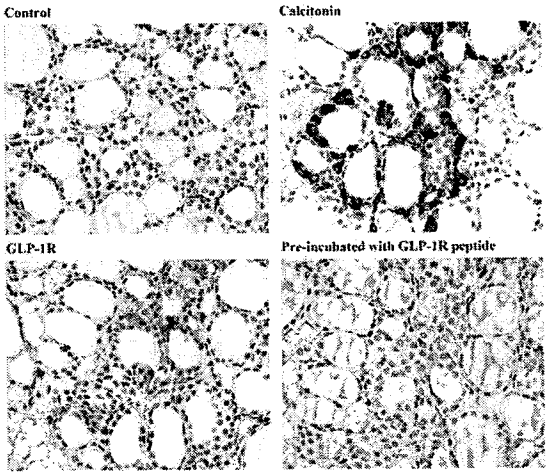
Figure 4 Pancreas serial sectioned, mouse



[N000 4.2.3.7.3 P22]

K100B cell staining in thyroid and pancreas tissue sections from GLP-1 receptor knockout mice, GLP-1R negative control tissues, was similar to wild type mice. GLP-1 receptor staining in GLP-1R knockout mice was attributed to expression of a non-functional GLP-1 receptor amino terminus. Figure 5 shows calcitonin immunoreactive cells in thyroid tissue sections from GLP-1R knockout mice, but GLP-1 receptor labeling with K100B was weak, although preabsorption of the antibody with the GLP-1 receptor peptide antigen did inhibit staining to a greater extent than in thyroid from wild-type mice.

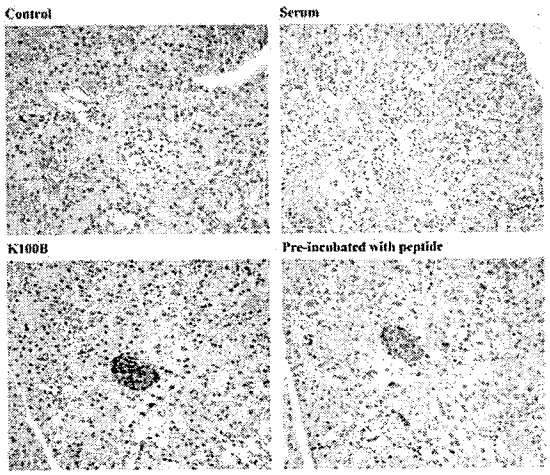
Figure 5 Thyroid gland serial sectioned. KO-mouse



[N000 4.2.3.7.3 P23]

K100B labeled cells in pancreas from GLP-1R knockout mice, but labeled cell types were not identified. Preabsorption of K100B with the peptide antigen partially decreased islet staining.

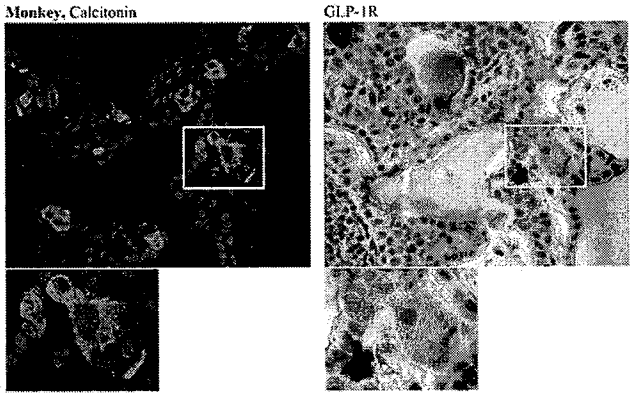
Figure 6 Pancreas serial sectioned. KO-mouse



[N000 4.2.3.7.3 P24]

Results of immunohistochemical studies colocalizing calcitonin and GLP-1 receptor immunoreactivity in thyroid tissue sections from cynomolgus monkeys to identify GLP-1 receptors on c-cells was equivocal. GLP-1 receptor immunoreactivity may not be confined to calcitonin immunoreactive cells and not all identified c-cells reacted with the anti-GLP-1 receptor antibody. A fluorescent Alexa488-labeled anti-human calcitonin polyclonal antibody and a primary rabbit anti-human GLP-1 receptor polyclonal antibody (antibody K100B directed against the receptor's amino terminus) labeled with chromogenic secondary goat anti-rabbit secondary antibody coupled to HRP (biotinylated antibody, avidin coupled HRP) were used for colocalization. Figure 2 shows specific calcitonin staining (left) and weak GLP-1 receptor staining (right). In some instances, calcitonin immunoreactive cells were not GLP-1 receptor immunoreactive and visa versa.

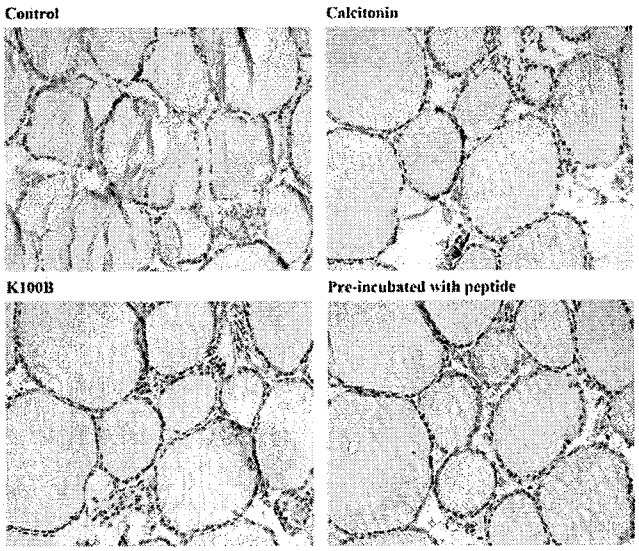
Figure 2 Cynomolgus Monkey and human thyroid gland, double staining



[N000 4.2.3.7.3 P20]

Figure 9 shows K100B labeled specific cells in monkey thyroid, but GLP-1 receptor labeling with the K100B polyclonal antibody was weak and specificity wasn't demonstrated because preabsorption of the antibody with the GLP-1 receptor peptide antigen had little effect on staining.

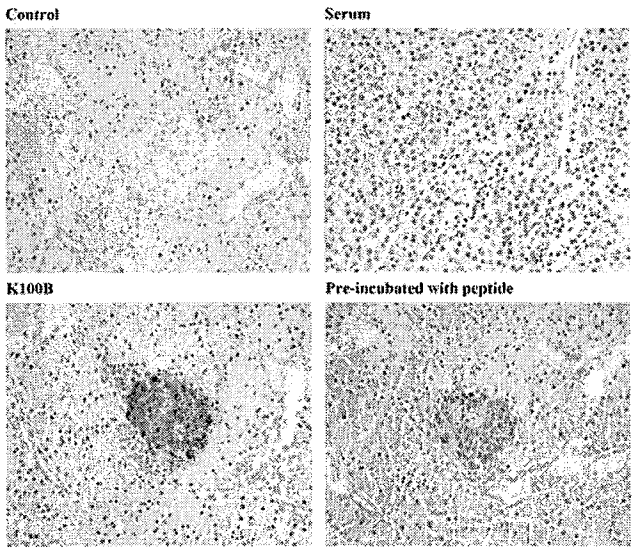
Figure 9 Thyroid gland serial sectioned, Cynomolgus monkey



[N000 4.2.3.7.3 P27]

Figure 10 shows K100B labeled specific cells in monkey pancreas, a GLP-1 receptor positive control. Pancreas cell types labeled by K100B were not identified. Specificity of K100B immunoreactivity was not clearly demonstrated because staining was only partially blocked by preincubating the antibody with the antigenic peptide.

Figure 10 Pancreas serial sectioned, Cynomolgus monkey



[N000 4.2.3.7.3 P28]

- 20040515PR4 / Investigation of GLP-1 receptor mRNA expression in mouse, rat cynomolgus monkey, and human thyroid C-cells and in pancreatic islets studied by in situ hybridization

In situ hybridization of species specific ^{35}S-labeled riboprobes to GLP-1 receptor mRNA was evaluated in paraffin-embedded thyroid tissue sections from mice, rats, cynomolgus monkeys, and humans. Thyroid c-cells were identified by indirect fluorescent microscopy after staining with an Alexa488-coupled anti-calcitonin antibody. In situ hybridization to pancreatic islets served as a positive control for GLP-1 receptor probes and hybridization of an ^{35}S-labeled riboprobes to calcitonin served as a control for mRNA quality in thyroid tissue. An ^{35}S-labeled probe to cyclophilin, a low to medium abundance transcript, served as a addition control for mRNA quality in samples of thyroid and pancreas.

Thyroid c-cells from mice, rat, cynomolgus monkeys, or humans have very low to undetectable levels of GLP-1 receptor transcript. Evidence of GLP-1 receptor mRNA in thyroid tissue was equivocal in all species tested. Results are summarized in Table 1 (below). GLP-1 receptor mRNA levels in calcitonin-positive thyroid cells (identified by immunofluorescence after staining with anti-calcitonin antibody) determined by autoradiography was weak in mice and rats and undetectable in monkeys and humans. In situ hybridization using a calcitonin mRNA probe showed thyroid cells stained with anti-calcitonin antibody contained calcitonin mRNA demonstrating thyroid tissue was suitable for in situ hybridization.

Table 1 Summary of results with GLP in situ hybridisation and control experiments

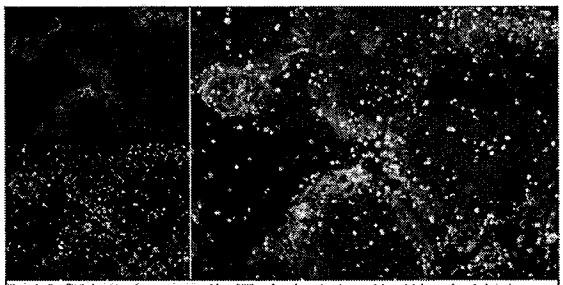
Species	In-situ hybridisation with ^{33}S labelled riboprobe to mRNA in tissue, with or without double labelling by immunofluorescent antibody to calcitonin in thyroids.									
	Tissue	GLP-1R mRNA (5-6 weeks exposure)			Calcitonin mRNA (3-6 days exposure)			Cyclophilin mRNA(2-3 weeks exposure)		
		as	s §	calc IFL	as	s	calc IFL	as	s	calc IFL
Mouse	pancreas*	very strong	OK					strong	OK	
	thyroid	weak	OK	strong	very strong	OK	strong	very strong	OK	strong
Rat	pancreas*	medium	OK					strong	OK	
	thyroid	weak	OK	strong	very strong	OK	strong	very strong	OK	strong
Cynomolgus monkey	pancreas	medium	OK					strong	OK	
	thyroid	below limit	-	strong	strong	OK	strong	strong	OK	strong
Human	pancreas	weak	OK					strong	OK	
	thyroid	below limit	-	strong	strong	OK	strong	strong	OK	strong

*) Pancreas sections from mice and rats were only exposed for 20-25 days with the autoradiographic emulsion before development

§) OK for hybridisations of sense ribobrobes means that the signal over islets in pancreas or C-cells in thyroids was similar to surrounding tissue elements.

[N000 4.2.3.7.3 P35]

Although the sponsor claims GLP-1 receptor mRNA was detectable in thyroid c-cells identified by anti-calcitonin antibody staining, Figure 3 shows colocalization of GLP-1 receptor mRNA and calcitonin was equivocal due to high background staining by the anti-calcitonin antibody and weak to undetectable autoradiographic signals in cells. As a positive control, anti-sense rat GLP-1 receptor radiolabeled probes labeled cells in rat pancreas (Figure 16, left) and an antisense calcitonin radiolabeled probe labeled anti-calcitonin immunoreactive cells (Figure 8, left) in rat thyroid tissue sections. Sense probes for GLP-1 receptor and calcitonin were inactive in both tissues (left panels in Figures 16 and 8).



Top left: Calcitonin demonstrated by IFL after incubation with rabbit anti-calcitonin-Alexa488 complex; lower left: silver grains in autoradiographs after in-situ hybridisation with ^{35}S labelled antisense riboprobe to ratGLP-1R; right: composite of the IFL and in-situ hybridisation frames. Cryostat sections.

Figure 3 Rat thyroid GPL-1R mRNA co-localisation with C-cell calcitonin IFL

[N000 4.2.3.7.3 P17]