CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-341

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO
NDA: 22-341
Submission date: May 23, 2008
Drug: liraglutide
Sponsor: Novo Nordisk Inc.
Indication: treatment of type 2 diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Background comments:
Liraglutide is a recombinant human GLP-1 analog. It is lipidated and, consequently, is resistant to peptidase degradation thus it has a prolonged half-life compared to unmodified GLP-1. The pharmacology/toxicology reviewer and supervisor recommend that liraglutide not be approved for the treatment of type 2 diabetes mellitus. The primary concern is that liraglutide produced increased thyroid C-cell tumors in rats and mice.

Carcinogenicity:
Nonclinical studies of liraglutide included assessment of carcinogenic potential in two-year rat and two year mouse studies. These studies were conducted by subcutaneous injection which is the route of administration in humans. The protocols for these studies were reviewed by the executive carcinogenicity assessment committee. The high doses were selected based on the maximum tolerated dose in male rats and on achieving an AUC in female rats and male and female mice of greater than 25 fold the human AUC. Male and female Sprague Dawley rats received doses of 0 (vehicle), 0.075, 0.25, or 0.75 mg/kg/day. Male and female CD-1 mice received doses of 0, 0.03, 0.2, 1, and 3 mg/kg/day.

Liraglutide significantly increased the incidence of thyroid C-cell adenomas in male and female rats at ≥0.25 mg/kg, C-cell carcinoma in males at 0.75 mg/kg, and combined C-cell adenomas and carcinomas in males and females at ≥0.25 mg/kg. The doses of 0.075, 0.25 and 0.75 produced systemic exposures in rats of approximately 0.5, 2 and 8 fold the human AUC.

Liraglutide significantly increased the incidence of thyroid c-cell adenomas at ≥ 1 mg/kg in male and female mice and C-cell adenomas and carcinomas (combined) at ≥ 1 mg/kg in females. The doses of 0.03, 0.2, 1 and 3 mg/kg produced systemic exposures in mice of approximately 0.2, 2, 10 and 45 fold the human AUC.

The executive carcinogenicity assessment committee reviewed these studies and although there was some doubt that AUCs of greater than 25 fold the human

AUC were achieved, the studies were found to be acceptable since the doses were clearly high enough to elicit a carcinogenic response.

The occurrence of the same tumors in both sexes of both species at relevant human exposures raises the possibility that patients may be at increased risk for this tumor. The applicant conducted a number of nonclinical studies to explore the mechanism of tumor formation and its relevance to humans. The applicant's initial theory was that liraglutide bound to GLP-1 receptors on thyroid C-cells and increased the synthesis and secretion of calcitonin from these cells. As a result of this activation, the applicant proposed that C-cells proliferated and eventually, with persistent activation, progressed to adenomas and carcinomas. The applicant believed that this mechanism was not relevant to primates including humans. The pharm/tox reviewer concluded that the data submitted by the applicant did not adequately establish this mechanism nor did the data demonstrate that such a mechanism would be irrelevant to humans. The executive carcinogenicity assessment committee agreed that the applicant had not shown convincingly that the tumor findings were irrelevant to humans.

The applicant also noted that, regardless of mechanism, no hyperplasia or tumors were observed in monkey studies of up to 87 weeks duration. However, the monkey data for liraglutide is not particularly reassuring. Although the data on mechanism provided by the applicant is not definitive, the highly specific nature of the tumor induction and the lack of genotoxicity suggest that the mechanism is most likely related to an exaggerated pharmacologic effect. The only systemic tumors that were increased in the studies were thyroid C-cell tumors and it is reasonable to expect at least some binding and activation of thyroid C-cells by GLP-1 receptor agonists based on the collective data. The applicant provided data that showed a lack of GLP-1 receptors in the thyroids of monkeys. However, published information has shown that the GLP-1 receptor can be detected in human thyroids and in human medullary thyroid tumors. If a compound was causing an increase in tumors in multiple tissues by some general mechanism then a lack of tumor findings in the monkey might be somewhat reassuring; however, liraglutide is producing a very specific signal which suggests that a very specific mechanism is the cause. The monkey data would be reassuring if it were clear that the monkey accurately represented the human with regard to the suspected mechanism. Since the currently available data suggests that at least a subset of humans have GLP-1 receptors in the thyroid while it is not clear that monkeys do, it does not appear that additional monkey studies would be informative unless it can be shown that they express GLP-1 receptors in their thyroids similarly to the subset of humans that express the receptor in the thyroid.

The concern about the tumorigenic potential of liraglutide would be diminished if it was clearly demonstrated that it produced tumors by a mechanism that was not relevant to humans. The concern of using liraglutide for a particular duration might also be diminished if it could be shown that there was a duration of treatment during and after which the probability of tumorigenesis was low.

Liraglutide is a nongenotoxic carcinogen therefore it is possible that early preneoplastic events induced by liraglutide could be reversible. Studies of up to 26 weeks in rats did not show preneoplastic effects in the thyroid; however, thyroid C-cell hyperplasia was noted in mouse studies of 4 to 13 week duration. The reversibility of the hyperplasia was assessed in mice treated for 9 weeks. The hyperplasia was mostly although not completely reversed after a 15 week recovery period. Whether any of the lingering thyroid effects would have progressed to adenoma or carcinoma is unknown.

Conclusions:
The thyroid C-cell tumors observed in the mouse and rat raises the concern that patients taking liraglutide may be at increased risk for this tumor. Relevance to humans is not firmly established but it appears possible that at least a segment of the population could be at increased risk. Additional nonclinical studies may help better define the risk. An understanding of the mechanism by which the tumors are induced and of its relevance to humans could assist in determining risk. In addition, demonstration that tumors did not develop in a responsive animal species after subchronic exposure followed by long term observation may help determine whether the risk of tumorigenesis might be relatively low for patients treated for a limited duration even if the mechanism of tumor formation is relevant to humans.

The pharm/tox reviewer and supervisor concluded that liraglutide should not be approved unless the sponsor can demonstrate that the tumors are not of human relevance.

Options for approval:
I agree that the tumor findings are significant enough to warrant further evaluation of the risk. One option would be to require this evaluation prior to approving the drug. Once additional studies were completed, the relevance and risk could be reassessed. This would prevent any risk to patients until the drug was later approved, if it was approved at all.

If clinical benefit is considered great enough, then another possible option would be to approve the drug with postmarketing commitments to further assess the relevance and risk. Once additional studies were completed, the relevance and risk could be reassessed and further regulatory action considered, if any. This would potentially place some patients at an increased risk of tumor development. The exact number of patients at risk and level of risk would be unknown. It may be possible that the risk would be relatively low for the period of time during which further studies were being conducted even for those patients expressing GLP-1 receptors in the thyroid given that liraglutide is a nongenotoxic carcinogen in animals. In addition, limiting the maximum duration that patients should be treated with liraglutide and excluding patients with pre-existing thyroid disease could further reduce the risk. Enhanced clinical monitoring for thyroid tumor development until the issue of human risk is further resolved might also permit

liraglutide use while minimizing the number of patients that develop malignant tumors. However, it is acknowledged that it would be challenging to incorporate, as a part of routine therapy, adequate and clinically acceptable monitoring for preneoplastic thyroid effects and thyroid tumors.

Possible additional nonclinical studies:
A number of nonclinical studies might be conducted to address the relevance and risk of the thyroid C-cell tumors and the division has been considering these possibilities. A plan would need to be developed with the applicant. The primary areas to consider are:
1. Additional studies that establish a mechanism of action and the relevance of the mechanism to humans.
2. Studies that establish that the thyroid GLP-1 receptor expression in the thyroid is an appropriate model of human thyroid GLP-1 receptor expression and tumor development for those humans that do express GLP-1 receptor in the thyroid. A lack of tumor development in monkeys expressing GLP-1 receptor in the thyroid could then be used to support a treatment duration of relatively low risk in humans.
3. Studies that establish a duration of treatment which does not result in tumor development during or after the period of treatment. That is, that the preneoplastic events do not progress in the absence of continued treatment. This might be demonstrated by treating mice for various durations and following lifetime tumor development.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22341	ORIG-1	NOVO NORDISK INC	VICTOZA (LIRAGLUTIDE)

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/s/

PAUL C BROWN
01/22/2010

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: July 13 , 2009
From: Karen Davis-Bruno PhD; Pharmacology Supervisor; DMEP
To: NDA 22-341 Victoza (liraglutide)
Re: Supervisor's Memo Pharmacology/Toxicology Review NDA 22-341; A. Parola; PhD

Glucagon-like peptide-1 (GLP-1) is a circulating endogenous peptide of 30 amino acid sequence (7-36 amide) that is secreted from epithelial L-cells in the distal small intestine and colon in response to food. GLP-1 improves glycemic control by stimulating glucose-dependent insulin secretion, increases insulin synthesis, inhibits glucagon secretion, slows gastric emptying and acid secretion and decreases food consumption through the GLP-1 receptor (GLP-1R). This receptor is widely distributed throughout the body (e.g. alpha, beta, delta cells of the pancreas, peripheral and central nervous system, heart, kidney, type II pneumocytes, parietal cells). Various signal transduction pathways have been associated with GLP-1R depending on the tissue. Systemic activity of GLP-1 is limited because it is readily degraded (t1/2<2 minutes) by a pervasive endopeptidase (membrane, soluble forms) known as dipeptidylpeptidase-4 (DPP-4) and subsequent renal clearance. Liraglutide is a GLP-1 analogue that is lipidated so that it is resistant to metabolism by DPP-4 (t1/2 =hours). Its chemical structure tends to promote self-association following subcutaneous injection slowing systemic absorption which in conjunction with high protein binding tends to further increase its elimination half-life particularly limiting its renal excretion.

Liraglutide has been evaluated in pharmacology, acute and chronic, genetic, and reproductive toxicology studies and carcinogenicity studies consistent with a development program for a new molecular entity for chronic use. A series of mechanistic studies were performed to explore liraglutide-induced thyroid C-cell tumors observed in rodent carcinogenicity studies.

Clinical Relevance of Rodent Thyroid C-cell Tumors

Liraglutide was negative in a series of genetic toxicity evaluations. Liraglutide has demonstrated dose-related, carcinogenic potential in both genders with multiple rodent species (rat, mouse) at multiple tissue sites (thyroid C-cell, skin) following life-time treatment with liraglutide at clinically relevant systemic exposures. In rats, thyroid C-cell adenomas and carcinomas occur at low multiples of the proposed clinical exposure. In mice thyroid C-cell adenomas occur at 10-times (males, females) and carcinomas 45-times (females) the clinical exposure. The earliest appearance of thyroid C-cell carcinoma occurred after 15 months treatment in an early decedent mouse from the carcinogenicity study. Mechanistic studies using adolescent (age 2 months) or adult (age 8 months) rats exhibit tumors after 7 months of treatment. Thyroid C-cell hyperproliferative changes (hyperplasia, adenoma, carcinoma) are rare findings (<1%) in mouse carcinogenicity studies. Diffuse and focal hyperplastic responses as well as adenomas are common findings in aging rats, however malignant C-cell carcinoma is a rare finding (<1%) in a rat carcinogenicity study.

Mechanistic studies were performed to establish a mode of action for the rodent tumors based on liraglutide-induced GLP-1 receptor mediated calcitonin synthesis and secretion. The thyroid C-cell hyperplasia with progression to tumors was attributed to an increased release of calcitonin in the thyroid. These mechanistic studies have been extensively reviewed by DMEP, CDER Executive Carcinogenicity Assessment Committee (ECAC) and discussed at an April 2009 Advisory Committee as well as a CDER Regulatory briefing June 2009, convened to evaluate the safety and efficacy of liraglutide. The outcome of these series of reviews is that the mechanistic studies were inadequate to support the sponsor's proposed mechanism of action and that there was insufficient evidence to demonstrate that the C-cell tumors were rodent specific and not therefore relevant to humans. NovoNordisk has acknowledged that a mechanism has not yet been established to explain the thyroid tumorigenicity.

Recent data under review from other GLP-1 receptor agonists with longer half-lives (t1/2) than endogenous GLP-1 peptide as well as sustained release formulation of short-acting GLP-1 analogues (i.e. exenatide), suggest that persistent receptor activation is associated with thyroid C-cell hyperproliferation in rodents. Monkeys have not shown proliferative thyroid C-cell lesions following liraglutide treatment up to 20 months at >60 times human exposure. Caution needs to be exercised in interpreting the relevance of monkey toxicities studies to address a potential human risk for carcinogenicity. These monkey studies were not powered nor designed to evaluate carcinogenicity. The duration of treatment in these primate studies was only 5% of a monkey's lifespan whereas in the rodent carcinogenicity studies many more animals were evaluated following lifetime exposure which more closely mimics the intended therapeutic use of liraglutide as anti-diabetic agent. Furthermore, liraglutide was immunogenic in monkeys but not in mice or rats. Anti-liraglutide antibodies were shown which cross react with GLP-1 after 12 months of liraglutide treatment and the neutralizing effects of these anti-drug antibodies were not assessed with regard to liraglutide exposure in these monkeys.

Dr. Parola's Pharmacology/Toxicology review of the available nonclinical data recommends not approving this marketing application. I agree with his recommendation based on the clear drug-related carcinogenicity signal observed in rodent life-time bioassays at relevant therapeutic exposures. He concludes that the mechanistic data presented regarding thyroid C-cell tumors were inconclusive in substantiating these findings as rodent specific. Therefore by inference these findings are potentially clinically relevant as there is an absence of supporting evidence otherwise. He has recommended that NovoNordisk perform further mechanistic studies to establish their claim that these findings are rodent specific. Suggestions for further studies have been discussed with NovoNordisk at the end of review meeting held June 2009. These suggestions are included in Dr. Parola's review under section I.B. Recommendations for nonclinical studies. The nonclinical deficiency lies in the inability to dismiss the rodent carcinogenicity findings as rodent specific and therefore of human relevance until proven otherwise by supportive studies of the sponsor's design and at their discretion. The sponsor suggests that clinical monitoring for calcitonin is an appropriate biomarker for hyperproliferative thyroid C-cell lesions. However calcitonin has not been established as

an adequate predictive biomarker for these lesions. Human medullary thyroid carcinoma (MTC) is mediated by activating rearranged during transfection (RET) mutations. There may be a correlative increase in plasma calcitonin in these patients, however the elevation is not considered predictive of the MTC diagnosis. It is unclear if drug-induced medullary carcinoma follows the same mechanisms as RET associated MTC.

Fibrosarcomas, Local Tolerance and Impurities Concerns

Liraglutide is administered by daily subcutaneous injection. Dose-related malignant fibrosarcomas were observed in male mice at the injection site as well as in the dorsal skin and subcutis. The incidence of injection site fibrosarcomas in high dose male mice exceeded concurrent and historical controls and reached statistical significance at 45-times clinical systemic exposure by trend test and not pair-wise comparisons. These tumors were not considered drug related by ECAC. Inflammation was not noted at the injection sites in mice above concurrent control incidence. Injection site reactions were described as subacute inflammation and fat necrosis in pigs and monkeys. In primates thickening at the injection site with inflammation, necrosis and fibrosis were noted with continued dosing. This has relevance in that Dr. Parola's Pharmacology/Toxicology review indicates that the mouse carcinogenicity study was performed using a liraglutide dosing solution that was 10-times less concentrated than the clinical formulation. Other repeat dose toxicology studies used dilute dosing solutions of liraglutide relative to the clinical concentration suggesting that the local toxicity of liraglutide may not have been thoroughly assessed in the nonclinical program. Furthermore, different drug lots were used in the various nonclinical studies which may have diverse impurity profiles. NovoNordisk groups the impurities in liraglutide and specifications as a group, rather than on the individual impurity. Since the sponsor has structurally identified these impurities (see pg. 15 Table 1 of review) it isn't clear why the specification was established for groups of impurities.

Subchronic animal studies qualify the general toxicity profile up to the proposed drug product specifications. However impurities were not qualified in stand alone genetic toxicology studies (in vitro) and the carcinogenicity studies establish a positive drug-related thyroid C-cell tumorigenic response in both species at low clinical exposures. A statistically significant incidence by trend analysis for skin/subcutis fibrosarcomas are seen in male mice at 45-times clinical exposure only. This might reflect the concentration of impurity or this may be coincidental. The sponsor has identified the grouped impurities components as [redacted]. These are unlikely to have a structural alert for genotoxicity. [redacted] are generally exempt from genotoxicity testing according to ICHS6 guidelines on biologics. Liraglutide is a lipidated peptide synthesized by acylating a recombinant peptide. Subcutaneously injected anti-diabetic agents (i.e. insulin) are commonly associated with local injection site reactions under clinical use. While an extensive evaluation of injection site reactions may not have been assessed in the nonclinical development program the clinical formulation has been evaluated in the clinical trials without significant concern and therefore additional nonclinical testing of local toxicity and impurities genotoxicity may not be necessary. The fibrosarcoma incidence and thyroid C-cell tumors are described in the labeling comments.

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Karen Davis-Bruno
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22,341**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**5/23/2008**
PRODUCT:	**Victoza® (liraglutide for injection)**
INTENDED CLINICAL POPULATION:	**Adults with Type 2 Diabetes Mellitus**
SPONSOR:	**Novo Nordisk Inc., Princeton, NJ**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Metabolic and Endocrine Products (HFD-510)**
PHARM/TOX REVIEWER:	**Anthony Parola, PhD**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, PhD**
DIVISION DIRECTOR:	**Mary Parks, MD**
PROJECT MANAGER:	**John Bishai, PhD**

Date of review submission to Division File System (DFS): July 10, 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. **Recommendation on approvability:** Based on the nonclinical data, this application is not approvable because there is insufficient nonclinical information about liraglutide to determine if it is safe for chronic use. In 2-year lifetime exposure carcinogenicity studies, liraglutide caused thyroid C-cell tumors in mice and rats at clinically relevant exposures. The human relevance of liraglutide-induced rodent C-cell tumors is unknown, and mechanistic studies performed by the applicant did not mitigate this risk.

B. **Recommendation for nonclinical studies:**

1. The proposed mode-of-action for liraglutide-induced rodent thyroid C-cell tumors based on drug-induced calcitonin secretion driving C-cell hyperplasia and tumor formation was not supported by mechanistic studies. The applicant should determine a mode-of-action for liraglutide-induced rodent C-cell tumors and evaluate the human relevance of rodent C-cell tumors based on this mode-of-action. These studies may include evaluating:
    a. the effect of liraglutide on REarranged during Transfection (RET) protooncogene signaling in normal and focal hyperplastic and/or neoplastic thyroid C-cells in mice and/or rats. Consider determining if liraglutide alters phosphorylation of tyrosine residues in RET important for C-cell proliferation / transformation, such as Y1062. In humans, RET mutations constitutively activating its tyrosine kinase activity are a common cause of spontaneous and inherited medullary thyroid carcinoma.
    b. GLP-1 receptor expression in normal, focal hyperplastic, and neoplastic C-cells in liraglutide treated mice and/or rats. A published study showed 100% of rats, but only 60% of mice had detectable GLP-1 receptor in their thyroid, but liraglutide induces C-cell focal hyperplasia and tumors in both rats and mice. Whether or not a thyroid GLP-1 receptor is required for liraglutide's proliferative effects on C-cells in mice or rats is unknown.
    c. GLP-1 receptor dependence of liraglutide-induced thyroid C-cell focal hyperplasia and/or neoplasia in mice and/or rats. Consider determining if liraglutide-induced C-cell focal hyperplasia occurs in mice or rats treated with a GLP-1 receptor antagonist or in GLP-1 receptor knockout mice.

2. Provide evidence that local toxicity after repeat subcutaneous injection with liraglutide was adequately assessed in nonclinical studies. In chronic repeat-dose toxicity studies, liraglutide caused irreversible injection site reactions in monkeys using drug formulations that were at least 3-times more dilute than the clinical formulation. Liraglutide caused fibrosarcomas in the dorsal skin and subcutis in high dose male mice in a 2-year subcutaneous dose carcinogenicity study and these tumors were attributed to local toxicity due to high drug concentration at or near the injection site. The concentration of liraglutide in the high dose drug formulation in the mouse study was 0.6 mg/mL, 10-times more dilute than the clinical formulation (6 mg/mL).

3. Some liraglutide impurities were not qualified in genetic toxicity studies. Evaluate the *in vitro* genetic toxicity of liraglutide impurities at impurity levels consistent with drug substance and drug product acceptance criteria.

C. Recommendations on labeling

8.1 Pregnancy

Pregnancy Category C

Liraglutide has been shown to cause abnormalities in fetal rats at maternal systemic exposures 0.8 times the human exposure resulting from the maximum recommended human dose (MRHD) of 1.8 mg/day based on AUC. Liraglutide has been shown to cause reduced fetal growth, fetal abnormalities and it increased total major abnormalities in fetal rabbits at maternal systemic exposures 0.2 times the human exposure at the MRHD. There are no adequate and well-controlled studies in pregnant women. Liraglutide should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Female rats given s.c. doses of 0.1, 0.25 and 1.0 mg/kg liraglutide once a day beginning 2 weeks-before mating to gestation day 17 had estimated systemic exposures 0.8, 3, and 11 times the human exposure at the MRHD of 1.8 mg/day based on AUC. The number of early embryonic deaths slightly increased in the 1 mg/kg high dose group. Fetal abnormalities in kidneys and blood vessels, irregular ossification in the skull, and a more complete state of ossification in bones occurred at all doses. Mottled liver and minimally kinked ribs occurred at the highest dose. The incidence of malformations, including misshapen oropharynx or narrowed larynx at 0.1 mg /kg and umbilical hernia at 0.1 and 0.25 mg/kg, exceeded concurrent and historical controls.

Pregnant rabbits given s.c doses of 0.01, 0.025 and 0.05 mg/kg liraglutide from gestation day 6 through day 18 had maternal systemic exposure less than 1 times the human exposure at the MRHD of 1.8 mg/day based on AUC, at all doses. Liraglutide decreased fetal weight and dose-dependently increased total major fetal abnormalities at all doses. The incidence of malformations exceeded concurrent and historical controls at 0.01 mg/kg (kidneys, scapula), $\geq$ 0.01 mg/kg (eyes, forelimbs), 0.025 mg/kg (brain, tail and sacral vertebrae, major blood vessels and heart, umbilicus), $\geq$ 0.25 mg/kg (sternum), and at 0.05 mg/kg (parietal bones, major blood vessels). Irregular ossification and/or skeletal anomolies occurred in the skull and jaw, vertebrae and ribs, sternum, pelvis, and scapula. Visceral anomalies occurred in blood vessels, lung, liver, esophagus, tail, and gall bladder.

In pregnant rats given doses of 0.1, 0.25 and 1.0 mg/kg liraglutide from gestation day 6 through lactation day 24 (weaning), liraglutide delayed parturition to gestation day 22 in the majority of treated rats, and decreased maternal food consumption and body weight gain during gestation, but not during lactation, at all doses. F_1 generation body weight decreased at all dose levels from postpartum day 7 to week 16 in males and from day 7 to week 10 in females. Bloody scabs and agitated behavior occurred in F_1 generation males descended from 1 mg/kg liraglutide treated rats. Body weight of F_2 generation rats descended from liraglutide-treated females trended lower than controls from birth to postpartum day 14, but differences never reached statistical significance.

8.3 Nursing mothers

It is not known whether liraglutide is excreted in human milk. Because many drugs are excreted in human milk, the potential for clinically significant adverse reactions in nursing infants from liraglutide exists. A decision should be made whether to discontinue nursing or to discontinue liraglutide, taking into account the importance of

liraglutide to the lactating woman. Studies in lactating rats have demonstrated that liraglutide is excreted unchanged in milk at levels approximately 50% of those in maternal plasma. Use caution when administering liraglutide to nursing women.

13 Nonclinical Toxicology

13.1 Carcinogenicity, Mutagenicity, Impairment of Fertility

A 104-week carcinogenicity study was conducted in male and female CD-1 mice at doses of 0.03, 0.2, 1, and 3 mg/kg/day liraglutide administered by s.c. bolus injection yielding systemic exposures 0.2, 2, 10, and 45 times the human exposure, respectively, resulting from the maximum recommended human dose of 1.8 mg/day liraglutide based on plasma AUC comparison. A dose-related increase in benign thyroid C-cell adenomas was seen in the 1 and 3 mg/kg groups with an incidence of 13% and 19% in males and 6% and 20% in females, respectively. C-cell adenomas did not occur in control groups or 0.03 and 0.2 mg/kg groups. The incidence of combined C-cell adenomas and carcinomas increased in 1 and 3 mg/kg group females because carcinomas occurred in 2 of 76 females in the 3 mg/kg dose group. A treatment-related increase in fibrosarcomas was seen on the dorsal skin and subcutis, the body surface used for drug injection, in males in the 3 mg/kg group. This effect was attributed to a high local concentration of injected drug. The liraglutide concentration in the clinical formulation (6 mg/mL) is 10 times higher than the concentration in the formulation used to administer 3 mg/kg liraglutide to mice in the carcinogenicity study (0.6 mg/mL).

A 104-week carcinogenicity study was conducted in male and female rats at doses of 0.075, 0.25 and 0.75 mg/kg/day liraglutide administered by s.c. bolus injection yielding systemic exposures of 0.5, 2, and 8 times, respectively, the human exposure resulting from the maximum recommended human dose of 1.8 mg/day, based on plasma AUC comparison. A treatment-related increase in benign thyroid C-cell adenomas was seen in the 0.25 and 0.75 mg/kg males and in all female dose groups. An increase in thyroid C-cell carcinomas was observed in all liraglutide dose groups of males and in females at 0.25 and 0.75 mg/kg. The incidence of combined C-cell adenomas and carcinomas was increased at 0.25 and 0.75 mg/kg in males and at all doses in females.

Liraglutide was negative with or without metabolic activation in the Ames test for mutagenicity or in a human peripheral blood lymphocyte chromosome aberration test for clastogenicity. Liraglutide was negative in repeat dose *in vivo* micronucleus tests in rats.

In a rat fertility study, subcutaneously injected doses of 0.1, 0.25 and 1 mg/kg liraglutide were administered to males 4 weeks prior to and throughout mating and the same doses were administered to females for 2 weeks prior to mating until gestation day 17. No direct adverse effects on male fertility were observed at up to 1 mg/kg, a systemic exposure 11 times the human exposure resulting from the MRHD of 1.8 mg liraglutide daily, based on AUC. An increase in early embryonic deaths occurred at 1 mg/kg in female rats. Reduced body weight gain and food consumption were observed in females at the 1 mg/kg dose.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Liraglutide (NN2211 or NNC 90-1170) is a lipidated human GLP-1 analog with prolonged pharmacologic activity after subcutaneous bolus injection. Liraglutide's

extended duration of action is due to it's resistant to peptidases that inactivate it, dipeptidyl peptidase IV (DPP-4) and neutral endopeptidases (NEPs), it's highly plasma protein bound which decreases renal excretion and provides further protection from peptidases, and because self-association at subcutaneous injection sites slows its absorption. Biologic effects of liraglutide are mediated by the GLP-1 receptor, a G-protein coupled receptor. *In vitro*, liraglutide is a selective GLP-1 receptor agonist active at subnanomolar concentrations in the absence of plasma proteins and at nanomolar concentrations in their presence. *In vitro*, liraglutide is pharmacologically active at cloned GLP-1 receptors from mice, rats, rabbits, pigs, monkeys, and humans, and *in vivo*, in animal models of type 2 diabetes and obesity. Chronically administered liraglutide did not cause hypoglycemia in rats treated for up to 6 months at human exposure multiples (HEM) up to 8X based on plasma liraglutide AUC comparison at the maximum recommended human dose (MRHD) of 1.8 mg/day or cynomolgus monkeys treated for up to12 months at human exposure multiples up to 72X.

Liraglutide was formulated as a solution for subcutaneous injection. Bioavailability of subcutaneously injected liraglutide was 53% in monkeys and 55% in humans. Peak and total liraglutide exposure generally increased linearly with dose with no appreciable accumulation in mice, rats, or monkeys and with some accumulation in humans due to its longer elimination half life (~1.5 fold). Liraglutide was highly plasma protein bound in all species (> 98%). Liraglutide did not readily cross the blood brain barrier and only very low levels were found in the CNS. Liraglutide circulates as the intact parent drug without forming any major circulating human metabolite. Liraglutide is primarily eliminated by peptidase metabolism (neutral endopeptidase and DPP IV) with further extensive and rapid catabolism of intermediate metabolites prior to excretion in urine, feces, and expired air. Only low levels of liraglutide cross the placenta in rats or rabbits. Intact liraglutide was secreted in milk from rats. Levels of liraglutide in rat milk were ~50% of maternal plasma levels.

Safety and toxicity of liraglutide was evaluated in safety pharmacology studies, single and repeat dose toxicity studies, genetic toxicity studies, 2 year carcinogenicity studies in rats and mice, reproductive and developmental toxicity studies, local tolerance studies, and mechanistic studies of liraglutide-induced thyroid C-cell tumors in rodents. A 4 week repeat dose toxicity study in rats was performed to qualify liraglutide drug substance impurities.

In safety pharmacology studies, liraglutide increased heart rate in isolated rabbit hearts and in conscious rats. In rats, liraglutide increased arterial blood pressure and decreased body temperature. Liraglutide had no effect on the QTc interval in conscious telemetered monkeys. In rats, liraglutide had a diuretic effect. Liraglutide weakly inhibited acetylcholine-induced smooth muscle contraction of isolated guinea pig ileum and *in vivo*, it delays gastric emptying in minipigs.

Liraglutide was well tolerated in chronic repeat dose toxicity studies multiples of human exposure up to 8X in rats and up to 72X in monkeys. Clinical signs of toxicity and reduced food consumption were dose-limiting in rats. A dose limiting toxicity was not observed in mice. Transiently reduced food consumption and decreased body weight gain were dose limiting in rabbits and monkeys. In clinical studies, gastrointestinal adverse effects including nausea, diarrhea, vomiting, and dyspepsia were dose-limiting and the most common cause of subject withdrawal from clinical studies.

Liraglutide was not immunogenic in mice or rats, but anti-liraglutide antibodies that cross-reacted with GLP-1 occurred in one mid-dose monkey and several high dose monkeys in chronic repeat dose studies. Neutralizing effects of anti-liraglutide antibodies were not characterized. Immunogenicity in animal studies is not predictive for immunogenicity in humans.

Liraglutide toxicity occurred in thyroid (mice and rats), at injection sites (mice, pigs, and monkeys) and it induced a mild anemia (mice, rats, and monkeys). In thyroid, liraglutide caused ultimobranchial cysts and/or C-cell focal hyperplasia, a preneoplastic lesion, in 4 & 13 week mouse studies, a low incidence of inflammatory cell infiltrate in mice in the carcinogenicity study, and C-cell focal hyperplasia and tumors at clinically relevant exposures in carcinogenicity studies in both mice and rats. Human relevance of drug-induced C-cell tumors in rodents is unknown, and the applicant was unable to establish a mode of action for liraglutide-induced rodent C-cell tumors. Injection site reactions were described as subacute inflammation and fat necrosis in pigs, and in monkeys, thickening at the injection site with inflammation, necrosis, and fibrosis with continued dosing. Although inflammation didn't occur at injection sites in mice, fibrosarcomas in the dorsal skin and subcutis, the body surface used for injection, occurred in high dose male mice in the carcinogenicity study using a liraglutide dosing solution that was 10 times more dilute than the clinical formulation. Mild anemia, characterized by decreased RBC count, hemoglobin, and hematocrit occurred at clinically relevant exposures in some repeat dose studies in mice, rats, and monkeys. In the 13-week mouse toxicity study, anemia was likely due to hemolysis. Liraglutide thyroid toxicity was attributed to its GLP-1 receptor agonist activity, but injection site reactions and anemia were not.

Liraglutide was not mutagenic or clastogenic *in vitro* with or without metabolic activation in an Ames test or a chromosomal aberrations assay in human peripheral blood lymphocytes and it was not clastogenic *in vivo* in erythrocyte micronucleus assays in rats.

Two-year carcinogenicity studies in mice and rats showed liraglutide is a multi-sex, multi-species carcinogen causing thyroid C-cell tumors at clinically relevant exposures in male and female mice and rats and fibrosarcomas on the dorsal skin and subcutis of male mice. Human relevance of rodent tumor findings is unknown.

Liraglutide did not affect fertility of male rats, but in female rats treated with liraglutide from 2 weeks prior to mating through organogenesis, liraglutide increased the number of early embryonic deaths at maternal plasma exposures 11 times the human exposure, based on AUC comparison. In fetal rats, liraglutide caused fetal abnormalities of displaced kidneys, displaced azygous vein, and irregular ossification in the skull and a more complete state of ossification at all doses yielding maternal plasma human exposure multiples ≥ 0.8. At the highest dose, mottled liver and minimally kinked ribs occurred at 11-times the human exposure. Major abnormalities of misshapen oropharynx or narrowed larynx occurred at 0.8-times human exposure and umbilical hernia occurred at 0.8 and 3-times human exposure. Pregnant rabbits were treated with liraglutide during organogenesis using doses yielding maternal plasma exposures < 1X human exposure. Liraglutide decreased fetal weight and increased the incidence of total major fetal abnormalities at all doses, (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 liraglutide, respectively). Irregular ossification and/or skeletal abnormalities occurred in the skull and jaw, vertebrae and ribs, sternum, pelvis, and scapula. Visceral abnormalities occurred in blood vessels and gall bladder.

In a prenatal and postnatal toxicity study of 0, 0.1, 0.25, or 1 mg/kg liraglutide administered to parental F_0 rats from gestation day 6 through weaning on lactation day 24, liraglutide delayed delivery to day 22 in the majority of treated rats and decreased F_1 generation pup weight at all doses during the lactation period.

B. Pharmacologic activity

Endogenous glucagon-like peptide-1 (GLP-1) is a 30- or 31-amino acid peptide incretin that improves glycemic control by stimulating glucose-dependent insulin

secretion (incretin effect), increasing insulin synthesis, inhibiting glucagon secretion, slowing gastric emptying and acid secretion, and decreasing food consumption and body weight gain. The 30-amino acid amidated form and the 31-amino acid glycine extended form of GLP-1 are equipotent, but in humans, GLP-1(7-36)amide is the predominant circulating active GLP-1. Effects of GLP-1 are mediated by a G-protein coupled receptor, the GLP-1 receptor. GLP-1 increases glucose-dependent pancreatic beta cell insulin secretion and synthesis by a cAMP-dependent mechanism. Systemic effects of GLP-1 are limited by rapid metabolism to GLP-1(9-36)amide by DPP-4, further degradation by neutral endopeptidase, and excretion in urine.

Liraglutide is a lipidated human GLP-1(7-37) analog with an extended duration of action permitting once a day dosing in humans. Liraglutide improves glycemic control by increasing glucose-dependent insulin secretion, inhibiting glucagon secretion at high glucose levels, increasing pancreatic beta cell mass in hyperglycemic animals, inhibiting cytokine or fatty acid induced beta cell apoptosis, and slowing gastric emptying. Like GLP-1, liraglutide decreases food consumption and body weight gain.

In vitro, liraglutide is a potent, selective, GLP-1 receptor agonist in cells expressing cloned GLP-1 receptors from mice, rats, rabbits, monkeys, and humans. Liraglutide dose-dependently stimulates glucose-dependent insulin secretion from perifused mouse islets and inhibits cytokine or fatty acid-induced apoptosis of neonatal pancreatic beta cells from rats. In animal models of type 2 diabetes, liraglutide improves glycemic control by increasing glucose-dependent insulin secretion, inhibiting glucagon secretion at high glucose levels, increasing pancreatic beta cell mass in hyperglycemic animals, inhibiting cytokine or fatty acid induced beta cell apoptosis, decreasing gastric emptying, and decreasing food consumption and body weight gain. Although its effects on gastric emptying, food consumption, and body weight gain are independent of blood glucose concentration, liraglutide's hypoglycemic effects only occur at elevated blood glucose concentrations. In subchronic nonclinical studies of liraglutide using models of type 2 diabetes, liraglutide improved glycemic control in *ob/ob* mice, *db/db* mice, Zucker diabetic fatty (ZDF) rats, sand rats fed a high calorie diet, and streptozotocin-induced diabetic minipigs and it improved glucose disposal during glucose tolerance tests in ZDF rats, female pigs, and streptozotocin-induced diabetic minipigs. Since liraglutide doesn't affect insulin sensitivity, it was not effective in insulin resistant, non-diabetic Zucker obese rats. Although liraglutide didn't lower blood glucose in non-diabetic rats, it stopped the development of diabetes in pre-diabetic ZDF rats. Liraglutide had only transient effects on beta cell mass in normoglycemic Sprague Dawley rats and did not affect beta cell mass in normoglycemic monkeys treated with high doses for 52 weeks (HEM 72X).

C. Nonclinical safety issues relevant to clinical use

Liraglutide is a nongenotoxic, multisex, multispecies carcinogen. Liraglutide caused thyroid C-cell tumors at low multiples of human exposure in mice (HEM 10X) and rats (HEM < 1X). The NOAEL for thyroid tumor was 0.2 mg/kg liraglutide in mice (HEM 2X), but a NOAEL was not established in rats using a low dose yielding plasma exposures 0.5-times human exposure. To determine human relevance of liraglutide-induced rodent thyroid C-cell tumors, the applicant proposed a novel mode of action based on drug-induced, GLP-1 receptor-mediated calcitonin secretion and synthesis driving C-cell hyperplasia with progression of hyperplasia to tumors. However, mechanistic studies did not adequately support the mode of action and a large majority of members from an April 2009 Advisory Committee convened to evaluate the safety of liraglutide and CDER's Executive Carcinogenicity Assessment Committee (December 2008 meeting) concluded there was insufficient evidence to determine that liraglutide-induced C-cell tumors in mice and rats are not relevant to humans. Recent data from

other long-acting GLP-1 receptor agonists and sustained-release formulations of short-acting agonists suggest persistent GLP-1 receptor activation causes C-cell tumors in rodents. Proliferative C-cell lesions did not occur in monkeys treated for up to 20 months at high multiples of human exposure to liraglutide (HEM 64X).Human relevance of liraglutide-induced thyroid C-cell tumors in rodents is unknown. Plasma calcitonin was a biomarker for liraglutide-induced proliferative C-cell lesions in mice, but not in rats.

Injection site reactions after subcutaneous dosing were characterized by inflammation with or without necrotic or fibrotic changes in pigs after single doses and after repeat dosing in monkeys with increased severity with increased treatment duration. In monkeys, injections site reactions were not reversible. In a 2-year mouse carcinogenicity study, fibrosarcomas in the dorsal skin and subcutis occurred at or near the drug injection site in high dose male mice. The concentration of liraglutide in dosing formulations in repeat dose toxicity studies in monkeys was 2 mg/mL in the 52-week monkey study and 0.6 mg/mL in the mouse carcinogenicity study, 3-fold and 10-fold lower than the liraglutide concentration in the clinical formulation. Injection site reactions occurred in ~2% of clinical trial subjects treated for $\geq$ 26 weeks.

In rats, single doses of liraglutide increased arterial blood pressure and heart rate, and repeat dosing decreased heart weight and caused sporadic increases in plasma CPK at clinically relevant exposures. Liraglutide increased heart weight in male monkeys at clinically relevant exposures and sporadically increased plasma CPK. Changes in heart weight occurred in the absence of correlative histopathology in rats or monkeys. In clinical trials, liraglutide caused a small decrease in systolic blood pressure and a small increase in heart rate.

Liraglutide was immunogenic in monkeys, but not in mice or rats. Anti-liraglutide antibodies that cross-react with GLP-1 occurred in monkeys after 12 or 20 months of drug exposure, and neutralizing effects of the antibodies were not determined. Immunogenicity in nonclinical studies is not predictive of immunogenicity in humans. Approximately 8.6% of clinical trial subjects develop antibodies, but antibody formation was not associated with reduced efficacy.

Liraglutide is excreted intact in rat milk. Liraglutide increases early embryonic deaths, it decreases fetal weight in rabbits, and it causes fetal abnormalities in rats and rabbits at clinically relevant exposures. In a multigeneration prenatal and postnatal toxicity study in rats, liraglutide delayed delivery from pregnant F_0 rats (from day 21 to day 22), decreased pup weight in the F_1 generation during the nursing period, and it was associated with bleeding scabs and agitated behavior in F_1 males descended from 1 mg/kg liraglutide-treated F_0 females.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22,341
Sequence number/date/type of submission:
000/May 23, 2008/Original NDA
035/June 22, 2009/Response to Nonclinical Information Request

Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Novo Nordisk Inc., 100 College Road West, Princeton, NJ 08540
Manufacturer for drug substance: Novo Nordisk A/S Halla Alle, DK-4400 Kalundborg, Denmark

Reviewer name: Anthony L Parola, PhD
Division name: Metabolism and Endocrinology Products
HFD #: 510
Review completion date: July 8, 2009

Drug:

Trade name: Victoza
Generic name: Liraglutide
Code name: NNC 90-1170, NN2211
Chemical name: Glycine, L-histidyl-L-alanyl-L-α-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-α-aspartyl-L-valyl-Lseryl-L-seryl-L-tyrosyl-L-leucyl-L-α-glutamylglycyl-Lglutaminyl-L-alanyl-L-alanyl-N^{6}-[N-(1-oxohexadecyl)-L-γ-glutamyl]-L-lysyl-L-α-glutamyl-L-phenylalanyl-L-isoleucyl-L-alanyl-L-tryptophyl-L-leucyl-L-valyl-L-arginylglycyl-Larginyl-

OR

$N^{\varepsilon 26}$-(N-hexadecanoyl-L-γ-glutamyl)-[34-L-arginine] glucagon-like peptide 1-(7-37)-peptide

CAS registry number: 204656-20-2
Molecular formula/molecular weight: $C_{172}H_{265}N_{43}O_{51}$ 3,751.20 Da
Structure:

b(4)

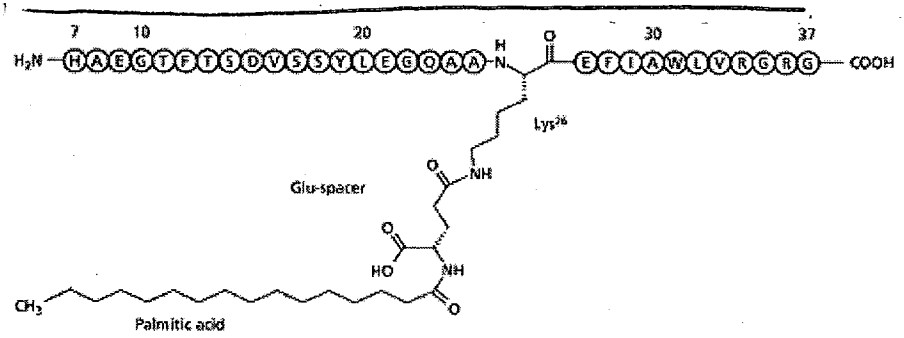


Figure 1 The chemical structure of liraglutide

[N000 3.2.S.1.2 P3]

Drug class: GLP-1 receptor agonist
Intended clinical population: Adults with type 2 diabetes mellitus
Clinical formulation: liraglutide solution for injection
Route of administration: Subcutaneous injection
Sponsor's Maximum Proposed Clinical Dose: 1.8 mg liraglutide/day (Cmax 44 nM, AUC_{0-24h} 809 nM.h) from Table 11-16 from clinical trial report NN2211-1608.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Pivotal safety and toxicity studies reviewed within this submission:

Safety Pharmacology
- 990263 / Modified Irwin screen test in the mouse
- 980091 / Evaluation of NNC 90-1170 on respiration in conscious rats
- 205242 / Effect on hERG tail current recorded from stably transfected HEK293 cells
- 980422 / Effects on QT interval and MAP duration in isolated perfused rabbit hearts
- 990264 / Effect on cardiovascular function in the telemetered rat
- 980092 / Evaluation of NNC 90-1170 on cardiovascular function in conscious cynomolgus monkeys
- 990262 / Effect on the renal function in the rat
- 201207 / Effect on the isolated ileum in the guinea pig

Single Dose Toxicity
- 980178 / Acute subcutaneous toxicity test in mice
- 980175 / Acute subcutaneous toxicity test in rats

Repeat Dose Toxicity
- 204082 / 13 week toxicity study in mice with subcutaneous administration
- 980189 / 13 week subcutaneous toxicity study in rats with recovery period
- 200239 / 26 week subcutaneous toxicity study in rats
- 990191 / 13 week subcutaneous toxicity study in the cynomolgus monkey with a recovery period
- 200241 / 52 week subcutaneous toxicity study in cynomolgus monkeys with a 4 week recovery period

Genetic Toxicity
- 980191 / NNC 90-1170 glipacyl reverse mutation in four histidine-requiring strains of Salmonella typhimurium and two tryptophan-requiring strains of Escherichia coli – Ames
- 203114 / Induction of chromosome aberrations in cultured human peripheral lymphocytes
- 980192 / Induction of micronuclei in the bone marrow of treated rats
- 990072 / Assessment of micronucleus frequencies on microscope slide preparations from rats

Carcinogenicity
- 204229 / 104 week carcinogenicity study in mice with subcutaneous administration (Appendix A)

- 200240 / 104 week carcinogenicity study in rats with subcutaneous administration (Appendix B)

Reproductive and Developmental Toxicity

- 990284 / Main segment I/II subcutaneous reproduction study in rats
- 990055 / Developmental toxicity study in rabbits
- 201109 / Pre and post natal study in rats (subcutaneous administration)

Local Tolerance

- 204291 / Local toxicity of 3 Phase 3 formulations with pH 7.7, 7.9, and 8.15 two and 5 days after subcutaneous injections in pigs

Special Toxicity

Mechanistic Studies:Rodent Thyroid C-cell tumors (Appendix C)

Impurities

- 205092 / 4 week toxicity study in rats with subcutaneous administration (bridging study)

Drug Substance

Liraglutide is produced from recombinant DNA technology by expressing the Arg^{34}GLP-1[7-37] precursor in yeast (Saccharomyces cerevisiae), purifying the peptide precursor from the [redacted] then acylating Arg^{34}GLP-1[7-37] [redacted] Lys^{26}. Liraglutide is purified by [redacted]. The reader is referred to the Chemistry Review for detailed review of drug substance and drug product manufacturing, characterization, and quality control. b(4)

Drug substance bioactivity of liraglutide and related impurities was assessed in a cell-based assay measuring its potency to increase intracellular cAMP in BHK cells expressing a recombinant human GLP-1 receptor (GLP-1R). Log-dose response curves for the test material and a liraglutide reference material were fit using a 4 parameter logistic curve, and bioactivity of the test material was expressed relative to the reference sample. Specific bioactivity is expressed as the ratio of bioactivity / RP-HPLC assay value (mg active material / mg determined by HPLC.). The reference sample has a specific bioactivity of 1.

Drug substance acceptance criteria are shown in Table 2.

Table 2 Parameters and Limits

Parameters	Analytical Procedure and No	Limits/Acceptance criteria
Appearance	Visual Inspection A3105b	
Identity	Peptide Mapping A3678a	
Identity	RP-HPLC A3678a	
Content	RP-HPLC A3678a	
Content based on a dried basis	RP-HPLC A3678a/Calculation	
Specific bioactivity	cAMP assay B2002a	
Sum of liraglutide related impurities	RP-HPLC A3678a	
	RP-HPLC A3678a	
	RP-HPLC A3678a	
	RP-HPLC A3678a	
	RP-HPLC A3678a	
	RP-HPLC A3678a	
	RP-HPLC A3678a	
High Molecular Weight Protein	SE-HPLC A6005b	
Bacterial Endotoxins	Ph.Eur. Method D, USP, JP Kinetic chromogenic Method	
Total Viable Count	Pour Plate Method Ph.Eur., USP, JP	
Host Cell Protein[1]	B813a	

[1]

[N000 3.2.S.4.5 P9]

b(4)

Description of Liraglutide-Related Impurities

b(4)

[N000 3.2.S.1.3 P4]

b(4)

The applicant used drug substance and drug product acceptance criteria based on impurity groups and not individual impurities, and this was accepted (see Chemistry Reviews).

4 Page(s) Withheld

✓ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

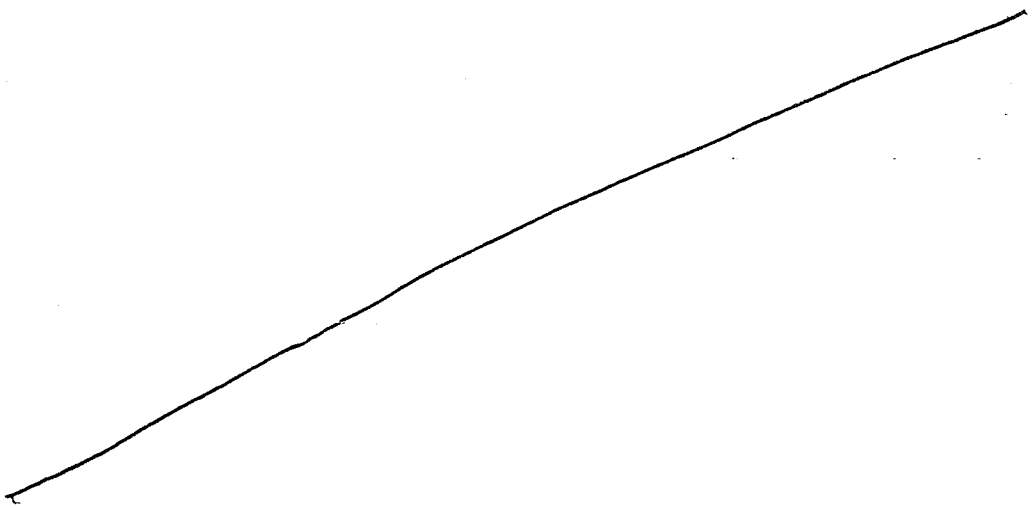
_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox-1

Table 2 Impurity profile in Drug Substance or Drug Product Batches used in genetic toxicity studies.

Study No.	NN980191	NN203114	NN980192	NN990072 (specimens from NN980183)	Acceptance criteria



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[N035 P8]

The toxicity of impurities was adequately evaluated in repeat-dose nonclinical studies, but not in genetic toxicity assays.

Drug Product

Liraglutide 6.0 mg/mL Solution for Injection, 3 mL Cartridge

Table 1 shows the composition of liraglutide solution, including active ingredients and excipients.

Table 1 Composition of Liraglutide 6.0 mg/ml, 3 ml cartridge

Name of ingredients	Quantity per mL	Function	Reference to standards
Active substance			
Liraglutide	6.0 mg	Active drug substance	Novo Nordisk A/S
Excipients			
Disodium phosphate, dihydrate	1.42 mg		Ph. Eur., USP
Phenol	5.5 mg *Note 1*		Ph. Eur., USP, JP
Propylene glycol	14.0 mg		Ph. Eur., USP, JP
			Ph. Eur., USP, JP
			Ph. Eur., USP, JP
Water for Injections	To make 1.0 ml	Solvent	Ph. Eur., USP, JP

Note 1:

b(4)

[N000 3.2.P.1 P4]

All excipients were listed in the CDER's inactive ingredients database in products for injection at concentrations equal to or higher than in the drug product or in repeat dose toxicity studies.

Drug product acceptance criteria are shown in the summary table below.

Test	Method of Analysis	Acceptance Criteria
Characters:		
Macroscopy	Visual inspection No. A3196e	Complies[1]
Identity of liraglutide	RP-HPLC No. A6016e	Complies[2]
Content of liraglutide	RP-HPLC No. A6016e	Release: [redacted] Shelf life: [redacted]
pH	USP	Release: [redacted] Shelf life: [redacted]
High molecular weight proteins	SE-HPLC No. A6005e	Release: [redacted] Shelf life: [redacted]
Sum of liraglutide related impurities	RP-HPLC No. A6016e	Release: [redacted] Shelf life: [redacted]
Of these:		
Other [redacted] liraglutide related impurities	RP-HPLC No. A6016e	Release: [redacted] Shelf life: [redacted]
Liraglutide related impurities A	RP-HPLC No. A6016e	Release: [redacted] Shelf life: [redacted]
Liraglutide related impurities B	RP-HPLC No. A6016e	Release: [redacted] Shelf life: [redacted]
Liraglutide related impurities C	RP-HPLC No. A6016e	Release: [redacted] Shelf life: [redacted]
Other [redacted] liraglutide related impurities	RP-HPLC No. A6016e	Release: [redacted] Shelf life: [redacted]
Bacterial endotoxins	USP Kinetic Chromogenic method	[redacted]
Sterility	[redacted]	Complies
Identity of preservatives	RP-HPLC No. A6002e	Complies[3]
Phenol	RP-HPLC No. A6002e	Release: [redacted] Shelf life: [redacted]
Freezing point depression	Cryoscopy No. A2495e	[redacted]
Particulate matter [redacted] µm µm	USP	[redacted] container [redacted] container
Dose accuracy	Weighing A2400le	Test of max. dose: 190 - 210 mg* 285 - 315 mg**

b(4)

* [redacted]

** [redacted]

b(4)

[redacted]

[1]: *Complies means verified as liraglutide.*

[2]: *One International Unit (IU) of enkonavin is equal to one Endotoxin Unit (E.U.).*

[3]: *Complies means verified as phenol*

[N000 3.2.P.5.1 P2-3]

Photostability testing shows the drug product (6 mg/mL liraglutide solution in the cartridge) is not photostable, and it should be protected from light by wrapping the cartridge in aluminum foil or inserting it into the pen-injector.

Liraglutide solution is packaged in a 3 mL glass cartridge closed with a [redacted] rubber disk consisting of bromobutyl rubber (in contact with the solution) and polyisoprene rubber. The cartridge is assembled in a pen injector (Figures 1). [redacted] Pen injectors for liraglutide are modified [redacted] delivery systems for subcutaneous injection of insulin and insulin analogs. [redacted] b(4)

b(4)

Formulation 4: 6.0 mg/mL liraglutide solution (measured by reversed phase HPLC) with sodium phosphate [redacted], propylene glycol [redacted], and phenol [redacted] at pH 8.15 in 3 mL [redacted] cartridges. b(4)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Glucagon-like peptide-1 (GLP-1) is a 30- or 31-amino acid peptide primarily secreted from epithelial L-cells in the distal small intestine and colon in response to ingesting food. The 30-amino acid amidated form and the 31-amino acid glycine extended form of GLP-1 are equipotent, but in humans, GLP-1(7-36)amide is the predominant circulating active GLP-1. GLP-1 improves glycemic control by stimulating glucose-dependent insulin secretion (incretin effect), increasing insulin synthesis, inhibiting glucagon secretion, slowing gastric emptying and acid secretion, and decreasing food consumption and body weight gain. The effects of GLP-1 are mediated by a single family B 7 transmembrane G-protein coupled receptor, the GLP-1 receptor (GLP-1R), that is widely expressed; in the pancreas (alpha, beta, and delta cells), peripheral and central nervous systems, heart, kidney, lung (surfactant-secreting type II pneumocytes), and stomach (parietal cells). In pancreatic beta cells, 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 GDP for GTP in the $G\alpha_s$ subunit. The $G\alpha_s$-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 insulin secretion by increasing intracellular calcium (activating L-type calcium channels and ryanodine receptor-dependent and IP_3 receptor-dependent intracellular calcium release) and inhibiting efflux of intracellular potassium through K_{ATP} channels and Kv channels. In pancreatic beta cells, GLP-1R agonists increase glucose-dependent, but not glucose-independent insulin release. Because GLP-1R agonists don't increase the sensitivity of beta cells to glucose, the risk of inducing hypoglycemia is low. Beta cell GLP-1Rs are linked to MAPK signaling, a pathway regulating mitosis, differentiation, and cell survival / apoptosis.

Systemic activity of GLP-1 is limited because dipeptidyl peptidase 4 (DPP-4 or CD26), a widely expressed enzyme with membrane bound and soluble forms, rapidly metabolizes it by cleaving off an N-terminal dipeptide to yield GLP-1(9-36)amide. GLP-1(9-36)amide has weak

insulinotropic effects, but it potently and directly inhibits hepatic glucose production. Neutral endopeptidases also metabolize GLP-1, but to a lesser extent. Because the half-life of GLP-1 in systemic circulation is short, < 2 minutes, the clinical utility of GLP-1 is limited. Exenatide, a subcutaneously injected peptidase-resistant GLP-1 mimetic, is the only GLP-1R agonist approved for marketing in the US.

Liraglutide (NN2211 or NNC 90-1170) is a lipidated GLP-1 analog with prolonged pharmacologic activity after subcutaneous (sc) administration due to its long elimination half-life. Resistance to metabolism by DPP-4 or NEPs, reduced renal excretion, and delayed absorption resulting from self association of the attached lipid improve liraglutide's pharmacokinetic profile and prolong its activity. Liraglutide is highly protein bound in systemic circulation further increasing its resistance to DPP-4 or NEP-mediated hydrolysis and reducing renal excretion.

In vitro, liraglutide was pharmacologically active in all species used in nonclinical studies. In radioligand binding assays, liraglutide binds to the GLP-1R (species unspecified) with subnanomolar potency in the absence of albumin (IC_{50} 0.52 nM) but in the presence of albumin, protein binding reduces the concentration of free drug and decreases its potency 35-fold (IC_{50} 18 nM). Liraglutide is a GLP-1R agonist increasing cAMP accumulation in cells expressing cloned recombinant mouse, rat, rabbit, pig, monkey and human GLP-1Rs in the presence of plasma proteins (EC_{50} 5 – 60 nM). *In vitro* pharmacology screening studies showed liraglutide is GLP-1R selective and it did not interact with off-target sites including ion channels, enzymes, or other G-protein coupled receptors, including the cloned human glucagon receptor. However, its affinity or agonist potency at the human GLP-2 receptor was not determined. Liraglutide(9-37), a hypothetical product of DPP-4 mediated metabolism, was 235-fold less potent than liraglutide at stimulating hGLP-1R mediated cAMP formation, but liraglutide(9-37) was not a major human metabolite *in vitro* or *in vivo*. Liraglutide dose-dependently increased GLP-1R-mediated, cAMP-dependent proliferation of cultured neonatal pancreatic beta cells with a maximal effect occurring at 100 nM. Liraglutide dose-dependently inhibited cytokine or fatty acid-induced apoptosis of neonatal pancreatic beta cells from rats, and the inhibitory effect was GLP-1R-mediated and cAMP-dependent. Liraglutide's glucose-dependent effect on insulin secretion was demonstrated in perifused islets from mice. Nanomolar concentrations of liraglutide increased insulin secretion at 8 and 10 mM glucose, but not at lower glucose concentrations.

In vivo, liraglutide was active in animal models of type 2 diabetes and obesity. Liraglutide decreased food consumption and body weight gain in normal animals, and importantly, it did not cause hypoglycemia in normal or diabetic animals. Liraglutide inhibited the development of diabetes in prediabetic rats. Liraglutide was less effective at lower blood glucose in insulin resistant models of type 2 diabetes.

In normal male Sprague Dawley rats treated with 0.2 mg/kg liraglutide twice a day, liraglutide transiently increased beta cell mass 19% after 1 week, but the effect was absent after 6 weeks of treatment. Liraglutide (0.15 mg/kg) had no effect on energy expenditure or substrate oxidation in normal fasted Sprague Dawley rats. In a 7-day repeat dose study of 0.2 mg/kg liraglutide administered twice a day in normal Sprague Dawley rats, liraglutide reduced body weight gain and food consumption without affecting the respiratory exchange ratio, energy expenditure, bone mineral content, bone area, or bone mineral density. In Wistar rats with monosodium glutamate-induced deficits in hypothalamic arcuate nucleus GLP-1R signaling, sc injection of 0.2 mg/kg liraglutide twice a day for 7 days decreased food consumption, water consumption, body weight, and adiposity.

Liraglutide improved glycemic control in mouse, rat, and pig models of type 2 diabetes, slowed the onset of diabetes in pre-diabetic rats, and reversed diabetes symptoms in diabetic sand rats. Single sc doses of 0.03 to 1 mg/kg liraglutide dose-dependently decreased food intake, blood glucose, and body weight gain in obese, insulin resistant, diabetic, leptin-deficient, ob/ob mice. In female ob/ob mice treated with liraglutide or liraglutide + metformin for 15 days, liraglutide lowered blood glucose and increased insulin, but adding metformin had no additional beneficial

effect over liraglutide alone. In obese diabetic, leptin-receptor deficient db/db mice treated with liraglutide for 15 days, liraglutide increased pancreatic beta cell proliferation resulting in increased cell volume, density, and beta cell fraction. The absence of Pdx-1 expression in the pancreas of liraglutide treated mice suggested increased beta cell mass was not due to transdifferentiation of pancreatic exocrine cells into beta cells. In Zucker diabetic fatty (ZDF) rats, single sc doses of 0.007 to 6.6 mg/kg liraglutide dose-dependently decreased food consumption and blood glucose. In ZDF rats treated with 0.03 or 0.15 mg/kg liraglutide twice a day for 6 weeks, the higher dose decreased food intake and improved glycemic parameters (decreased plasma glucose, decreased HbA1c, increased insulin secretion, and increased the volume of pancreatic beta cells and islets), but paradoxically increased body weight. In candy-fed diet-induced obese Sprague Dawley rats, a model of type 2 diabetes, 0.2 mg/kg liraglutide administered subcutaneously twice a day shifted food preference to normal chow, decreased food consumption, decreased body weight, reversed body weight gain, and reversed body fat gain while maintaining energy expenditure. However, liraglutide had no beneficial effect on glycemic control or pancreatic beta cell mass. In female pigs challenged with an intravenous glucose load 5 hours after a single sc injection of 0.003 – 0.01 mg/kg liraglutide, liraglutide dose-dependently decreased the glucose AUC 20 – 70 minutes after glucose loading in the absence of any substantive increase in plasma insulin. In streptozotocin-induced diabetic Gottingen minipigs, a model of impaired glucose tolerance without insulin resistance, treatment with 0.0033 mg/kg/day liraglutide for 4 weeks increased insulin and decreased plasma glucose. In glucose-clamped Gottingen minipigs with streptozotocin-induced diabetes, 0.002 mg/kg liraglutide increased plasma insulin and decreased plasma glucagon. In 8 week old pre-diabetic ZDF rats, sc injection of 0.2 mg/kg liraglutide twice a day for 15 days inhibited the development of elevated blood glucose, triglycerides, and cholesterol, but without increasing pancreatic beta cell proliferation or mass. Liraglutide lowered body weight, food intake, basal insulin and fructosamine and in an oral glucose tolerance test, liraglutide decreased glucose AUC and increased basal insulin. In Zucker obese (ZO) rats, a model of insulin resistance, single daily sc injections of 0.15 mg/kg liraglutide for 7 days increased plasma insulin, but without effecting resting blood glucose, or without effecting glucose excursions during an oral glucose tolerance test. In sand rats fed a high energy diet, a insulin resistant model of type 2 diabetes, liraglutide dose-dependently reversed symptoms of type 2 diabetes during a 4 week treatment period. During the treatment period, 0.0125 – 0.3 mg/kg/day liraglutide dose-dependently decreased blood glucose in high energy fed sand rats, and treated rats became diabetic 15 days after treatment was stopped.

Liraglutide decreased food consumption, body weight gain, and adiposity in animal models of obesity. In diet-induced obese Sprague Dawley rats treated for 4 weeks with 0.2 or 0.3 mg/kg liraglutide twice a day, liraglutide decreased subcutaneous fat (inguinal, epididymal, mesenteric, and perirenal), decreased plasma triglycerides, elevated free fatty acids, and in an oral glucose tolerance test, it decreased glucose $AUC_{0\text{-}180min}$ and increased insulin secretion. Single daily sc injections of 0.003 – 0.007 mg/kg liraglutide for 7 weeks decreased food consumption, by decreasing the number, duration, and size of meals, and decreased body weight of Gottingen minipigs, a model of extreme hyperphagia.Liraglutide's effects were reversed after treatment was stopped. Food consumption in non-diabetic glucose intolerance rhesus monkeys with middle-age onset obesity was dose-dependently decreased over a 16 day period by 0.01 or 0.03 mg/kg liraglutide with recovery to baseline when treatment was stopped.

The effect of liraglutide on infarcted myocardium was assessed in pigs. In a pig model of myocardial infarction, 0.01 mg/kg liraglutide ad ministered for 3 days prior to infarct had no effect on infarct size, but it slightly increased heart rate and cardiac output after reperfusion.

In pharmacodynamic drug interaction studies, 0.3 and 3 nM liraglutide dose-dependently increased insulin secretion from isolated perfused rat pancreas elicited by 30 nM glipizide, a sulfonylurea. In diabetic male ZDF rats, 6 weeks of treatment with 0.2 mg/kg liraglutide + 5 mg/kg pioglitazone twice a day improved glycemic control (decreased blood glucose, decreased

HbA1c, increased plasma insulin) and improved glucose tolerance better than either treatment alone, but the combination increased weight gain and subcutaneous fat deposits. In ZDF rats treated for 6 weeks, 0.2 mg/kg liraglutide + 30 mg/kg atorvastatin twice a day decreased HbA1c, increased plasma insulin, decreased plasma triglycerides, and decreased plasma cholesterol to a greater extent than liraglutide or atorvastatin alone. Liraglutide (0.1 mg/kg liraglutide twice a day) normalized olanzepine-induced increased food consumption, body weight, subcutaneous fat deposits, fasting plasma glucose, and plasma cholesterol in female Sprague Dawley rats.

Liraglutide was evaluated in neurobehavioral, pulmonary, cardiovascular, renal, and gastrointestinal safety pharmacology studies. Single sc doses up to 2 mg/kg liraglutide in mice had no neurobehavioral effects up to 24 hours after dosing. Liraglutide doses up to 2 mg/kg in mice had no effect on the time to sleep onset or duration induced by 5 mg/kg hexabarbitone or 4.5 mg/kg ethanol. In a pulmonary safety pharmacology study in Sprague Dawley rats, up to 2 mg/kg liraglutide did not affect respiratory parameters including penh, respiratory rate, tidal volume, or minute volume. In cardiovascular safety pharmacology studies, liraglutide did not inhibit hERG channel current *in vitro* and it was not considered proarrhythmogenic in an *ex vivo* study using isolated rabbit hearts treated with up to 1.43 μM liraglutide. In isolated rabbit heart, effects on shortening the QTc interval and decreasing MAP_{90} were attributed to the vehicle, but 1.43 μM liraglutide increased heart rate 6%. In conscious telemetered Sprague Dawley rats subcutaneously injected with a single dose of up to 2 mg/kg liraglutide, 0.2 and 2 mg/kg increased arterial blood pressure, increased heart rate, and decreased body temperature for up to 24 hours after dosing. In conscious telemetered cynomolgus monkeys, single sc doses up to 2 mg/kg had no significant effect on arterial blood pressure, heart rate, ECG intervals (including QTc), or body temperature for up to 22 hours after dosing. In a renal pharmacology study in water-loaded Sprague Dawley rats administered a single sc dose of up to 2 mg/kg liraglutide, proteinuria occurred at the highest dose during the 6 – 24 hour collection period. Liraglutide had a diuretic effect reducing urine osmolarity and specific gravity and increasing urine volume 2 – 6 hours after dosing at 0.02, 0.2, and 2 mg/kg. Urine volume, sodium, potassium, and chloride dose dependently increased at 0.2 and 2 mg/kg 0 – 2 hours after dosing, and in the 0.2 mg/kg group, the effect persisted for 24 hours. In the 2 mg/kg group, there was a rebound effect with increased urine specific gravity and osmolarity, and decreased excretion of sodium and chloride. In an *ex vivo* gastrointestinal safety pharmacology study using isolated strips of guinea pig ileum, the NOEL was 0.29 μM because the highest dose of 1.43 μM liraglutide weakly and reversibly inhibited acetycholine-induced smooth muscle contraction.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Liraglutide is an injectable, lipidated analog of human GLP-1(7-37) that is a GLP-1 receptor (GLP-1R) agonist active at low nanomolar concentrations *in vitro*. *In vivo*, GLP-1R agonists improve glycemic control in animal models and humans with type 2 diabetes mellitus by stimulating glucose-dependent insulin secretion (incretin effect), increasing insulin synthesis, inhibiting glucagon secretion, slowing gastric emptying and acid secretion, and decreasing food consumption and body weight gain. Liraglutide is longer acting than endogenous GLP-1 because it's more slowly absorbed from subcutaneous injection sites due to self-association of liraglutide's lipid moiety, it's resistant to inactivation by peptidases (DPP-4 and NEP), and renal clearance is reduced. Liraglutide is highly plasma protein bound, and protein binding decreases renal excretion and further enhances its resistance to peptidases.

Drug activity related to proposed indication:

In vitro

NNC 90-1170 binding to plasma proteins decreases its GLP-1R affinity and agonist potency. In a filtration format radioligand binding assay, varying concentrations of bovine or human serum albumin decreased affinity of NNC 90-1170, but not GLP-1. Two percent human serum albumin reduced the affinity of NNC 90-1170 by 35-fold from IC_{50} 0.52 nM in the absence of albumin to 18 nM in the presence of albumin. Human plasma decreased the potency of liraglutide to stimulate cAMP formation in BHK cells (fibroblasts derived from Syrian hamster kidneys) expressing the human receptor over 1000-fold from 0.06 nM in the absence of plasma to 100 nM in its presence.

In vitro, NNC 90-1170 was pharmacologically active in species used in nonclinical studies. The potency of NNC 90-1170 to stimulate cAMP formation (measured using the Amersham SPA kit or Perkin Elmer Flashplate assay) in baby hamster kidney (BHK) cells heterologously expressing cloned GLP-1Rs (membranes or whole cells) from mice, rats, rabbits, pigs, monkeys, or humans or in whole cells expressing the was determined in buffer or in plasma from the respective species. Receptor density determined by saturation radioligand binding with [^{125}I]GLP-1 showed receptor density was similar for each species. The potency of NNC 90-1170 to stimulate cAMP formation was 3 – 60-fold lower than GLP-1in all species, but liraglutide retained nanomolar agonist potency to activate the GLP-1. Estimates of GLP-1 binding affinity and agonist potency at the human GLP-1Rare approximately 10-fold higher (higher affinity and more potent) than those reported in published studies (~ 50pM).

GLP-1 Receptor Species	cAMP Accumulation EC_{50} (pM)	
	NNC 90-1170	GLP-1
Human	5 - 60	1 - 2
Mouse	20	2
Rat	24	6
Rabbit	15	3
Pig	9	3
Monkey	5	1

Liraglutide(9-37) was 235-fold less potent than liraglutide at stimulating cAMP formation in membranes from BHK cells expressing the cloned human receptor (EC_{50} 1,680 pM and 7 pM, respectively)(Figure 2, below). The potency of liraglutide(9-37) was similar to GLP-1(9-36)amide. Compared to GLP-1(7-36)amide, GLP-1 (9-36)amide is characterized as a GLP-1R partial agonist or antagonist in most assays. Because the metabolite was derived from liraglutide, it was uncertain if the activity of liraglutide(9-37) was due to contamination with the parent drug.

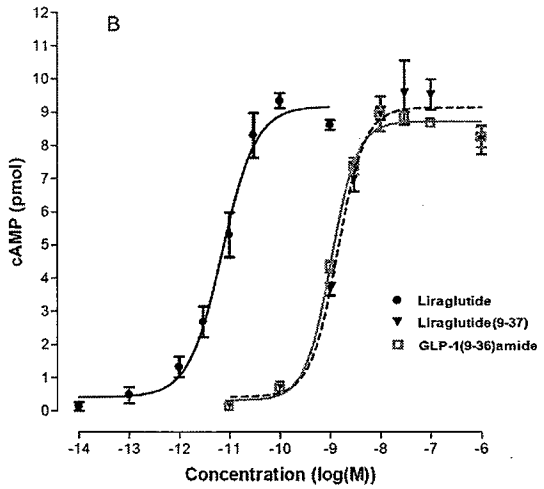


Figure 2 Receptor activity of liraglutide(9-37) and GLP-1(9-36)amide

[15964-045 P8]

[3-Iodo-Tyr19]liraglutide, non-radioactive iodinated liraglutide, was approximately 2-fold less potent than liraglutide at stimulating cAMP formation in human GLP-1R expressing cell membranes (EC_{50} 13 pM for iodinated liraglutide versus 7 pM for liraglutide). Liraglutide, iodinated liraglutide, GLP-1(7-36)amide, liraglutide(9-37) and GLP-1(9-36)amide were equally efficacious at stimulating cAMP accumulation.

NNC 90-1170 dose-dependently increased proliferation of cultured neonatal β-cells from 3 – 5 day old Wistar rats after a 24 hour exposure period. Replicating β-cells were labeled by incorporating BrdU and identified by as immunocytochemical staining for insulin and BrdU. Concentrations of 1, 10, and 100 nM NNC 90-1170 increased β-cell proliferation with a maximal effect occurring at 100 nM (Figure 2, below). The effect of 100 nM NNC 90-1170 was GLP-1R mediated because it was blocked by 1 μM exendin (9-39), a GLP-1R antagonist.. GIP and GLP-1 also caused β-cell proliferation, but glucagon did not. GLP-1 induced proliferation was cAMP-dependent because it was mimicked by 10 μM forskolin and inhibited by 10 nM wortmannin (PI3K inhibitor), 20 μM PD98059 (MEK inhibitor), and 10 μM H89 (PKA inhibitor), but it wasn't inhibited by 20 μM SB203580 (p38 MAPK inhibitor) (Figure 4, below). Treatment with both 500 ng/mL human growth hormone and 10 nM GLP-1 increased proliferation 2.8 fold, compared to ~ 2 fold for either treatment alone.

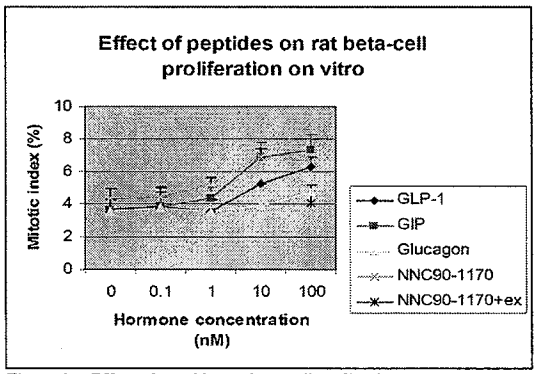


Figure 2 Effect of peptides on beta-cell replication

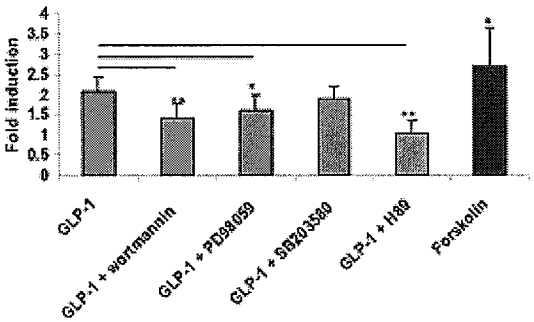


Figure 4 Signalling pathways involved in GLP-1-induced replication

[amp901170 P9,11]

Liraglutide inhibits cytokine or fatty acid-induced apoptosis of neonatal Lewis rat β-cells by cAMP-dependent and PI3K-mediated pathway. Liraglutide and GLP-1 dose-dependently blocked apoptosis induced after a 16 hour incubation with cytokines (Figure 2A, 100 U/mL IFNγ, 100 U/mL TNFα, and 40 U/mL IL-1β) or by fatty acids (Figure 2B, 1 mM 2:1 oleate:palmeate). The anti-apoptotic effects of 1 μM liraglutide was GLP-1R-mediated because it was blocked by 10 μM exendin(9-39) and it was cAMP-dependent because liraglutide's anti-apoptotic effect was mimicked by 10 μM forskolin. Liraglutide's anti-apoptotic effect was mediated by PI3K because it was blocked by the PI3K inhibitor, wortmannin (Figure 5A, B), but not be the MAP kinase inhibitor PD98059 (Figure 5A is cytokine induced apoptosis and 5B is fatty acid induced apoptosis).

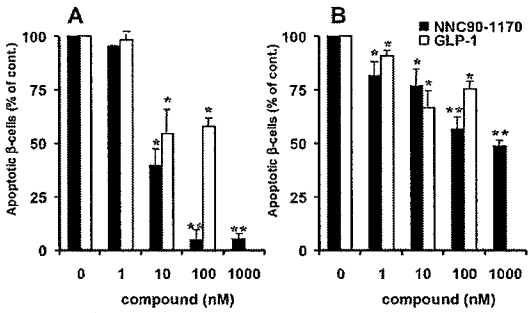


Figure 2 NNC90-1170 prevents cytokine and fatty acid-induced apoptosis in beta-cells

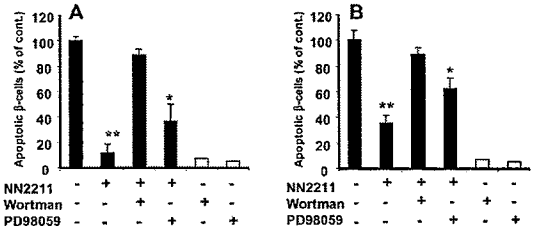


Figure 5 PI3 kinase is involved in the NNC90-1170 signaling cascade

[sqbr231100 P12,15]

Ex vivo

Liraglutide increased glucose-dependent insulin secretion from perifused islets from male NMRI mice. Liraglutide and GLP-1(7-37)amide increased insulin secretion at 8 – 10 mM glucose (Figure 3). In the presence of 10 mM glucose, 1 to 100 nM NNC 90-1170 or GLP-1(7-36)amide dose-dependently increased insulin secretion (Figure 2). The effects of 100 nM NNC 90-1170 or GLP-1(7-36)amide to increase insulin secretion elicited by 10 mM glucose persisted for at least 110 minutes (data not shown).

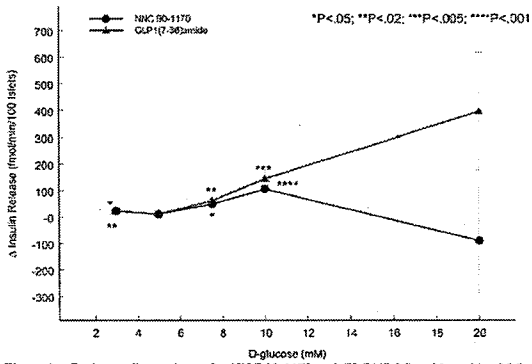


Figure 3 D-glucose Dependency for NNC 90-1170 and GLP1(7-36)amide at 20 mM D-glucose

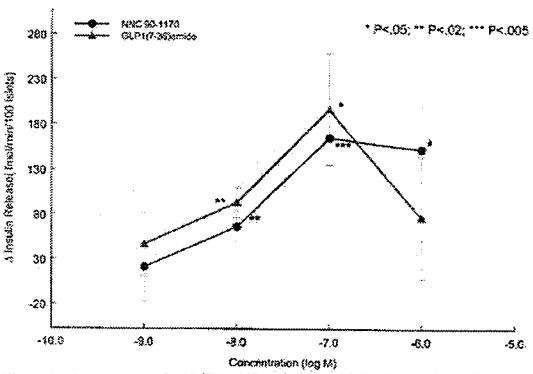


Figure 2 Dose-response for NNC 90-1170 and GLP1(7-36)amide at 10 mM D-glucose

[Jn51998024 P10,11]

Analysis of liraglutide solutions (0.001 – 1.2 mM) by ultracentrifugation, circular dichroism, and NMR spectroscopy indicates the fatty acid side chain drives self-association of liraglutide into heptamers. The apparent K_d for heptamer formation was 0.1 μM.

In Vivo, Normal Animals

Liraglutide increased pancreatic beta cell mass 19% in normal male Sprague Dawley rats treated for 1 week, but the effect was transient because it didn't occur in rats treated for 6 weeks. Effects on pancreatic beta cell mass, alpha cell mass, islet volume, or total pancreas mass were determined after 1 or 6 weeks of treatment with 0 (vehicle) or 0.2 mg/kg liraglutide injected subcutaneously twice a day. Pancreatic beta cells were stained using a guinea pig anti-swine insulin antibody and alpha cells were stained using a rabbit anti-swine glucagon antibody to estimate beta and alpha cell mass and total islet volume (visualized by counterstaining with Meyers hematoxylin). Results are summarized in the table below.

Table 1 All treatment data

	Vehicle 7 days (n=6)	NNC90-1170 7 days (n=6)	Vehicle 42 days (n=6)	NNC90-1170 42 days (n=6)
Body weight before treatment (g)	235 ± 4	231 ± 4 #	244 ± 2	234 ± 4 #
Body weight after treatment (g)	282 ± 2	252 ± 2 ***	437 ± 8	395 ± 8 **
Weight gain during study (g)	47 ± 3	21 ± 2 ***	194 ± 7	161 ± 8 *
Beta-cell mass (mg)	4.83 ± 0.22	5.76 ± 0.27 *	8.29 ± 0.67	7.74 ± 0.72 #
Beta-cell mass per body weight (mg/10 g)	0.17 ± 0.01	0.23 ± 0.01 ***	0.19 ± 0.01	0.20 ± 0.02 #
Alpha-cell mass (mg)	0.72 ± 0.08	0.76 ± 0.04 #	0.99 ± 0.04	1.00 ± 0.09 #
Alpha-cell mass per body weight (mg/10 g)	0.026 ± 0.003	0.030 ± 0.002 #	0.023 ± 0.001	0.025 ± 0.002 #
Volume-weighted mean islet volume ($\mu m^3 * 10^6$)	5.65 ± 0.42	6.12 ± 0.50 #	9.87 ± 0.88	8.37 ± 1.11 #
Pancreas mass (g)	1.17 ± 0.03	1.23 ± 0.04 #	1.81 ± 0.11	1.71 ± 0.08 #
Fasting blood glucose	NA	NA	5.2 ± 0.2	5.5 ± 0.1 #

Data are presented as mean ± sem. *** P<0.001 versus vehicle, ** P<0.005 versus vehicle. * P<0.05 versus vehicle, # NS. P>0.05 versus vehicle

[bock2002, P12]

Liraglutide caused a 19% increase in total beta cell mass and a 35% increase in relative beta cell mass (due to decreased body weight from liraglutide treatment) after 1 week, but the effect was transient because it was absent after 6 weeks of treatment.

Liraglutide had no effect on energy expenditure or substrate oxidation in fasted resting male Sprague Dawley rats. Rats (8/dose, 426 g) were subcutaneously administered a single dose of 0 (vehicle, PBS), 0.15 mg/kg liraglutide (0.5 mL/kg), or 2,4-dinitrophenol diluted in 0.9% saline (12.5 mg/kg, 10 mg/mL, positive control) with O_2 consumption, CO_2 production, substrate oxidation (respiratory exchange ratio, RER, vCO_2/vO_2), and resting energy expenditure (EE) measured by indirect calorimetry.

Table 1 Effects of NNC 90-1170 on metabolic activity in the male rat.

The effects, expressed as AUC0-193min (baseline: 0), of vehicle, NNC 90-1170, and DNP on O_2 consumption, CO_2 production, RER, and EE were determined by indirect calorimetry.

	Vehicle	NNC 90-1170	DNP	One way ANOVA
O_2 consumption	190200±18210	193500±9007	258600±11530**	p<0.0001
CO_2 production	163500±16830	161500±12310	218500±9299**	p<0.0001
RER	166.0±6.4	163.0±2.4	163.4±2.3	p=0.32
EE	390.1±44.3	395.8±36.11	540.6±31.7**	p<0.0001

Mean ±Std, n = 8 rats/ group, **, p<0.01 vs. vehicle.

[cfle423-4016, P13]

In a 7 day repeat dose study, liraglutide reduced body weight gain and food consumption in 12 week old male Sprague Dawley rats (7/dose, 421.9 g mean body weight) without affecting the respiratory exchange ratio or energy expenditure. During the treatment period, food intake of vehicle treated rats was restricted to the amount consumed by the liraglutide treated group. Metabolic parameters were measured by indirect calorimetry and body composition and bone mineral content was measured by dual energy x-ray absorptometry (DEXA). 2,4-Dinitrophenol (12.5 mg/kg sc on day 7) was used as a positive control for indirect calorimetry studies.

Table 1 Body composition

	Vehicle		Vehicle (pf)		NNC 90-1170 Vehicle (pf)*			
Body weight (g)	417.3	± 15.3	378.5	±10.6**	373.3	±14.7**	378.5	±10.6**
BMC (g)	10.2	± 0.8	10.2	±0.3	9.8	±0.6	9.8	±0.4
Bone area (cm^2)	70.4	± 6.4	75.1	±2.0	68.7	±3.0	69.5	±2.5
BMD (g/cm^2)	0.145	± 0.009	0.137	±0.004	0.143	±0.002	0.141	±0.004
Lean tissue mass (g)	372.3	± 18.9	321.2	±8.2**	339.6	±17.4**	329.8	±9.3**
Lean tissue mass (% of BW)	89.2	± 2.5	84.9	±2.5*	91.0	±3.5	87.2	±2.3
Fat tissue mass (g)	35.5	± 9.0	45.9	±9.7	24.6	±13.1	38.1	±9.0
Fat tissue mass (% of BW)	8.5	± 2.2	12.1	±2.3*	6.5	±3.3	10.0	±2.2
Fad pad+*	3.9	± 0.9	3.8	±0.8	4.6	±0.8	4.6	±0.8

*) Body composition determined using manual histogram settings.
+*) Epididymal fat.

[cfle010402-423, P14]

Rats were administered 0 (vehicle, PBS) or 0.2 mg/kg liraglutide subcutaneously twice a day for 7 days. Liraglutide decreased body weight 11% and reduced food intake 27% with the main effect seen within the first 3 days of dosing.

The effect of subcutaneously administered 0, 0.003, 0.0055, or 0.010 mg/kg NNC 90-1170 on plasma glucose, insulin, and glucagon was determined in female pigs (6/dose) challenged with a 2 mg/kg iv glucose load 5 hours after dosing with glucose parameters measured 90 minutes after glucose loading. Immunoreactive plasma GLP-1 increased with NNC 90-1170 dose (Figure 4). A reactive hypoglycemic effect occurred in all groups and there were no substantive differences in plasma insulin. Liraglutide treatment increased AOC determined 20 – 70 minutes after glucose loading (Figure 5).

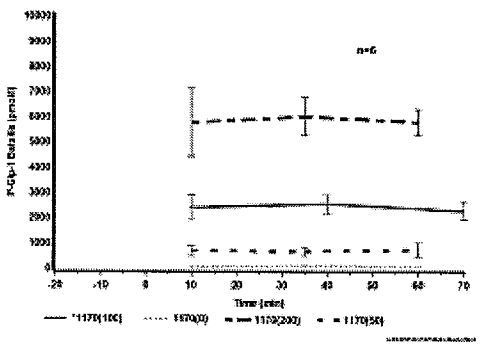


Figure 4 Effect of GLP-1 analogue 90-1170 5 hours after s.c. administration in fed pigs. A dose response study

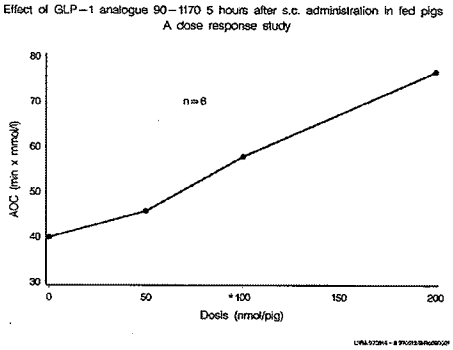


Figure 5 Effect of GLP-1 analogue 90-1170 5 hours after s.c. administration in fed pigs. A dose response study

[U1R8970915 P13, 14]

Plasma glucagon in the high dose group was lower than controls (Figure 3)

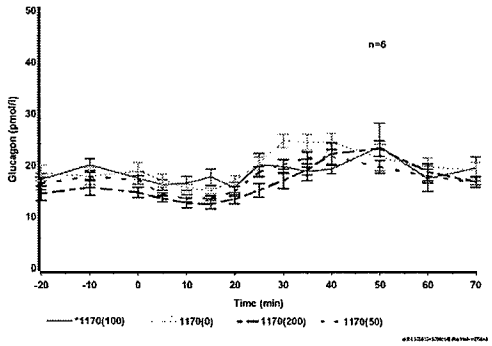


Figure 3 Effect of GLP-1 analogue 90-1170 5 hours after s.c. administration in fed pigs. A dose response study

[U1R8970915 P12]

In Vivo, Type 2 Diabetes Models

Liraglutide inhibited the development of elevated blood glucose, triglycerides, and cholesterol in prediabetic male Zucker diabetic fatty (ZDF) rats (8 weeks old at the start of treatment), but without increasing pancreatic beta cell proliferation or mass. ZDF rats are a model of type 2 diabetes developing hyperglycemia and insulin resistance on a diet with slightly increased fat content. Rats (10/group) were subcutaneously injected with 0 (vehicle, PBS) or 0.2 mg/kg liraglutide (6 mL/kg) twice a day for 15 days. Half the rats in each dose group were administered a single intraperitoneal injection of 100 mg/kg BrdU (10 mL/kg) 4 hours prior to sacrifice. During an oral glucose tolerance test on study days 1 and 13, liraglutide significantly decreased glucose AUC (Figure 1) and increased basal insulin (Figure 2) after 13 days of treatment. Liraglutide lowered body weight (Figure 9), food intake (Figure 8), basal insulin, fructosamine, and triglyceride levels and did not affect glucagon or total cholesterol.

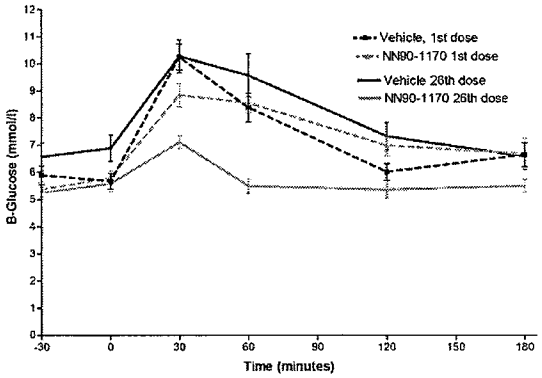


Figure 1 Blood glucose during OGTT 4 hours after the 1st and 26th dose

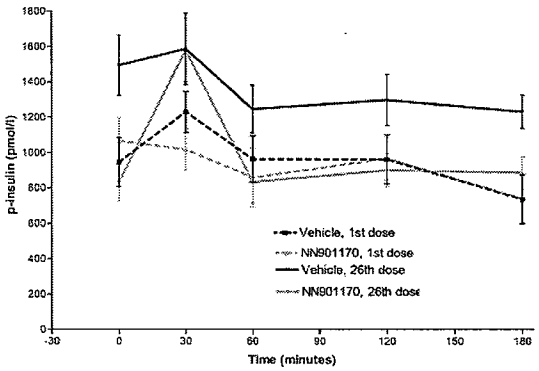


Figure 2 Plasma insulin during OGTT 4 hours after the 1st and 26th

[bidr000804a, P14-15]

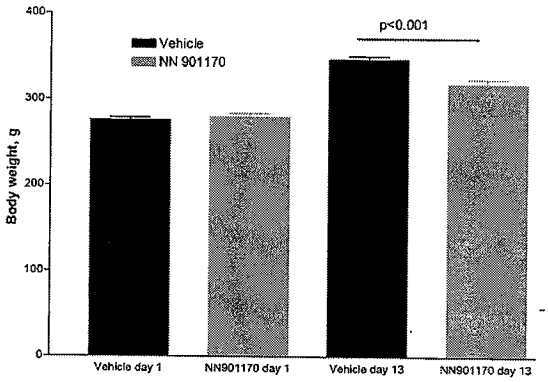


Figure 9 Body weight before and after 13 days of dosing

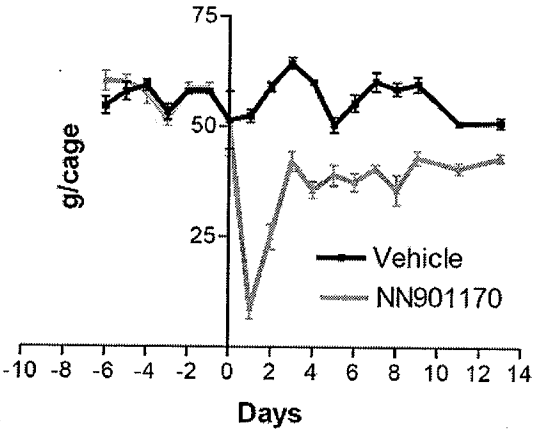


Figure 8 Food intake per cage before and after dosing

[bidr000804a, P18]

Liraglutide inhibited the development of hyperglycemia, elevated triglycerides, and elevated cholesterol in prediabetic ZDF rats, but without inducing pancreatic beta cell proliferation or beta cell mass. Liraglutide increased relative weight of pancreas, but not absolute weight, so the effect relative pancreas weight was attributed to decreased body weight. Liraglutide reduced pancreatic beta cell volume, total beta cell mass, and beta cell to body weight ratio.

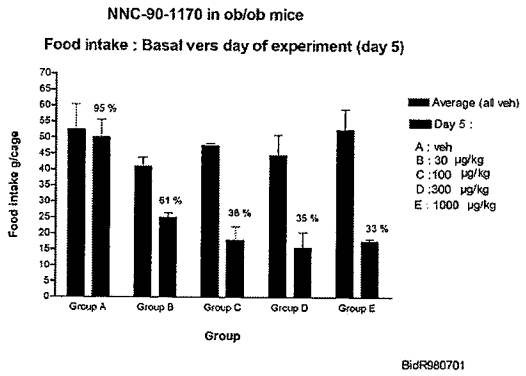
Table 1 Body and pancreas weights at sacrifice and beta-cell quantitation

	Vehicle	Liraglutide
Body Weight, g	346.9 ± 3.8	318.6 ± 5.4[a]
Pancreas Weight, g	1.00 ± 0.03	1.01 ± 0.02
Panc/Body Weight, mg/g	2.87 ± 0.06	3.17 ± 0.06[b]
Vvol beta, %	0.90 ± 0.04	0.66 ± 0.06[b]
Total beta, mg	9.0 ± 0.6	6.2 ± 0.6[b]
Beta, mg/kg b.w.	25.9 ± 1.7	20.8 ± 2.0[c]

[a] P<0.001, [b] P<0.01; [c] P<0.05

[bidr000804a, P13]

Compared to vehicle treated controls, single subcutaneous doses of 0.03, 0.1, 0.3, or 1 mg/kg liraglutide in diabetic, obese, insulin resistant, leptin-deficient female ob/ob mice (5-6/dose), a model of type 2 diabetes, dose-dependently decreased food intake, body weight gain, and blood glucose with the duration of the blood glucose lowering effect increased at higher doses. Within 24 hours after dosing with 1 mg/kg liraglutide, food intake was reduced 67% and body weight was reduced 6% at the highest dose. Blood glucose was decreased at all doses with a peak effect occurring 10 hours after dosing Figure 4, below).



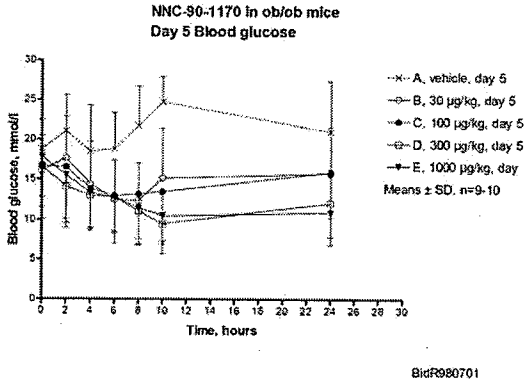


Figure 2 Food intake: Basal vers day of experiment (day 5) **Figure 4 Day 5 Blood glucose**

Figure 9 Group E day 5 vers Group E day 4

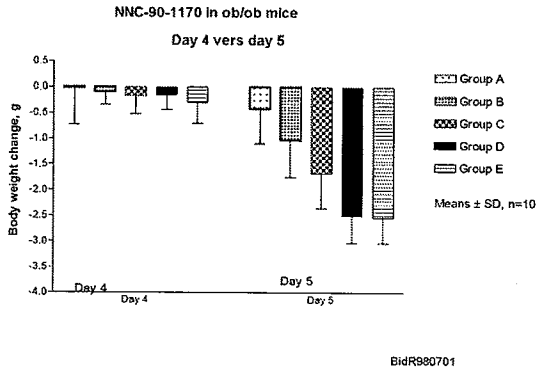


Figure 10 Day 4 vers day 5

[bidr980701 P14, 15, 18]

Liraglutide attenuated the development of diabetes in male Zucker diabetic fatty rats. Male ZDF rats were subcutaneously administered 0 (vehicle), 0.03, or 0.15 mg/kg liraglutide twice a day for 6 weeks. After 6 weeks, 0.15 mg/kg/day twice a day significantly decreased food intake (Table 2), increased body weight (Table 3, presumably due to glucosuria in diabetic vehicle and low dose groups), and decreased hematocrit (Table 4).

Table 2 JStu990301. Food and water intake

	Total food intake (kg/cage)	Food intake final week (kg/cage)	Water intake during final week (g/cage)
Vehicle	2.69 ± 0.02 (31.2 g)	0.43 ± 0.004 (30.5 g)	193 ± 8
Low dose	2.34 ± 0.07* (27.2 g)	0.38 ± 0.017 (27.4 g)	138 ± 24
High dose	2.12 ± 0.02§# (24.7 g)	0.36 ± 0.01& (25.6 g)	50 ± 4**
ANOVA	p<0.0005	p<0.02	p<0.02

Numbers in parentheses represent the equivalent average daily food intake per animal.*p<0.01 vs vehicle, $p<0.05 vs low dose, # p<0.001 vs vehicle, & p<0.02 vs vehicle, **p<0.05 vs vehicle and low dose, Tukey's post hoc test.

[jstu990301, P16]

Table 3 JStu990301. Body weights

	Initial body weights (g)	Body weight increase after 10 days treatment (g)	Final body weights (g)
Vehicle	247 ± 6	71 ± 4	371 ± 5
Low dose	239 ± 6	61 ± 3	368 ± 11
High dose	248 ± 5	41 ± 1*§	407 ± 8**
ANOVA	p=0.54	p<0.0001	p<0.01

Data are expressed as mean±SEM

* p<0.001 vs low dose, § p<0.0001 vs vehicle, ** p<0.01 vs low dose and vehicle. Tukey's post-hoc test

[jstu990301, P17]

Table 4 JStu990301. Hematocrit values during experiment

	Hematocrit (%) after 10 days	Hematocrit (%) after 41 days
Vehicle	51.6 ± 0.6	52.4 ± 0.3
Low dose	47.7 ± 1.2*	52.6 ± 0.9
High dose	47.1 ± 1.5*	50.2 ± 1.4**
ANOVA	p<0.005	p<0.05

Data are expressed as mean±SEM
*p<0.02 vs vehicle, Tukey's post hoc test
**p<0.05 vs low dose, Tukey's post hoc test, p<0.05 vs vehicle, Duncan's post-hoc test

[jstu990301, P17]

Liraglutide improved glycemic control. High dose liraglutide decreased plasma glucose, decreased HbA1c, and increased insulin secretion (Table 1), and it increased the volume of pancreatic beta cells and islets (Figure 5).

Table 1 Liraglutide treatment prevented diabetes development and increased insulin levels in diabetic ZDF rats

	AUC glucose (M×min)	AUC insulin (μM×min)	HbA_{1c} (%)
Lean vehicle	8.3±0.1	0.6±0.02	3.9±0.04
ZDF vehicle	30.4±0.6*	0.8±0.1	8.6±0.2*
ZDF liraglutide	16.2±0.2#§	2.5±0.4*#	5.5±0.4§#
ANOVA	p<0.0001	p<0.0001	p<0.0001

* p<0.001 vs lean vehicle; § p<0.01 vs lean vehicle; # p<0.001 vs ZDF vehicle; & p<0.01 vs ZDF vehicle.

[Pharmacology Written Summary, P9]

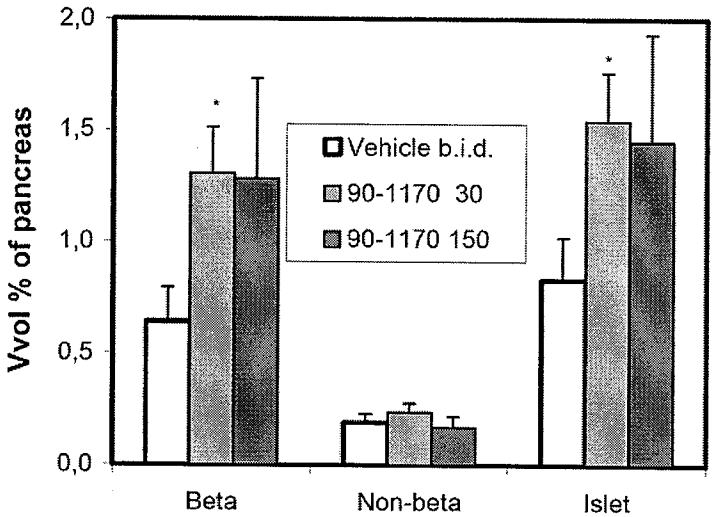


Figure 5 Beta-cell and non-beta-cell relative volume of pancreas volume in ZDF rats treated with vehicle or with 30 or 150 ug/kg bid of NNC 90-1170

[jstu990301b, P22]

Acute and subchronic anti-diabetic effects of NNC 90-1170 were determined in Zucker diabetic fatty (ZDF) rats and in Zucker obese (ZO) rats, a model of insulin resistance. In male ZDF rats administered single sc doses of 0, 0.007, 0.07 0.66 or 6.6 mg/kg NNC 90-1170, blood glucose dose-dependently decreased up to 5.3 mM compared to concurrent controls at ≥ 0.66 mg/kg from 2 – 6 hours after dosing, but with adverse effects on physical signs and appearance. Food consumption was reduced at ≥ 0.07 mg/kg. Treatment of insulin resistant male ZO rats with 0.15 mg/kg NNC 90-1170 injected subcutaneously once a day for 1 or 7 days had no effect on

resting blood glucose of insulin levels or no effect on glucose excursion during an oral glucose tolerance test (OGTT), despite treatment-related increased plasma insulin after 7 days of treatment.

NNC 90-1170 lowered blood glucose and increased insulin in female ob/ob mice, but coadministration with metformin had no addition beneficial effect. Pharmacodynamic parameters, including blood glucose, insulin, food intake and body weight were determined in female diabetic ob/ob mice (10/dose) administered vehicle (po, s.c.), 0.1 mg/kg NNC 90-1170 sc+ vehicle po twice a day, 125 mg/kg metformin po + vehicle po twice a day, or 0.1 mg/kg NNC 90-1170 sc + 125 mg/kg metformin po twice a day for 15 days. Figure 1 shows NNC 90-1170 only lowered blood glucose on day 1, and the effect was diminished on days 8 and 15 ((AUC) for blood glucose (baseline=0) was significantly reduced by NNC 90-1170 compared to vehicle on day 1 (235 ± 17 vs. 427 ± 17 mmol/l*h $p<0.001$), 8 (417 ± 15 vs. 556 ± 20 mmol/l h, $p< 0.01$) and 15 (378 ± 24 vs. 525 ± 14 mmol/l *h, $p< 0.001$). NNC 90-1170 increased plasma insulin 60%. NNC 90-1170 decreased food intake, but only during the first day after dosing and without affecting body weight. Coadministering a single dose of metformin and NNC 90-1170 had no additive effect to decrease blood glucose.

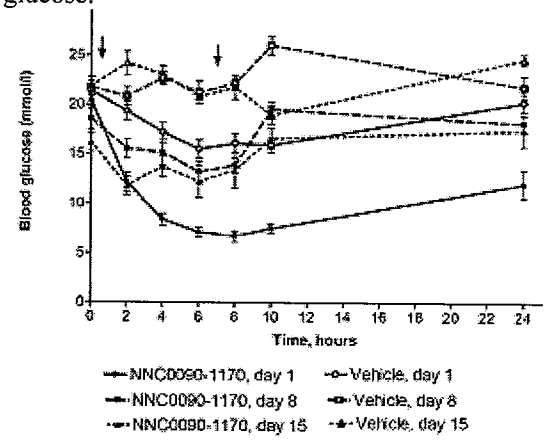


Figure 1 Blood glucose, arrows indicates time of dosing

[BidR981101, b P11]

In diabetic, leptin receptor-deficient db/db mice, the anti-diabetic effects of NNC 90-1170 are in part due to increased beta cell mass. Genetically obese and diabetic female db/db mice were treated with vehicle, 0.2 mg/kg/injection NNC 90-1170, or 0.1 mg/kg/injection exenatide by subcutaneous injection twice a day for 15 days to determine their effects on blood glucose, body weight, and food and water consumption. NNC 90-1170 plasma exposure was confirmed 4 hours after the last dose (243800 ± 68840 pM NNC 90-1170). Compared to controls, exenatide and NNC 90-1170 significantly reduced food consumption on day 1 (Figure 2), and significantly increased food consumption during the last week of dosing (Figure 3). During the last week of treatment, mean group body weight was lower in NNC 90-1170 (45.4 ± 0.2 g) and exenatide groups (45.1 ± 0.2 g) compared to the control group (46.4 ± 0.4 g), but differences in weight compared to controls were < 3% for either treated group. NNC 90-1170 lowered blood glucose on study days 1, 8 and 15, and exenatide significantly lowered blood glucose on days 1 and 15, but not on day 8. On day 1 (Figure 4), mean AUC was 389 ± 16 mM.hour in the vehicle group, 193 ± 24 mM.hour in the NNC 90-1170 group, and 233 ± 20 mM.hour in the exendin-4 group ($p<0.0001$). The glucose lowering effect of NNC 90-1170 persisted for 24 hours after dosing on day 1. Water intake decreased during days 10 – 14 in both NNC 90-1170 and exenatide treated groups.

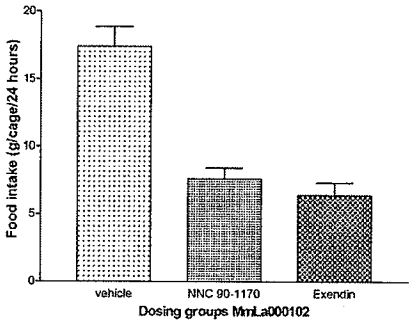


Figure 2 Food intake day 1 of dosing

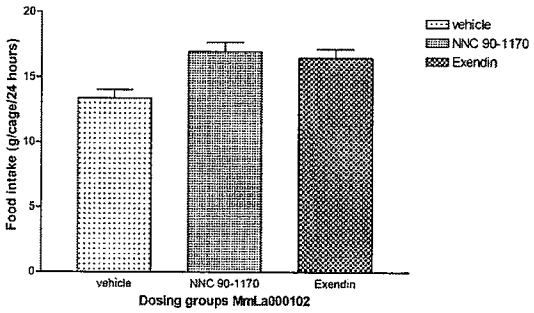


Figure 3 Food intake day 7-14 of dosing

[MmLa000102, b P17, 18]

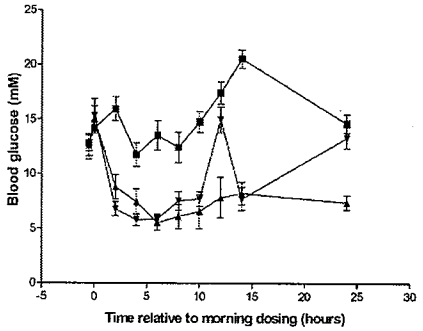


Figure 4 Blood glucose day 1 of dosing

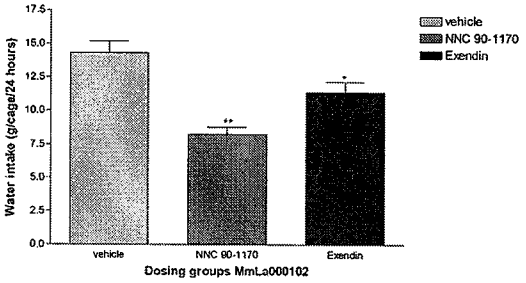


Figure 8 Water intake day 10-14 of dosing

[MmLa000102 P20, 18]

Four hours prior to sacrifice, mice were administered an ip dose of BrdU to label proliferating cells. Formalin fixed pancreas section were stained for BrdU, insulin, glucagon, somatostatin, pancreatic polypeptide, and Pdx-1. After 2 weeks of dosing, NNC 90-1170 significantly increased beta cell volume and density, but not absolute or relative weight of pancreas (Table 1). Exenatide increased absolute and relative weight of pancreas, without significantly increasing beta cell volume or density. The beta cell fraction in NNC 90-1170 and exenatide treated groups was 2 – 4 fold higher than in the control group. However, the exocrine pancreas was devoid of Pdx-1 stained cells (a transcription factor contributing to transformation of acinar cells into ductal cells that can further differentiate into beta cells) suggesting increased beta cell fraction in NNC90-1170 or exenatide treated mice was not due to transdifferentiation of the exocrine pancreas.

Table 1 Pancreas weight, relative pancreas weight, and beta-cell mass measures.

Groups	Body Weight (g)	Pancreas Weight (g)	Pancreas/Body W (mg/g)	Beta-Cells (Vvol %)	Beta-Cells (mg/pancreas)
Vehicle	45.5 ±1.2	0.27 ± 0.01	5.86 ± 0.17	1.94 ± 0.19	5.19 ± 0.58
NN2211	44.6 ±0.7	0.28 ± 0.01	6.18 ± 0.13	2.89 ± 0.14[c]	8.03 ± 0.53[d]
Exendin-4	43.8 ±0.7	0.30 ± 0.01[a]	6.86 ± 0.11[b]	2.26 ± 0.28	6.84 ± 0.89

[a] P<0.05 vs. vehicle, [b] P<0.001 vs. vehicle and NN2211, [c] P<0.001 vs. vehicle, and [d] P<0.01 vs. vehicle

[MmLa000102b P23]

Beta cell proliferation occurred in both exenatide and NNC 90-1170 treated groups (Figure 5).

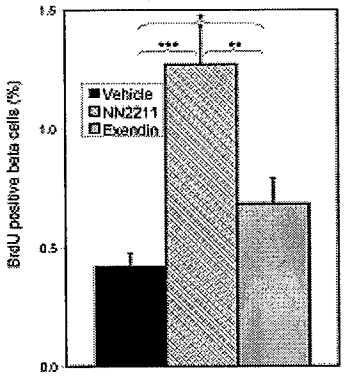


Figure 5 Beta-cell proliferation as BrdU index in db/db mice pancreata at sacrifice. A mean of 1800 insulin positive cells in 2 sections 250 µm apart were analysed for black BrdU positive nuclei.

[MmLa000102b P21]

The effects liraglutide or vildagliptin (LAF237, a DPPIV inhibitor) on food consumption, food preference, body weight gain, and body composition were determined in a rat model of type 2 diabetes, candy-fed diet-induced obese (DIO) female Sprague Dawley rats (4 month old), treated for 12 weeks. Fourteen weeks prior to drug treatment, rats were fed chow *ad libitum* supplemented with candy (Group A, n = 45, chocolate Droste, chocolate Toffifee, chocolate Marabou, grape sugar, and chocolate biscuit with candy changed daily) to induce obesity or they were fed chow ad libitum only (Group B, n = 15, lean). Rats were divided into 6 treatment groups including the following 4 groups shown in Figure 7:

0.2 mg/kg liraglutide sc BID (DIO rats, group 1), candy supplemented diet
10 mg/kg vildagliptin po BID (DIO rats, group 2), candy supplemented diet
Vehicle sc BID (DIO rats, group 3A), candy supplemented diet
Vehicle po BID (lean rats, group 3B), chow only diet

Figure 7 shows liraglutide, but not vildagliptin, decreased body weight to a level similar to lean control group maintained on chow. Liraglutide shifted food preference by increasing chow intake and decreasing candy consumption, decreased total food consumption, reversed body weight gain, reversed body fat gain (measured by DEXA scanning), but maintained energy expenditure. In this study, liraglutide did not have significant beneficial effects on glycemic control (measured in an OGTT) or pancreatic beta cell mass.

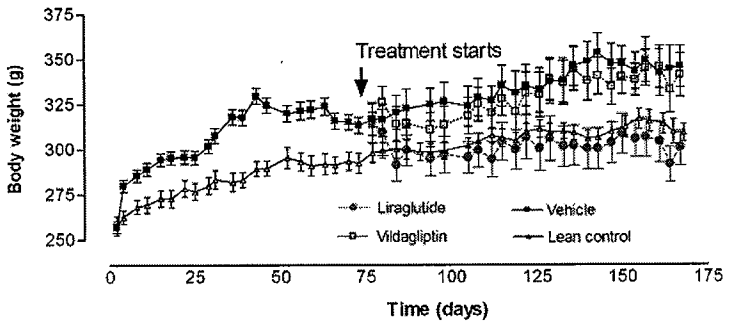


Figure 7 Liraglutide normalised body weight in candy fed rats though a mechanism involving shifted food preference

[Pharmacology Written Summary, P 15]

Male Gottingen minipigs made diabetic by reducing their beta cell mass with 43 mg/kg nicotine and 125 mg/kg streptozotocin administered iv are a model of impaired glucose tolerance and elevated fasting glucose in the absence of insulin resistance. An oral glucose tolerance test performed 1 week after streptozotocin treatment showed 2 pigs were diabetic and 10 had impaired glucose tolerance. Pigs (6/dose with both diabetic pigs allocated to the liraglutide group) were treated with 0 (vehicle) or 0.0033 mg/kg liraglutide administered sc once a day for 4 weeks. Liraglutide increased insulin release, delayed gastric emptying (Figure 5, assessed by paracetamol plasma levels after oral dosing), and decreased plasma glucose (Figure 4).

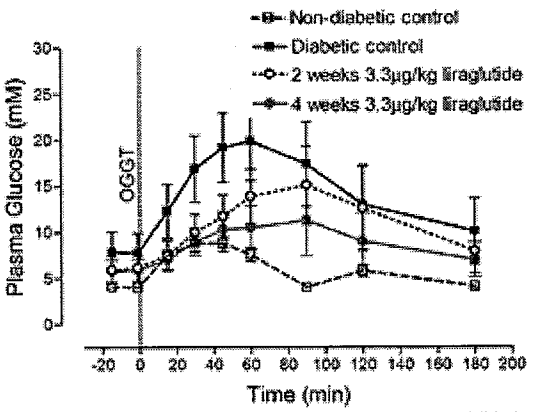


Figure 4 Liraglutide lowered blood glucose in streptozotocin (STZ)-induced diabetic Goettingen minipigs

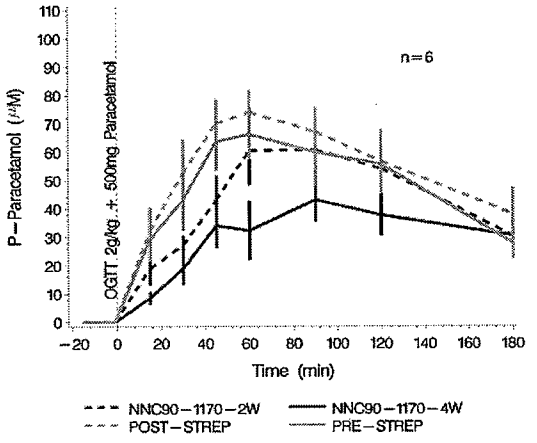


Figure 5 Plasma paracetamol concentrations before and after streptozotocin, and after 2 and 4 weeks treatment with NNC 90-1170 (3.3 µg/kg s.c.)

[Pharmacology Written Summary, P12] [ulr8-001103, P16]

Liraglutide stimulates glucose-dependent insulin secretion, suppresses glucose-dependent glucagon production, and increases glucose utilization in male Gottingen minipigs (n = 6) with drug-induced impaired glucose tolerance. Pigs treated with 100 mg/kg nicotine amide and 125 mg/kg streptozotocin developed mild impaired glucose tolerance (n = 5) or type 2 diabetes (n = 1) one month after treatment. Blood glucose was clamped 1.5 – 2 mM above fasting levels (by iv infusion) in pigs fasted for 18 hours and administered a single sc injection of 0 (vehicle) or 0.002 mg/kg liraglutide 80 minutes before starting the glucose clamp. Blood samples were analyzed for levels of liraglutide, glucose, insulin, and glucagon.

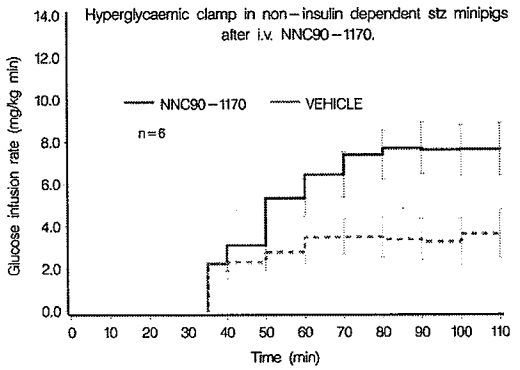


Figure 1 Glucose infusion rate after i.v. injection of NNC 90-1170 or vehicle

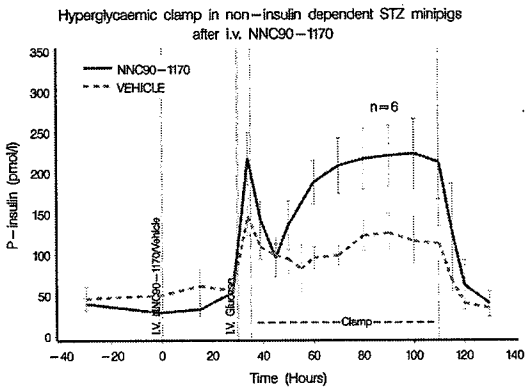


Figure 3 Plasma insulin profiles after i.v. injection of NNC 90-1170 or vehicle

[ulr4-990426, P12-14]

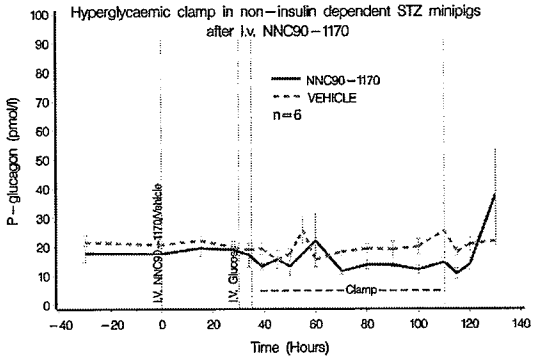


Figure 5 Plasma glucagon profiles after i.v. injection of NNC 90-1170 2 ug/kg (15 nmol/pig) or vehicle

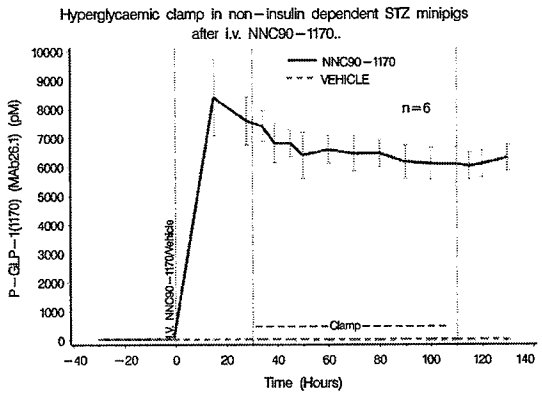


Figure 4 Plasma concentration of NNC 90-1170 after i.v. injection of 2 ug/kg (15 nmol/pig) or vehicle

[ulr4-990426, P16, 15]

Figure 1 shows a significant increase in the glucose infusion rate was required to clamp glucose levels ~ 2 mM above fasting levels during liraglutide treatment. The plasma insulin profile, shown in Figure 3, shows insulin was markedly increased in liraglutide treated pigs during the glucose clamp. During the glucose clamp, liraglutide decreased the area under the plasma glucagon curve (832 nM.min, 70 – 110 min) compared to control (531 nM.min), but glucagon levels increased after the clamp was terminated (Figure 5). Liraglutide levels remained constant during the glucose clamp (Figure 4).

The efficacy of liraglutide to reverse type 2 diabetes was evaluated in sand rats (Psammomys obesus or dessert gerbil) considered a less insulin resistant model of type 2 diabetes because they loose pancreatic beta cell function when they are fed a high energy diet. Liraglutide dose-dependently improved glycemic parameters in diabetic sand rats. Male and female sand rats (6-7/dose) made diabetic on a high energy diet (3.1 kcal/g) for 22 days were treated with 0 (vehicle), 0.0125, 0.025, 0.05, 0.1, 0.15, or 0.3 mg/kg/day liraglutide administered sc once a day for 4 weeks. Figure 3 shows liraglutide dose-dependently decreased morning blood glucose levels. On day 49 or 50, doses of 0.1 and 0.3 mg/kg liraglutide significantly lowered blood glucose compared to control and dose-dependently increased the number of normoglycemic animals (summary table below) with reversion to diabetes 15 days after treatment was stopped. Liraglutide doses of 0.05, 0.1, or 0.3 mg/kg significantly lowered HbA1c on day 59, and in the high dose group, the effect on HbA1c persisted to the end of the follow-up period.

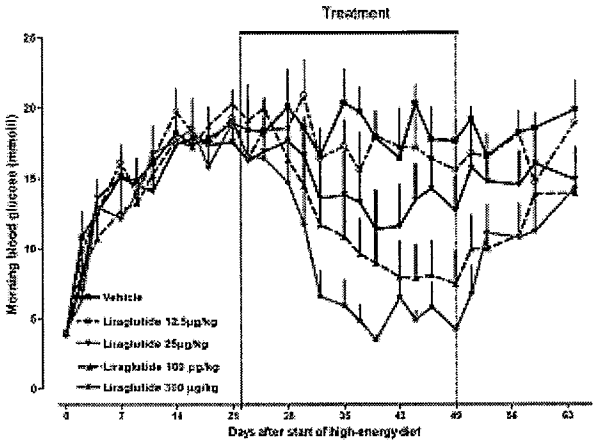


Figure 3 Liraglutide dose-dependently normalised blood glucose in high-energy diet induced diabetic Psammomys Obesus (sand rats)

[Pharmacology Written Summary, P11]

Morning blood glucose before, after and during the treatment period

	Blood glucose at start of treatment (mM)-Day 21	Blood glucose at end of treatment (mM) Day 49	Blood glucose at end of follow up period (mM) Day 64	% of animals normoglycemic at end of treatment-Day 49	% of animals normoglycemic at end of follow up period Day 64
Vehicle	18.9 ± 1.2	17.7 ± 1.5	19.9 ± 2.1	0	0
Liraglutide, 12.5µg/kg	19.3 ± 0.9	15.6 ± 3.3	19.0 ± 2.5	1/4	0
Liraglutide, 25µg/kg	17.6 ± 1.0	12.7 ± 2.4	14.9 ± 2.3	2/7	1
Liraglutide, 50µg/kg	18.0 ± 1.7	8.8 ± 2.7*	12.9 ± 2.2	4/6	3
Liraglutide, 100µg/kg	20.3 ± 1.0	7.4 ± 2.3*	13.9 ± 1.7	5/6	1
Liraglutide, 150µg/kg	19.5 ± 3.0	10.7 ± 2.5	14.1 ± 2.6	3/7	2
Liraglutide, 300µg/kg	18.9 ± 0.8	4.2 ± 0.3**	14.3 ± 1.8	6/6	3

Data are mean±SEM

*, denotes p< 0.05 and **, denotes p<0.01, as compared to vehicle treated animals in an Anova with Dunett multiple comparison test

HbA_{1C} before, after and during the treatment period

	HbA_{1C} before start of treatment (%) Day 16	HbA_{1C} at end of treatment (%) Day 59	% difference from before start of treatment	HbA_{1C} at end of follow up period (%)	% difference from before start of treatment
Vehicle	6.78 ± 0.17	10.81 ± 0.40	59 ± 10	11.38 ± 0.50	68 ± 14
Liraglutide, 12.5µg/kg	6.83 ± 0.29	10.26 ± 1.25	49 ± 28	10.11 ± 1.72	29 ± 22
Liraglutide, 25µg/kg	6.81 ± 0.19	8.86 ± 1.08	29 ± 37	9.58 ± 0.95	43 ± 10
Liraglutide, 50µg/kg	6.80 ± 0.34	7.58 ± 1.02*	10 ± 27*	7.71 ± 0.94	15 ± 9**
Liraglutide, 100µg/kg	6.82 ± 0.30	7.46 ± 1.04*	13 ± 31*	7.89 ± 0.90	3 ± 12**
Liraglutide, 150µg/kg	6.69 ± 0.11	8.17 ± 0.79	22 ± 30	8.56 ± 0.92	35 ± 11
Liraglutide, 300µg/kg	6.73 ± 0.16	6.41 ± 0.45**	-5 ± 14***	6.95 ± 0.43*	-2 ± 4**

Data are mean±SEM

*, denotes p< 0.05 and **, denotes p<0.01, as compared to vehicle treated animals in an Anova with Dunett multiple comparison test

[tora040802, P9-10]

In Vivo, Obesity and Hyperphagia Models

Effects of liraglutide on body weight and food consumption were evaluated in male Wistar rats and rats subjected to neonatal monosodium glutamate (MSG) treatment resulting in neuroendocrine dysfunction (chronic HPA-axis activation) characterized by stunted growth, adiposity, short tail (self-mutilation) and blindness. MSG induces irreversible hypothalamic arcuate nucleus lesions causing defects in GLP-1R signaling and they do not have an anorexic response to centrally administered GLP-1. In normal rats, a single iv dose of 0.1 or 0.5 mg GLP-1 decreased food and water consumption 30 and 60 minutes after the onset of feeding and increased diuresis. Effects on food and water consumption were completely abolished by prior administration of 0.1 mg exendin (9-39), a GLP-1R antagonist, but the diuretic effect was only partially blocked. Single sc doses of liraglutide (0.01, 0.05, or 0.2 mg/kg) dose-dependently decreased overnight food and water intake and increased diuresis in both normal and MSG-treated rats (Figure 7).

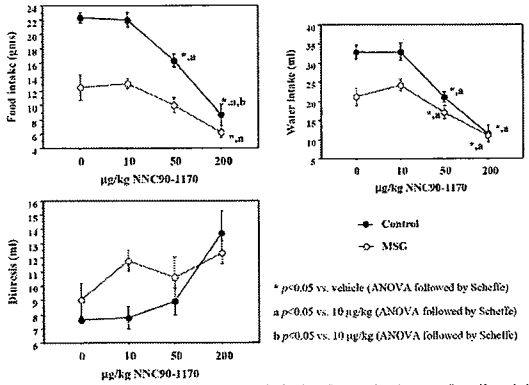


Figure 7 Dose response curves for 720 min food and water intake as well as diuresis in normal and MSG rats receiving a single sc dose of NNC90-1170

[pjl90-1170, P37]

SC injection of 0.2 mg/kg liraglutide twice a day (but not 0.1 mg/kg) in normal or MSG-treated rats (n = 5 – 8) for 7 days decreased body weight (Figure 8) and food consumption (data not shown). Decreased body weight persisted even after treatment was stopped on day 8 and even though food consumption and water intake gradually recovered. Decreased leptin levels in liraglutide treated rats were consistent with decreased adiposity. Therefore, 0.2 mg/kg/day liraglutide twice a day for 7 days had a long lasting anorectic effect and decreased adiposity, without any overt effect on fluid homeostasis.

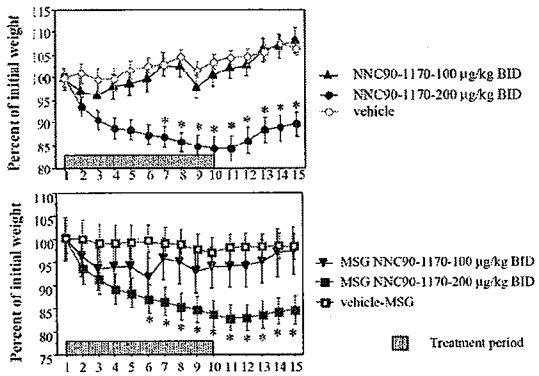
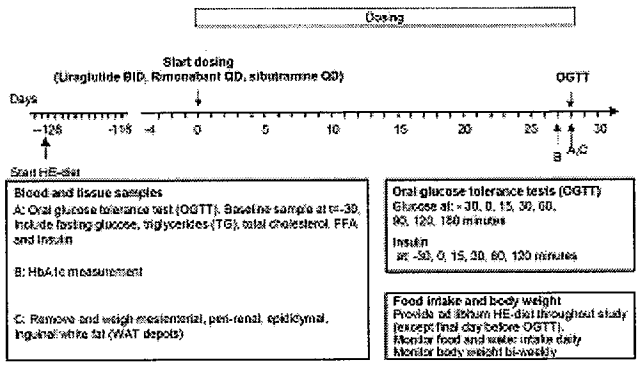


Figure 8 Subchronic administration of NNC90-1170 dose-dependently decreases body weight in both normal adult male Wistar rats and MSG rats.

[pjl90-1170, P38]

Twenty-eight days of treatment with liraglutide reduced food intake, body weight, subcutaneous fat, and improved glucose tolerance in diet-induced obese male Sprague Dawley rats, a rat model of obesity. In this study, effects of liraglutide were compared to sibutramine and rimonabant in rats made obese by feeding them a high fat diet (32% of calories derived from fat). A schematic of the study design and parameters is shown below.

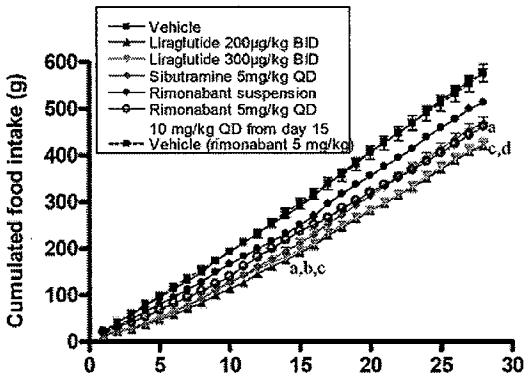


[rhs2005-007, P10]

Treatment groups1 – 6 are shown below (10 rats / group). The composition of vehicle 1 was 0.5% Natrosol (w/v), vehicle 2 was phosphate buffered saline, and vehicle 3 was 0.5% ethanol, 10% tween 80 in water. In a separate experiment, vehicle 3 had no effect on food consumption or body weight gain. Liraglutide doses were started at 0.1 mg/kg BID and escalated by 0.05 mg/kg BID every second day until the final dose was reached; 0.2 mg/kg BID on study day 4 and 0.3 mg/kg BID on study day 8.

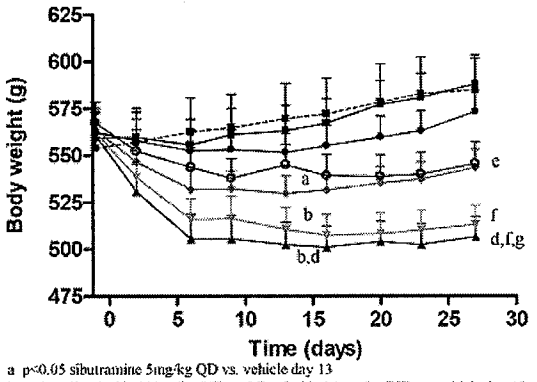
Group 1: vehicle 1 QD po + vehicle 2 BID sc
Group 2: vehicle 1 QD po + 0.2 mg/kg liraglutide BID sc
Group 3: vehicle 1 QD po + 0.3 mg/kg liraglutide BID sc
Group 4: 5 mg/kg sibutramine QD po + vehicle 2 BID sc
Group 5: 10 mg/kg rimonabant sus QD po + vehicle 2 BID sc
Group 6: 5 mg/kg rimonabant QD po + vehicle 2 BID sc (days 0 – 14)
10 mg/kg rimonabant QD po + vehicle 2 BID sc (days 15 – 28)

Because 5 mg/kg rimonabant in vehicle 1 (group 5) had a smaller effect on food consumption or body weight gain, a sixth group was added to the study using a different vehicle and a higher dose of 10 mg/kg rimonabant from study days 15 – 28. Figure 2 shows all treatment and both liraglutide doses significantly reduced cumulative food consumption. By the end of the study, liraglutide, sibutramine, and 10 mg/kg rimonabant decreased body weight compared to vehicle controls (Figure 4), but liraglutide had a significantly greater effect than the other drugs.



a p<0.001 all treatments vs. vehicle day 14 and 28
b p<0.05 liraglutide 200µg/kg BID vs. sibutramine 5mg/kg QD day 14
c p<0.01 liraglutide 200µg/kg BID vs. rimonabant 5mg/kg QD day 14 and 28
d p<0.01 liraglutide 200µg/kg BID vs. sibutramine 5mg/kg QD day 28

Figure 2 Food intake

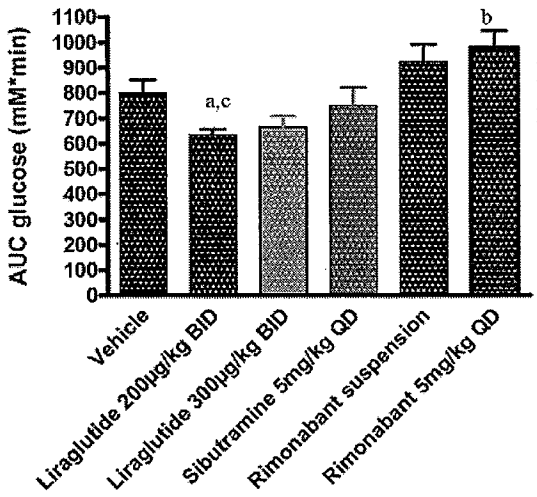


a p<0.05 sibutramine 5mg/kg QD vs. vehicle day 13
b p<0.01 liraglutide 200µg/kg BID and liraglutide 300µg/kg BID vs. vehicle day 13
d p<0.05 liraglutide 200µg/kg BID vs. rimonabant 5mg/kg QD day 13 and 27
e p<0.01 sibutramine 5mg/kg QD and rimonabant 5mg/kg QD vs. vehicle day 27
f p<0.0001 liraglutide 200µg/kg BID and liraglutide 300µg/kg BID vs. vehicle day 27
g p<0.05 liraglutide 200µg/kg BID vs. sibutramine 5mg/kg QD day 27

Figure 4 Body weight

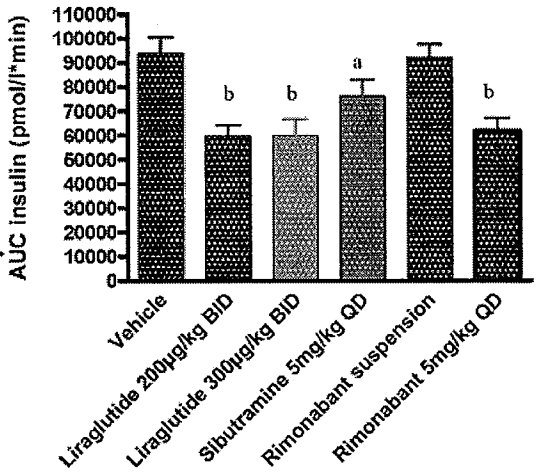
[rhs2005-007, P16-17]

Liraglutide and sibutramine decreased inguinal, epididymal, mesenteric, and perirenal subcutaneous fat, and decreased plasma triglycerides, but elevated plasma free fatty acids. During an OGTT performed at the end of the study, the high dose of liraglutide (0.2 mg/kg BID) decreased glucose $AUC_{0-180\ min}$ (Figure 6) and increased insulin secretion (measured as $AUC_{0-180min}$, Figure 7) compared to control.



a p<0.05 liraglutide 200µg/kg BID vs. vehicle
b p<0.05 rimonabant 5mg/kg BID vs. vehicle
c p<0.001 liraglutide 200µg/kg BID vs. rimonabant 5mg/kg QD QD

Figure 6 Oral glucose tolerance test. Plasma glucose and AUC after 28 days of treatment



a p<0.05 sibutramine 5mg/kg QD vs. vehicle
b p<0.001 liraglutide 200µg/kg BID, liraglutide 300µg/kg BID and rimonabant 5mg/kg QD vs. Vehicle

Figure 7 Oral glucose tolerance test. Plasma insulin and AUC after 28 days of treatment

[rhs2005-007, P20-21]

Liraglutide decreased food consumption and body weight of Gottingen minipigs, a model of extreme hyperphagia, by decreasing the number, duration, and size of meals. Healthy obese female Gottingen minipigs (6 pigs, 18 – 19 months old, 87.7 kg) were administered 0.003 – 0.007 mg/kg liraglutide sc once a day for 7 weeks to determine the effect of liraglutide on food

consumption and body weight. Figure 6 shows liraglutide markedly reduced daily food consumption during the 4 week steady state treatment period to 7.3 MJ from 18.4 MJ before treatment and 19.2 MJ after. Liraglutide decreased the number, size, and duration of meals, and the effect was reversed within 4 days after treatment was stopped. Body weight decreased 4.3 kg during the 7 week treatment period and increased 7.0 kg during the 7 weeks after treatment.

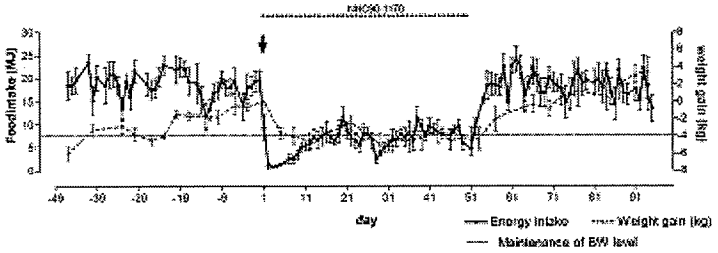


Figure 1 Food intake(MJ) and Body weight change in obese, ad libitum fed, hyperphagic, Göttingen female minipigs, during a 7 week NNC 90-1170 treatment period and the surrounding two 7 weeks basal period. (data presented as mean±S.E.M, n=6).

[kira011004-70, P12]

In adult, non-diabetic, glucose-intolerant rhesus monkeys with middle-age onset obesity (15.8 kg, > 25% body fat), liraglutide caused a reduction in food intake during the 8 hour feeding period and reduced body weight 0.4 kg during a 16 day treatment period. Monkeys (n = 5) were subcutaneously administered vehicle for 3 weeks, 0.03 mg/kg liraglutide twice a day for 4 days followed by a washout period because monkeys stopped eating, then 0.01 mg/kg/day liraglutide for 16 days (except the dose was reduced to 0.005 mg/kg on day 6 for one monkey due to excessive reduced food intake). Figure 2 shows treatment-related changes in food consumption during the treatment period with recovery to baseline levels after treatment was stopped.

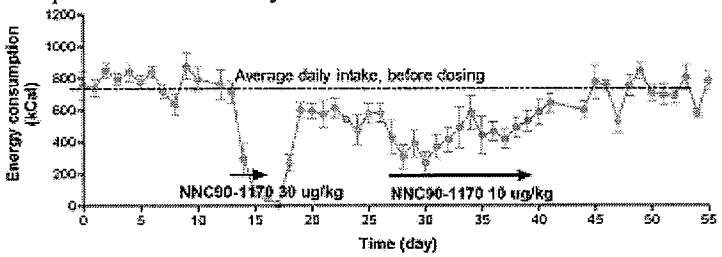


Figure 2 30 ug/kg NNC90-1170 reduced food intake to less than 10%. 10 ug/kg NNC90-1170 dosed for 16 days caused a sustained decrease (38% below baseline) in food intake in glucose-intolerant obese rhesus monkeys

[ks-1-004, P10]

In Vivo, Other Animal Models

Liraglutide had no effect on infarct size in a pig model of myocardial infarction. Six month old pigs (mixed breed, 70-80 kg) treated with 0.01 mg/kg liraglutide once a day for 3 days prior to induced infarction (with the last dose administered 12 hours before the procedure) were anesthetized. Myocardial infarction was induced by inflating an angioplasty balloon in the left anterior descending artery of the heart just distal to the 2nd diagonal branch for 40 minutes. The heart was reperfused for 2 hours before it was removed after fluorescein injection into the left auricle to identify the area at risk. The infarct size was measured in left ventricle slices perpendicular to the left anterior descending artery after incubating with 2,3,5-triphenyltetrazolium chloride. Figure 1 shows liraglutide had no effect on the area at risk, the infarct size, or the ratio of infarct size to area at risk.

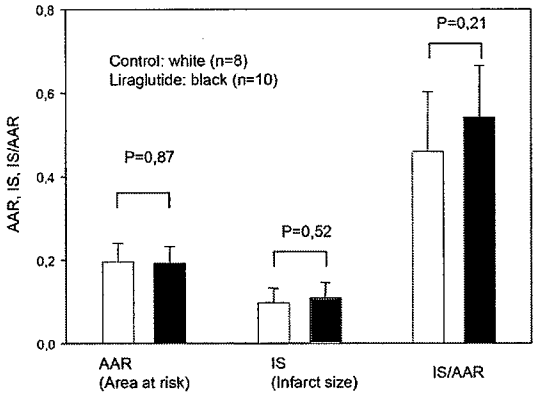
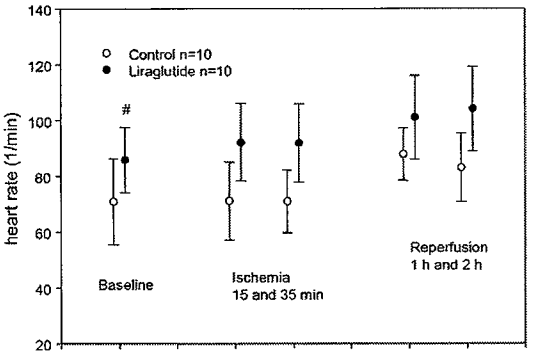


Figure 1 Infarct size (IS), area at risk (AAR) and the infarct size in relation to the area at risk (IS/AAR)



: The heart rate level is significantly larger in liraglutide group than control group throughout (p=0,03), while there is no significant difference in the change over time between groups (p=0,21)

Figure 4 Heart rate

[mm-01-2006, P15, 18]

There were no liraglutide treatment related effects on mean blood pressure, central venous pressure, mean pulmonary pressure, pulmonary capillary wedge pressure, or pulmonary or systemic vascular resistance. Heart rate was slightly higher in liraglutide treated pigs (Figure 4), and cardiac output was slightly higher in liraglutide treated groups (4.51 L/min vs 3.67 L/min for liraglutide and control groups 60 min after reperfusion and 4.79 L/min vs 4.19 L.min 120 min after reperfusion).

2.6.2.3 Secondary pharmacodynamics

In vitro pharmacology screening of NNC 90-1170 against a broad panel of receptors, enzymes, ion channels, and transporters in 3 separate studies showed no significant effects at 0.1 μM or 10 μM. NNC 90-1170 (10 μM.) inhibited radioligand binding to bombesin receptor (non-selective) in duplicate single point assays (58% inhibition), but the result was not confirmed in a second set of bombesin receptor assays (non-selective, and human BB2 and BB3 receptor assays) at a different CRO. Up to 1 μM NNC 90-1170 did not stimulate cAMP formation in BHK cells expressing the cloned human glucagon receptor. The affinity or agonist potency of liraglutide at human GLP-2 receptors was not determined.

2.6.2.4 Safety pharmacology

Neurological and behavioral effects:

Neurobehavioral effects of subcutaneously administered 0 (vehicle), 0 (saline), 0.02, 0.2, or 2 mg/kg NNC 90-1170 in male NMRI mice rats (6/dose) were assessed using a functional observational battery performed 0.5, 1, 2, 4, and 24 hours after dosing and observations in the home cage and during handling. Diazepam (20 mg/kg, 10 mL/kg po) was used as a positive control. There were no liraglutide-related findings. Increased incidence of exploratory activity at 0.02 mg/kg occurred 0.5 – 4 hours after dosing was considered equivocal because it didn't occur at higher doses. Diazepam, a positive control, decreased mobility in the home cage, alertness, startle response, body tone, grooming, and caused hunched posture, abnormal gait, and hypothermia within 4 hours after dosing.

	GROUP 3 VEHICLE	GROUP 4 0.2 MG/KG	GROUP 5 2 MG/KG	GROUP 6 0.02 MG/KG
NO FINDINGS				
BEFORE DOSING	4/6	5/6	5/6	5/6
0.5 HOUR AFTER DOSING	3/6	6/6	4/6	4/6
1 HOUR AFTER DOSING	2/6	5/6	5/6	3/6
2 HOURS AFTER DOSING	3/6	5/6	6/6	3/6
4 HOURS AFTER DOSING	3/6	6/6	5/6	4/6
24 HOURS AFTER DOSING	3/6	6/6	4/6	4/6
INCREASED EXPLORATORY ACTIVITY				
BEFORE DOSING	0/6	0/6	1/6	1/6
0.5 HOUR AFTER DOSING	0/6	0/6	2/6	2/6
1 HOUR AFTER DOSING	1/6	0/6	1/6	3/6
2 HOURS AFTER DOSING	0/6	0/6	0/6	3/6
4 HOURS AFTER DOSING	0/6	0/6	0/6	2/6
24 HOURS AFTER DOSING	1/6	0/6	1/6	1/6

[990263, P23]

Liraglutide had no significant effect on the time to sleep onset or sleep duration induced by hexabarbitone in male NMRI mice. Mice (6/dose, 5 weeks old) were subcutaneously injected with a single dose of 0 (saline), 0 (vehicle), 0.02, 0.2, or 2 mg/kg liraglutide or 5 mg/kg chlorpromazine (positive control) 120 minutes prior to intraperitoneal injection of 70 mg/kg hexabarbitone. The time to disappearance and reappearance of the righting reflux was determined for each mouse. Up to 2 mg/kg liraglutide had no effect on recovery of righting reflex after hexabarbitone treatment (Figure 1).

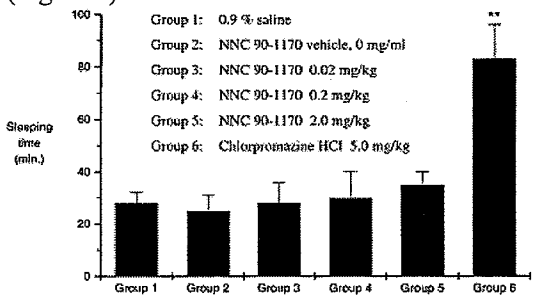


Figure 1 Time to reappearance of the righting reflex (sleeping time) following administration of hexobarbitone (Mean values + SD, n=6/group)

[990265, P19]

Liraglutide had no significant effect on the time to sleep onset or sleep duration induced by ethyl alcohol in male NMRI mice. Mice (6/dose, 5 weeks old) were subcutaneously injected with a single dose of 0 (saline), 0 (vehicle), 0.02, 0.2, or 2 mg/kg liraglutide or 5 mg/kg chlorpromazine (positive control) 120 minutes prior to intraperitoneal injection of 4.5 mg/kg ethanol. The time to disappearance and reappearance of the righting reflux was determined for each mouse. Up to 2 mg/kg liraglutide had no effect on recovery of righting reflex after hexabarbitone treatment (Figure 1).

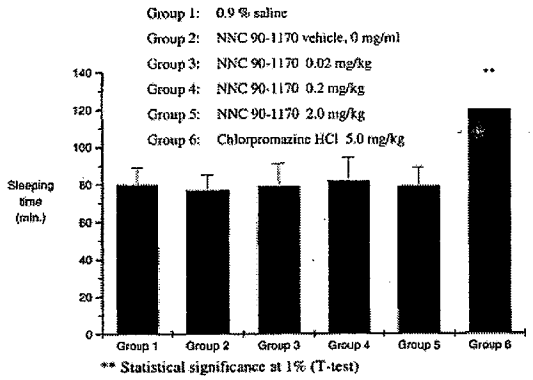


Figure 1 Time to reappearance of the righting reflex (sleeping time) following administration of alcohol (Mean values + SD, n=6/group)

[990266, P21]

Pulmonary effects:

Respiratory parameters in conscious male Sprague Dawley rats (10/dose) were monitored by whole body plethysmography with data collection up to 15 minutes prior to dosing and for up to 3 hours after subcutaneous injection with 0 (vehicle), 0 (0.9% saline), 0.02, 0.2, or 2 mg/kg liraglutide (1 mL/kg). Positive control groups were treated by a single subcutaneous injection with 5 mg/kg methacholine with or without 5 mg/kg terbutaline. Liraglutide had no significant effect of respiratory parameters including penh (enhanced pause, a controversial measure of pulmonary resistance), respiratory rate, tidal volume, or minute volume.

Cardiovascular effects:

Cardiovascular safety pharmacology studies of liraglutide were *in vitro* hERG channel inhibition, *ex vivo* monophasic action potential in isolated rabbit heart, and *in vivo* determining effects on blood pressure, heart rate, body temperature, and locomotor activity in conscious telemetered male Sprague Dawley rats and in male cynomolgus monkeys determining effects on blood pressure, ECG intervals, body temperature, and locomotor activity.

Liraglutide did not inhibit hERG channel current *in vitro*. In a whole-cell patch clamp assay of hERG channel tail current using HEK cells stably expressing hERG, 0.14, 0.29, or 1.43 μM liraglutide or 1 μM GLP-1 had no inhibitory effect. The positive control, 100 nM E-4031, inhibited 83.6% of hERG channel tail current (data not shown).

In an isolated female rabbit heart preparation, liraglutide was not considered proarrhythmogenic. Liraglutide and the vehicle shortened the QTc interval, without changing the ECG shape, and decreased $MAPD_{90}$ (monophasic action potential duration 90% at apex or base of left ventricle), but only liraglutide slightly increased the heart rate and coronary blood flow. Hearts from female Danish land rabbits were isolated, placed in an organ bath, and treated with vehicle (0, 0.27, 0.5, 0.268 mL/L), liraglutide (0, 0.14, 0.29, or 1.43 μM), or the positive control terfenadine (0, 1, 3, or 10 μM) using 4 hearts/treatment (vehicle, liraglutide, or terfenadine). Results are summarized in Table 1 below. 10 μM terfenadine caused ventricular tachycardia in 3/4 hearts, and these results were omitted from calculations. QTc interval was slightly reduced in liraglutide and vehicle treated hearts, but terfenadine increased QTc. Liraglutide (0.14 μM only) caused a minor increase in the magnitude of the T-wave, but higher doses did not, so the relation to liraglutide was equivocal. Major changes in ECG morphology were seen with terfenadine treatment including bigeminy (2 hearts) and torsades de pointes-like ventricular tachycardia (1 heart) after the addition of 3 μM terfenadine and torsades de pointes-like ventricular tachycardia (3 hearts) after the addition of 10 μM terfenadine. Consistent with their effects on QTc, liraglutide and vehicle shortened $MAPD_{90}$ measured at the base or apex of the left ventricle, but

terfenadine caused a minor decrease. At 1.43 µM, liraglutide caused a slight 6% increase in heart rate (12.4 bpm, from 207 to 219 bpm), but 10 µM terfenadine decreased HR (from 214 to 177 bpm). Increased coronary blood flow at 1.43 µM liraglutide compared to vehicle was due to decreased flow in the vehicle group.

Table 1 Mean table for all experiments

Vehicle	QT ms	SD	n	QTc ms	SD	n	Rate bpm	SD	n	$MAPD_{90}$ ms (A)	SD	n	$MAPD_{33}$ ms (B)	SD	n	Flow	SD	n
0 ml/L	155,0	12,0	4	275,4	8,6	4	192,5	26,8	4	125,9	18,8	4	131,4	11,6	4	34,1	8,2	4
0,27 ml/L	150,4	10,0	4	274,6	8,5	4	202,5	22,9	4	109,4	13,6	4	121,1	9,6	4	33,4	6,5	4
0,54 ml/L	149,2	7,3	3	268,5	1,8	3	196,3	20,9	3	104,4	14,4	3	111,1	7,0	3	27,4	6,4	3
2,68 ml/L	147,4	8,5	3	264,3	12,4	3	195,4	28,7	3	94,2	20,3	3	107,3	7,3	3	20,1	8,8	3

NNC 90-1170	QT	SD	n	QTc	SD	n	Rate	SD	n	$MAPD_{90}$	SD	n	$MAPD_{33}$	SD	n	Flow	SD	n
0 µM	155,9	1,9	4	288,0	16,9	4	207,0	25,5	4	119,3	4,5	4	120,9	3,0	4	36,5	4,4	4
0,14 µM	147,4	9,6	4	273,6	15,2	4	210,2	38,4	4	105,5	17,2	4	107,5	18,3	4	38,9	2,7	4
0,29 µM	148,6	6,0	4	278,2	19,2	4	215,1	45,3	4	108,3	4,9	4	110,4	3,1	4	38,5	4,8	4
1,43 µM	141,8	9,4	4	269,2	15,6	4	219,4	34,1	4	96,4	19,3	4	93,6	22,7	4	34,5	3,1	4

Terfenadine	QT	SD	n	QTc	SD	n	Rate	SD	n	$MAPD_{90}$	SD	n	$MAPD_{33}$	SD	n	Flow	SD	n
0 µM	159,2	20,7	4	297,2	19,1	4	214,1	34,2	4	121,7	19,9	4	124,0	19,2	4	43,4	13,0	4
1 µM	157,4	42,1	4	293,6	50,6	4	221,8	49,2	4	119,2	40,8	4	109,3	43,2	4	42,3	4,7	4
3 µM	162,6	21,3	3	315,0	26,4	3	178,7	19,2	3	88,9	35,2	3	102,6	26,8	3	30,0	15,8	4
10 µM	184,3N/A		1	314,8N/A		1	176,3N/A		1	82,6N/A		1	107,1N/A		1	16,4	7,2	4

[980422 P21]

Liraglutide increased arterial blood pressure and heart rate and decreased body temperature, but without affecting locomotor activity for up to 24 hours after dosing conscious, telemetered male Sprague Dawley rats. Rats (4 males) received a bolus subcutaneous injection of 0 (0.9% saline), 0 (vehicle), 0.02, 0.2, or 2 mg/kg liraglutide once a week for 5 weeks. At the end of the study, rats received a single oral dose of 10 mg/kg noradrenaline as a positive control. Compared to saline, vehicle (phosphate buffer, 38 mg/mL mannitol, 5 mg/mL phenol, pH 7.4) had no relevant effects. The NOAEL was 0.02 mg/kg liraglutide. At ≥ 0.2 mg/kg, liraglutide increased systolic, diastolic, and mean arterial blood pressure (Figures 1, 2, 3), increased heart rate (Figure 4), and decreased body temperature (Figure 5) with the onset and duration of effect related to dose, but not the magnitude. Liraglutide did not affect locomotor activity at any dose. The positive control, norepinephrine, increased blood pressure, decreased heart rate, and decreased body temperature.

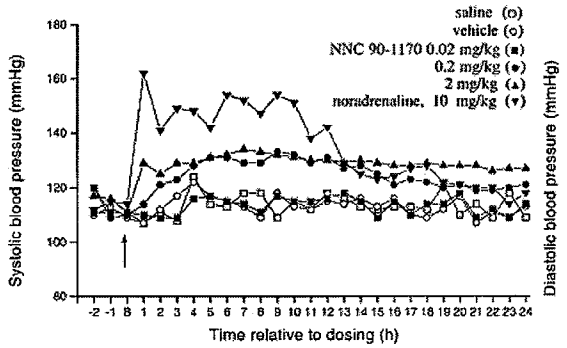


Figure 1 Effect of NNC 90-1170 on systolic blood pressure (mmHg) in the telemetered male rat.

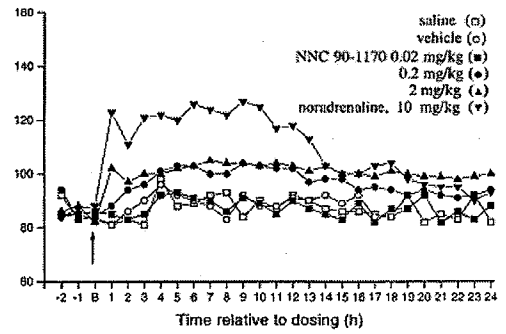


Figure 2 Effect of NNC 90-1170 on diastolic blood pressure (mmHg) in the telemetered male rat.

[990264 P23-4]

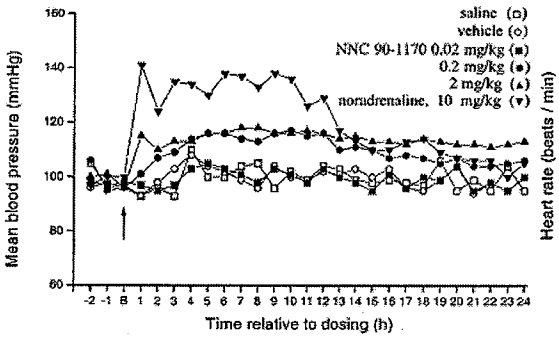


Figure 3 Effect of NNC 90-1170 on mean blood pressure (mmHg) in the telemetered male rat.

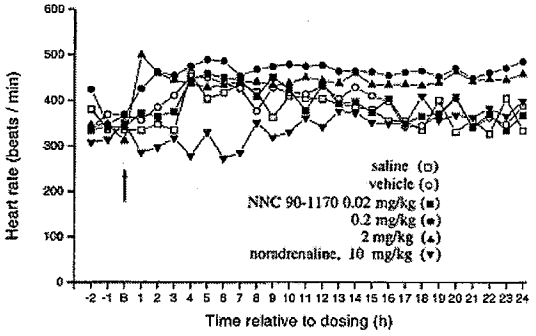


Figure 4 Effect of NNC 90-1170 on heart rate (beats/min) in the telemetered male rat.

[990264 P25-6]

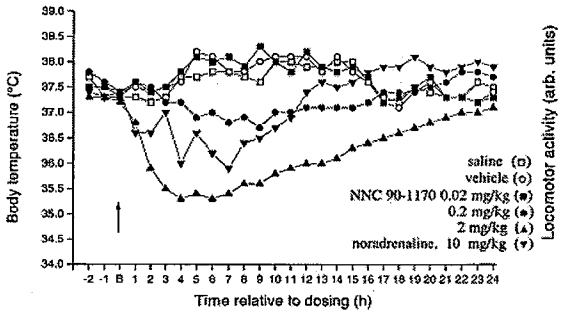


Figure 5 Effect of NNC 90-1170 on body temperature (°C) in the telemetered male rat.

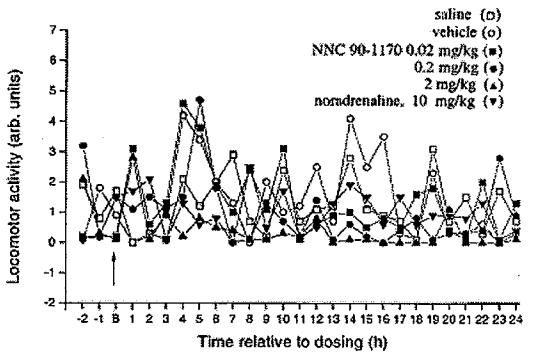


Figure 6 Effect of NNC 90-1170 on locomotor activity (arb. units) in the telemetered male rat.

[990264 P27-8]

Single bolus subcutaneous doses of up to 2 mg/kg liraglutide in conscious, telemetered male cynomolgus monkeys had no significant effect on systolic, diastolic, or mean arterial blood pressure, heart rate, ECG intervals including QT, body temperature, or locomotor activity. Monkeys (6/group) were subcutaneously injected with 0 (0.9% saline), 0 (vehicle) and one of 3 doses of liraglutide, 0.02, 0.2, or 2 mg/kg, and monitored for 30 minutes prior to dosing and up to 22 hours after dosing. At the end of the study, all monkeys were intravenously injected with 0.0003 mg/kg norepinephrine, a positive control that transiently elevates systolic and diastolic blood pressure and decreases heart rate and body temperature. Results for several key parameters after dosing with 0 or 2 mg/kg liraglutide are shown below.

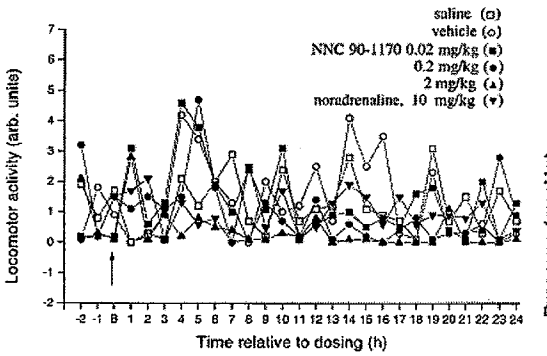
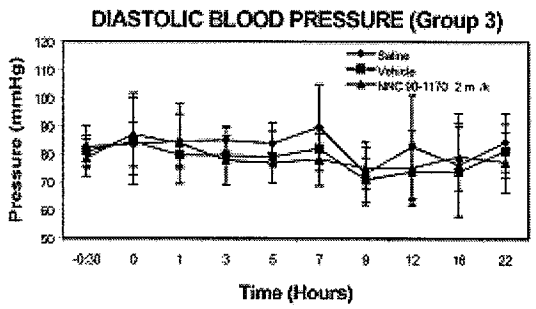
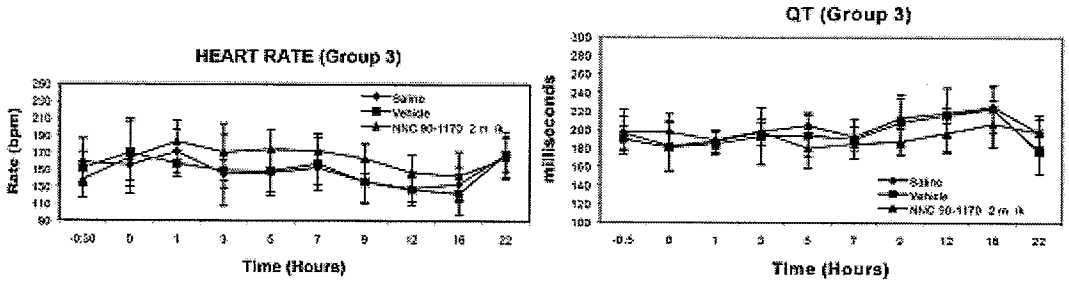


Figure 6 Effect of NNC 90-1170 on locomotor activity (arb. units) in the telemetered male rat.



[980092 P25-6]



[980092 P28, 32]

Renal Effects

Single subcutaneous doses of liraglutide had a dose-related diuretic effect beginning within 2 hours after dosing male Sprague Dawley rats, and at the high dose, caused proteinuria from 6 – 24 hours after dosing. Rats (6/group, nonfasted) were subcutaneous injected with 0 (0.9% saline), 0 (vehicle, 2 mL/kg), 0.02, 0.2, or 2 mg/kg liraglutide (1 mL/kg), water loaded with 20 mL/kg, then placed in a metabolism cage where urine was collected 0 – 2, 2 – 6, and 6 – 24 hours after dosing. Positive control group rats were treated with 0.005 mg/kg desmopressin (po, 10 mL/kg) or 2 mg/kg acetazolamide (po, 10 mL/kg) and water loaded with 10 mL/kg. Consistent with its antidiuretic effect, desmospressin reduced urine volume, sodium and potassium excretion, and increased osmolarity and specific gravity within 2 hours after dosing. Acetazolamide, a diuretic, increased urine volume and excretion of electrolytes and creatinine within 2 hours after dosing, and there was a mild rebound effect of decreased urine volume, increased osmolarity, and decreased sodium excretion from 6 – 24 hours after dosing. Compared to saline, vehicle (phosphate buffer, 38 mg/mL mannitol, 5 mg/mL phenol, pH 7.4) had no significant effect on urinalysis parameters. The NOEL for effects on renal function was < 0.02 mg/kg liraglutide due to reduced urine osmolarity and specific gravity and increased urine volume 2 – 6 hours after dosing at ≥ 0.02 mg/kg.

Urine Parameters	Post-Dose Sample Time (hours)	Liraglutide Dose (mg/kg)			
		0 (vehicle)	0.02	0.2	2
Volume (mL)	0 - 2	4.1	3.8	7.7*	12.6**
	2 - 6	1.5	3.5	9.4**	1.1**
	6 - 24	7.6	7.3	10.9	7.6
Spec. Grav.	0 - 2	1.013	1.012	1.01	1.008
	2 - 6	1.039	1.023**	1.01**	1.012**
	6 - 24	1.027	1.028	1.021*	1.035**
Osmolarity (mmol/kg)	0 - 2	429	417	375	389
	2 - 6	1261	857*	425**	489**
	6 - 24	896	953	725	1191**
Sodium (mmol)	0 - 2	0.203	0.2	0.513*	1.277**
	2 - 6	0.151	0.358	0.949**	1.017**
	6 - 24	0.333	0.417	0.606**	0.168
Potassium (mmol)	0 - 2	0.275	0.294	0.435	0.472*
	2 - 6	0.236	0.353	0.427*	0.478**
	6 - 24	0.995	1.107	0.897	0.895
Chloride (mmol)	0 - 2	0.200	0.211	0.512*	1.128**
	2 - 6	0.221	0.413	0.730**	0.542**
	6 - 24	0.435	0.562	0.744**	0.188**
Protein (mg)	0 - 2	0.767	0.876	0.811	0.884
	2 - 6	1.499	1.884	1.203	6.199
	6 - 24	5.280	8.080	8.120	23.55*

*/**: Dunnett-toat baaed on pooled variance sig. at 5% or 1% leval.

At ≥ 0.2 mg/kg, urine volume, sodium, potassium, and chloride dose-dependently increased and specific gravity and osmolarity decreased within 2 hours of dosing. The diuretic effect persisted from 2 – 6 hours after dosing in 0.2 and 2 mg/kg groups, and up to 24 hours in the 0.2 mg/kg group. At 6 – 24 hours after dosing in the 2 mg/kg group, increased urine specific gravity and osmolarity, decreased sodium and chloride, decreased urine pH (6.1 compared to 6.8 in control) and increased protein were attributed to a rebound effect from diuresis. However, it's unlikely that increased urine protein in the 2 mg/kg group was due to a rebound effect from diuresis.

Gastrointestinal Effects

At low micromolar concentrations, liraglutide reversibly reduced acetylcholine-induced contraction of smooth muscle in isolated guinea pig ileum strips. To determine the effects of liraglutide on ileal smooth muscle contraction, stretched, isolated longitudinal strips of ileum from fasted male albino guinea pigs (4 strips/agonist) maintained in a tissue bath were treated with 0 (Tyrodes solution, tissue bath medium), 0 (vehicle), 0.14, 0.29, or 1.43 µM liraglutide in the absence or presence of the agonists histamine, acetylcholine, or barium chloride. To verify the system, agonist effects were blocked with positive control antagonists of smooth muscle contraction; diphenhydramine, atropine, or papaverine. Up to 1.43 µM liraglutide had no effect on the baseline tension and it did not antagonize contraction elicited by histamine or barium chloride, but 1.43 mM liraglutide caused a slight reduction in the magnitude of the acetylcholine concentration-response curve (Figure 2).

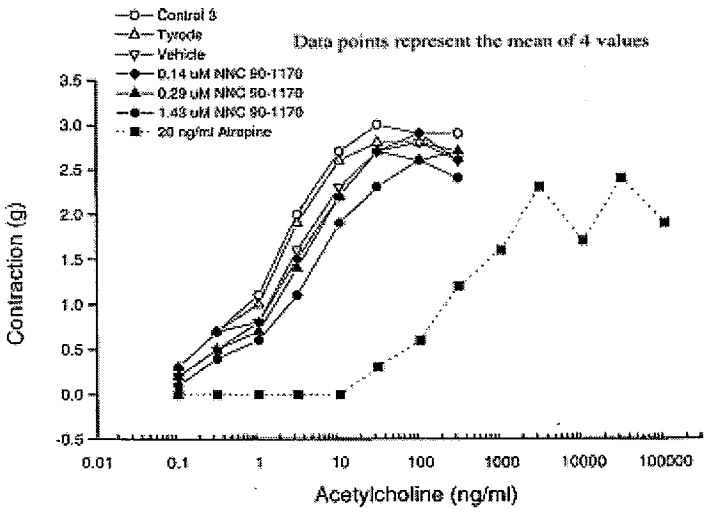


Figure 2 Effects of NNC 90-1170 on acetylcholine induced contractions in the isolated guinea pig ileum

[201207 P36]

2.6.2.5 Pharmacodynamic drug interactions

Liraglutide dose-dependently increased glucose-dependent insulin secretion from isolated, perfused pancreas from overnight fasted male Sprague Dawley rats, and the effect was enhanced by glipizide (Figure 1, below). 0.3 or 3 nM NNC 90-1170 dose-dependently increased insulin secretion, and the liraglutide response at both doses was enhanced by 30 nM glipizide. Glipizide had a similar effect on glucose-dependent insulin secretion enhanced by GLP-1(7-37) (Figure 1, below).

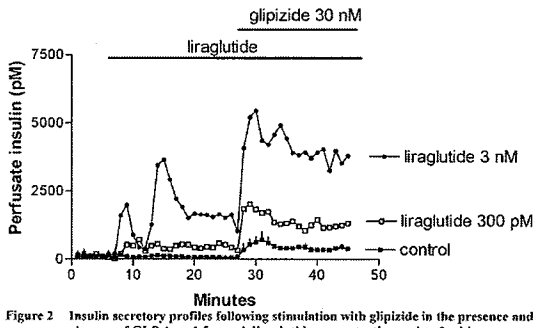


Figure 2 Insulin secretory profiles following stimulation with glipizide in the presence and absence of GLP-1, n=1 for each liraglutide concentration and n=3 without liraglutide

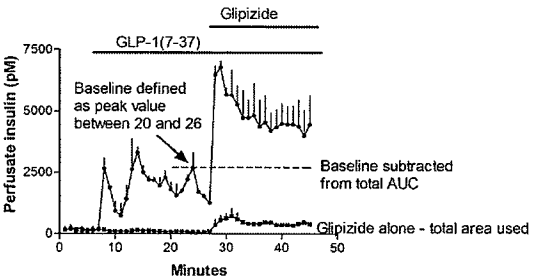


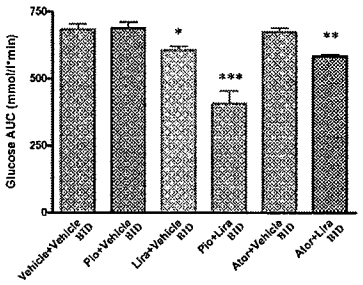
Figure 1 Insulin secretory profiles following stimulation with glipizide in the presence and absence of GLP-1, n=2 with GLP-1 and n=3 without GLP-1

[jstu020304 P12]

The effect of combining liraglutide with pioglitazone or atorvastatin was determined in severely diabetic male ZDF rats (blood glucose ~ 30 mM). The following treatment regimens were administered twice a day for 42 days:

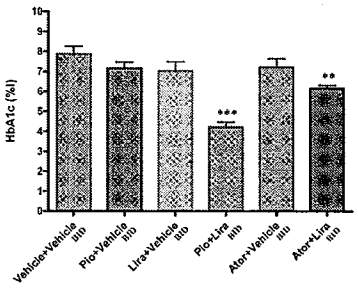
Placebo: vehicle 1 (po)+ vehicle 2 (sc)
Vehicle 1 + 0.2 mg/kg liraglutide
5 mg/kg pioglitazone + vehicle 2
5 mg/kg pioglitazone + 0.2 mg/kg liraglutide
30 mg/kg atorvastatin + vehicle 2
30 mg/kg atorvastatin + 0.2 mg/kg liraglutide

Pioglitazone and atorvastatin were administered orally (0.5 mL total volume) and liraglutide was injected subcutaneously (0.5 mL total volume). Vehicle 1 for pioglitazone or atorvastatin was 10% hydroxypropyl beta-cyclodextrin in water. Vehicle 2 was phosphate buffered saline. The combination of liraglutide with pioglitazone had a synergistic antidiabetic effect decreasing blood glucose (Figure 11) and HbA1c (Figure 16), increasing plasma insulin (Figure 12), improving glucose tolerance (day 42 OGTT), but the combination increased body weight gain (Figure 8), and subcutaneous inguinal, epididymal, and perirenal fat deposits.



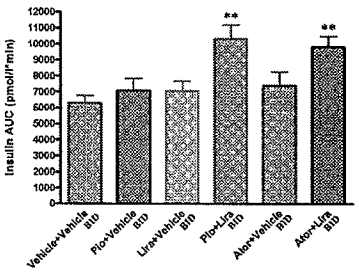
* p<0.05 Lira+Vehicle vs. Vehicle+Vehicle
** p<0.01 Ator+Lira vs. Vehicle+Vehicle
*** p<0.0001 Pio+Lira vs. Vehicle+Vehicle

Figure 11 24h plasma glucose, day 37



** p<0.01 Ator+Lira vs. Vehicle+Vehicle
*** p<0.0001 Pio+Lira vs. Vehicle+Vehicle

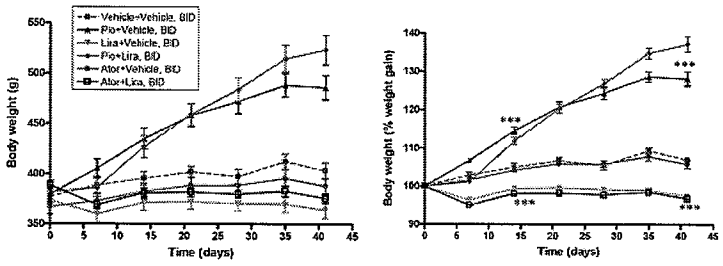
Figure 16 HbA1c after 42 days of treatment



** p<0.01 Ator+Lira vs. Vehicle+Vehicle

Figure 12 24h plasma insulin, day 37

[rhs2004-068b P34, 38, 34]

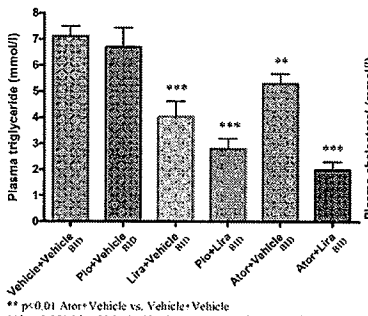


***p<0.0001 Lira+Vehicle and Ator+Lira vs. Vehicle+Vehicle day 14 and day 41
and Pio+Vehicle and Pio+Lira vs. Vehicle+Vehicle day 14 and day 41

Figure 8 Body weight

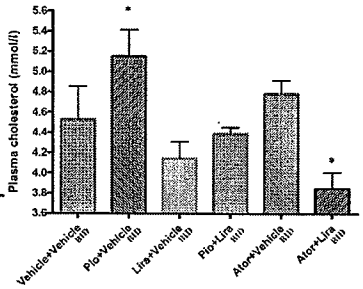
[rhs2004-068b P31]

The combination of liraglutide and atorvastatin improved glycemic control (attributed to effects of liraglutide) and blood lipid parameters including decreased plasma triglycerides (Figure 17), total cholesterol (Figure 18), and free fatty acids (Figure 19).



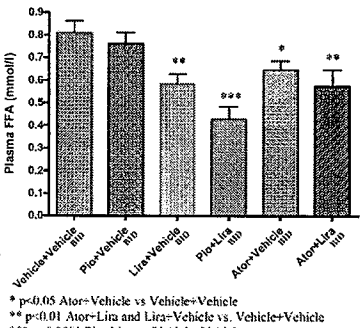
** p<0.01 Ator+Vehicle vs. Vehicle+Vehicle
*** p<0.001 Lira+Vehicle, Pio+Lira and Ator+Lira vs. Vehicle+Vehicle

Figure 17 Triglycerides after 42 days of treatment



* p<0.05 Ator+Lirae and Pio+Vehicle vs. Vehicle+Vehicle

Figure 18 Total cholesterol after 42 days of treatment



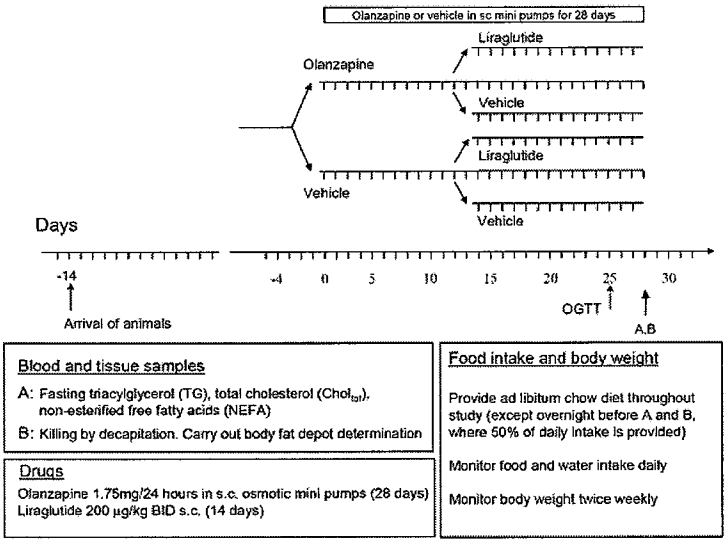
* p<0.05 Ator+Vehicle vs Vehicle+Vehicle
** p<0.01 Ator+Lira and Lira+Vehicle vs. Vehicle+Vehicle
*** p<0.0001 Pio+Lira vs. Vehicle+Vehicle

Figure 19 Free Fatty Acids after 42 days of treatment

[rhs2004-068b P339, 40]

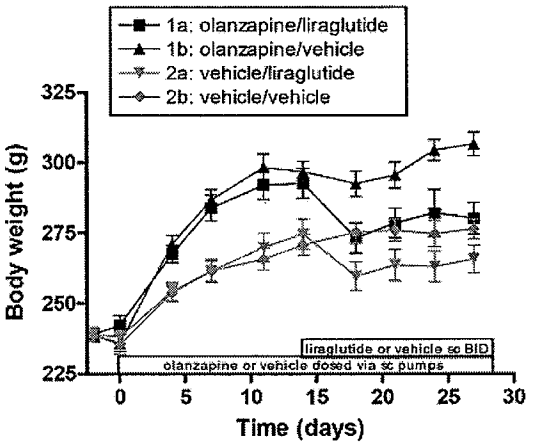
Combined atorvastatin + liraglutide decreased food intake, body weight gain, and subcutaneous inguinal fat deposits. Liraglutide alone improved glycemic control, decreased plasma triglycerides and free fatty acids, decreased food and water intake, and decreased body weight gain and subcutaneous epididymal fat deposits. There were no changes in beta cell mass in any liraglutide-treated group, but pioglitazone alone decreased beta cell mass. On study day 42, combined liraglutide and atorvastatin slowed conduction velocity in the caudal nerve, a sensory nerve.

In adult female Sprague-Dawley rats, liraglutide normalized olanzapine-induced increased food intake, body weight, subcutaneous fat deposits (mesenteric, retroperitoneal, inguinal), fasting plasma glucose, and plasma cholesterol. Vehicle (1.4% lactic acid phosphate buffered saline) or olanzepine was continuously dosed at a rate of 1.75 mg/24 hours using subcutaneous implanted osmotic minipumps in female rats. After 14 days, half of each vehicle or olanzepine sc infusion groups were treated with liraglutide (0.1 mg/kg sc bolus injection BID with the dose escalated by 0.05 mg/kg BID daily until reaching the final dose of 0.2 mg/kg sc BID 3 days after starting liraglutide), and the other half were treated with vehicle (50 mM PBS, 0.5 – 0.7 mL/dose, sc bolus injection BID). A schematic of the study design and parameters is shown below.



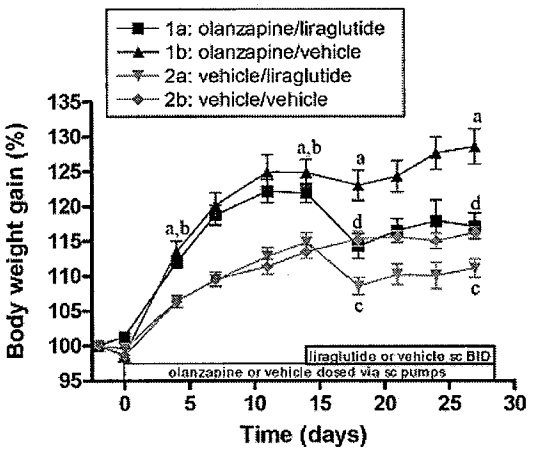
[rhs2005-104, P11]

Liraglutide decreased body weight and body weight gain in olanzepine or vehicle treated rats (Figures 1 and 2). Decreased body weight gain was due to decreased cumulative food intake between days 16 and 27 (the liraglutide treatment period).



[a] p<0.05 olanzapine/vehicle vs. vehicle/vehicle day 4, 14, 18 and 27
[b] p<0.05 olanzapine/liraglutide vs. vehicle/vehicle day 4 and 14
[c] p<0.05 vehicle/liraglutide vs. vehicle/vehicle day 18
[d] p<0.05 olanzapine/liraglutide vs. olanzapine/vehicle day 18 and 27

Figure 1 Body weight (g)



[a] p<0.05 olanzapine/vehicle vs. vehicle/vehicle day 4, 14, 18 and 27
[b] p<0.05 olanzapine/liraglutide vs. vehicle/vehicle day 4 and 14
[c] p<0.05 vehicle/liraglutide vs. vehicle/vehicle day 18 and 27
[d] p<0.05 olanzapine/liraglutide vs. olanzapine/vehicle day 18 and 27

Figure 2 Body weight gain (%)

[rhs2005-104, P16-17]

Liraglutide decreased mass of inguinal, mesenteric, and retroperitoneal fat (Table 7) and improved plasma glucose, triglycerides, and total cholesterol (Table 6) in olanzepine treated rats.

Table 7 Fat depots in grams (day 29)

	Olanzapine/ liraglutide	Olanzapine/ vehicle	Vehicle/ liraglutide	Vehicle/ vehicle
S.c. inguinal fat	2.4±0.2 [b,d]	3.4±0.3 [a]	1.5±0.1 [¶]	1.9±0.1
Mesenterial fat	1.8±0.1 [d]	3.1±0.2 [a]	1.2±0.1 [c]	1.7±0.2
Retroperitoneal fat	3.5±0.4 [d]	6.2±0.6 [a]	1.6±0.2 [c]	2.8±0.3

One-way ANOVA followed by Fisher PLDS

[a] p<0.05 olanzapine/vehicle vs. vehicle/vehicle

[b] p<0.05 olanzapine/liraglutide vs. vehicle/vehicle

[c] p<0.05 vehicle/liraglutide vs. vehicle/vehicle

[d] p<0.05 olanzapine/liraglutide vs. olanzapine/vehicle

[¶] p=0.084 vehicle/liraglutide vs. vehicle/vehicle

[rhs2005-104, P34]

Table 6 Blood biochemistry (day 25/28)

	Olanzapine/liraglutide	Olanzapine/vehicle	Vehicle/liraglutide	Vehicle/vehicle
Glucose (mM)	7.4±0.2	7.7±0.2 [a]	6.8±0.2	7.0±0.1
Insulin (pM)	40.6±6.6	49.3±6.1	38.7±5.4	48.6±11.4
Triacylglycerol (mM)	0.50±0.05	0.61±0.09 [¶]	0.52±0.03	0.48±0.02
Total cholesterol (mM)	2.30±0.08	2.33±0.11 [a]	1.86±0.10	1.88±0.10
NEFA (mEq/l)	0.71±0.06	0.64±0.06	0.76±0.04	0.66±0.06

One-way ANOVA followed by Fisher PLDS

[a] p<0.05 olanzapine/vehicle vs. vehicle/vehicle

[¶] p=0.068 olanzapine/vehicle vs. vehicle/vehicle

[rhs2005-104, P26]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Liraglutide was formulated as a solution for subcutaneous (sc) or intravenous (iv) injection. In nonclinical studies, liraglutide was dissolved in aqueous phosphate ——— (pH ——— 8.15) and the vehicle usually included phenol ——————— and ——————— (most nonclinical studies) or propylene glycol (104 week mouse carcinogenicity study and 87 week mechanistic study in monkeys) to make the solution ——————————— . Plasma pharmacokinetics were determined after single sc injections (mice, rats, rabbits, monkeys, pigs, and humans) and single iv injections (monkeys, pigs, and humans). Plasma toxicokinetics were determined in single dose studies in mice and rats and multiple dose studies in mice, rats (including pregnant rats), unmated and mated female rabbits, pigs, and monkeys. Plasma liraglutide concentrations were measured using a radioimmunoassay (RIA) that cross-reacted with endogenous GLP-1, and later during development, using an enzyme-linked immunosorbent assay (ELISA) that was more 'liraglutide-selective'. In general, plasma liraglutide concentrations determined by RIA were within 2-fold of concentrations measured by ELISA. Plasma levels of liraglutide determined by either method were expressed as nanomolar concentrations, but considering both methods only detect the peptide substructure of liraglutide, the assays may not distinguish between liraglutide and some of its metabolites, so drug concentrations should be expressed as liraglutide equivalents. Anti-liraglutide antibodies in blood samples were detected using a RIA precipitating protein-bound radioactivity from plasma incubated with ^{125}I-liraglutide. However, even nanomolar concentrations of liraglutide in blood samples interfere with detecting anti-drug antibodies by this method. The limit of sensitivity of the anti-drug antibody assay was > 1 µg/mL antibody at plasma liraglutide concentrations ≥ 20 nM. An assay to determine neutralizing activity of anti-liraglutide antibodies was not developed. Tissue distribution in albino rats and pigmented rats was determined by whole body autoradiography or scintillation / gamma counting tissue radioactivityafter single sc or iv dosing with 1 of 4 liraglutide radionuclides labeling the drug on the K34R hGLP-1 peptide (^{125}I- or ^{3}H-[tyrosine]), the glutamate linker (^{14}C-[glutamate]), or the lipid (^{3}H-[palmitate]). [3-iodo-tyr]Liraglutide, a non-radioactive version of ^{125}I-liraglutide, had a GLP-1R agonist potency similar to liraglutide in a cell-based assay. Tissue distribution was determined in male and female albino rats, male pigmented rats, pregnant albino Sprague Dawley rats, and pregnant New Zealand White rabbits. Protein binding was determined by equilibrium dialysis using plasma from mice, rats, rabbits, monkeys, and humans and human albumin and alpha-1 glycoprotein. Liraglutide metabolites were identified in plasma from mice, rats, monkeys, and humans, in urine from rats, monkeys, and humans, in feces from monkeys and humans, and in expired air from rats. *In vitro* metabolism was evaluated using hepatocytes from mice, rats, monkeys, and humans, kidney and liver slices from rats, perfused liver and kidney from rats, plasma from mice, rats, monkeys, and humans, and purified human dipeptidyl peptidase IV (DPP-4) and human neutral endopeptidase (NEP). *In vitro* CYP inhibitory potency of liraglutide was determined in human liver microsomes. Liraglutide effects on CYP activity were determined in rat liver samples taken from a 4-week repeat dose toxicity study. Mass balance after a single sc injection of radiolabeled liraglutide was evaluated in rats (^{125}I- or ^{14}C-liraglutide), cynomolgus monkeys (^{3}H-[pal]-liraglutide), and humans (^{3}H-[pal]-liraglutide) and after 7 days of repeat sc dosing in rats (^{125}I- or ^{14}C-liraglutide). Maternal transfer of radiolabeled liraglutide to fetuses was evaluated in rats and rabbits and secretion of liraglutide in milk was determined in lactating rats.

Bioavailability after subcutaneous (sc) injection was 53% in monkeys, 76% in pigs, and 55% in humans. Pharmacokinetics after sc dosing were linear with dose-proportional increases in

b(4)

peak (Cmax) and total (AUC) exposure in nonclinical species including mice, rats, rabbits, monkeys, and pigs. There were no substantive sex differences in exposure in any species. Although total 24 hour exposure ($AUC_{0\text{-}24h}$) was generally higher after repeat dosing, plasma accumulation was minimal in nonclinical species. Consistent with a longer elimination half-life, some accumulation occurred in humans (1.4 – 1.5 fold). Tmax occurred 6 – 8 hours after sc dosing. The plasma elimination half-life was shorter in rats (3.6 hours) than in mice, rabbits, or monkeys (6.7 – 7.1 hours), or pigs and humans (14 - 15 hours). Lower protein binding in rats compared to other species may account for the shorter plasma elimination half-life, at least in part. Liraglutide has a shorter half-life after iv dosing compared to sc dosing, and this is consistent with delayed absorption from sc injection sites contributing to its persistence in systemic circulation. The apparent volume of distribution (Vz 0.05 – 0.23 L/kg) after iv dosing was consistent with limited extravascular distribution of a highly plasma protein bound drug. Comparison of liraglutide exposure (AUC) versus body weight-based dose (mg/kg) after repeat dosing in different species showed liraglutide exposure was highly correlated with dose across species. Comparison of liraglutide plasma clearance (CL/f) versus body weight (kg) was consistent with allometric scaling with clearance being proportional to body weight across species. In cynomolgus monkeys, bioavailability of inhaled liraglutide was low based on plasma liraglutide AUC comparison from an inhaled dose of 0.133 mg/kg and from an iv dose of 0.120 liraglutide. Plasma exposure to liraglutide was ~ 70 fold lower after inhalation compared to iv dosing.

Anti-liraglutide antibodies were not detected in repeat sc dose studies in mice or rats, but they did occur in cynomolgus monkeys treated with 5 mg/kg/day liraglutide for 52 weeks and at 0.25 and 5 mg/kg/day liraglutide in monkeys treated for up to 87 weeks. Consistent with low nanomolar concentrations of liraglutide interfering with the anti-drug antibody assay, antibodies in two monkeys in the 5 mg/kg high dose group in the 52 week study were only detected in samples taken during a recovery period, several weeks after treatment was stopped.

Single sc dose tissue distribution studies in albino rats using liraglutide radiolabeled on the peptide, linker, or lipid substructures had similar tissue distributions at early time points ($\leq$ 4 hours, but distribution differed at later time points (> 4 hours), probably due to differences in the metabolic fate of different parts of liraglutide (amino acid, linker, or lipid). Plasma levels of radioactivity peaked 4 hours after dosing. At earlier time points (< 4 hours), higher amounts of radioactivity occurred at the injection site and in liver, kidney, brown fat, and adrenals. Despite high blood flow to the brain and reports of GLP-1 readily penetrating the blood-brain barrier in rats, liraglutide levels in brain were low suggesting it does not readily cross the blood-brain barrier. Thyroid is a target organ of toxicity in rats and mice, and levels of radioactivity in thyroid exceeded plasma levels at later time points in most studies using ^{14}C-liraglutide. Studies using ^{125}I-liraglutide were confounded by accumulation of free ^{125}I in thyroid. A microhistoautoradiography study of thyroid and pancreas from rats with samples taken up to 4 hours after administering a single intravenous dose of ^{3}H-[tyr]-liraglutide showed radioactivity occurred in pancreatic islets and blood vessel endothelium in thyroid and pancreas, but not in thyroid C-cells. Liraglutide did not bind to melanin-containing organs in pigmented Lister hooded rats and there were no substantive differences in tissue distribution between albino and pigmented male rats. *In vitro* protein binding studies showed liraglutide was highly bound to plasma proteins in CD-1 mice (99.5%), NZW rabbits (99.8%), cynomolgus monkeys (99.5%), and humans (99.0%) with slightly lower plasma protein binding in rats (97.0% in Sprague Dawley, 98.9% in ZDF). Liraglutide was highly bound to human serum albumin (99.4%) and human alpha-1 glycoprotein (99.3%).

Five-day repeat dose tissue distribution studies in pregnant New Zealand White rabbits and albino Sprague Dawley rats with liraglutide levels determined by ELISA showed liraglutide crossed the placenta into fetuses with excretion into amniotic fluid where drug levels were ~ 1% of maternal plasma levels. In rabbits, fetal plasma liraglutide levels were 1.5 – 4.2 % of maternal

plasma levels. Radiolabeled liraglutide was secreted in milk of lactating rats, primarily as the parent drug. Based on secretion of liraglutide-related radioactivity in milk, the sponsor estimates a pup would consume 0.3 – 3% of the total daily liraglutide dose administered to the dam. Liraglutide in rat milk was largely the intact drug, and its concentration in milk was ~50% of the maternal plasma concentration, but since liraglutide is a lipidated peptide, it probably has low oral bioavailability.

In vivo and *in vitro* metabolism studies show liraglutide circulates primarily as the intact parent drug that is metabolized by NEP and DPP-4 with further extensive metabolism of constituent amino acids and palmitic acid. No major human metabolites were identified, but HPLC characterization of radiolabeled metabolites from humans administered a single sc dose of lipid-labeled ^{3}H-[pal]-liraglutide showed only 2 metabolites occurred (Rt_R 1.05 and Rt_R 1.15). Both metabolites were < 10% of total drug-related peptide-containing material (based on AUC). In animals administered ^{3}H-[pal]-liraglutide, Rt_R 1.05 was also found in plasma from mice, rats, and monkeys and Rt_R 1.15 occurred in plasma from rats. In an *in vitro* metabolism study using purified peptidases, albumin increased the resistance of liraglutide to DPP-4 and NEP, with a much larger effect on NEP-mediated metabolism. *In vitro* metabolism studies using hepatocytes from mice, rats, monkeys, and humans, kidney and liver slices from rats, perfused liver and kidney from rats, and plasma from mice, rats, monkeys, and humans were consistent with NEP and DPP-4 mediated metabolism. Liraglutide does not inhibit CYP drug-metabolizing enzymes. Four weeks of treatment with 1 mg/kg/day liraglutide decreased CYP2A1 (hepatic steroid hydroxylase, testosterone 7 alpha-hydroxylase) activity ~2-fold in liver of male rats, but didn't significantly affect other liver CYPs including CYPs 1A2, 2B1/2, 2C11, 2E1, 3A1/2 and 4A.

Excretion of radiolabeled liraglutide was evaluated in rats, monkeys, and humans with recovery of excreted radioactivity and routes of excretion depending on the radiolabeled site. *In vivo* metabolism and excretion studies indicate liraglutide was not excreted intact, its metabolism and excretion were not dependent on kidney or liver, and it was extensively metabolized to amino acids and lipids with further metabolism to water and CO_2. Only 18.9 – 31.2% of total administered radioactivity from a single sc dose of lipid-labeled ^{3}H-[pal]-liraglutide in cynomolgus monkeys (0.05 or 5 mg/kg) or humans (0.75 mg) was recovered with 13.5 – 21.7% in urine and 5.4 – 8.8% in feces. The sponsor believes radioactivity from ^{3}H-[pal]-liraglutide was fully metabolized to $^{3}H_2O$ and excreted in urine, sweat, and expired air. In rats administered 1 or 7 daily sc doses of 1 mg/kg ^{14}C-liraglutide labeled in the glutamate linker, 91.7 – 93.2% of the administered radioactivity was recovered with 71.3 – 72.2% in expired air ($^{14}CO_2$), 8.0 – 8.7% in feces, 4.4 – 6.9% in urine, and 8.1% in the carcass. In rats administered 1 or 7 daily sc doses of 1 mg/kg ^{125}I-liraglutide or a single sc dose of 1 mg/kg ^{125}I-liraglutide (labeled on tyrosine-19 of K34R hGLP-1(7-37) in liraglutide), 93.9 – 100.6% of the administered radioactivity was recovered with 77.5 – 89.4% in urine, 3.1 – 7.4% in feces, and 7.0 - 10% in the carcass. ^{125}I was primarily excreted in urine as free ^{125}I or ^{125}I not associated with protein (not precipitated by TCA).

In contrast to liraglutide, which is highly protein bound, stable in plasma, resistant to DPP-4 and NEP, and largely extensive metabolized prior to excretion, GLP-1 is not bound to plasma protein and it's rapidly metabolized by DPP-4, with GLP-1 peptide metabolites excreted in urine with or without further metabolism by NEP prior to excretion.

2.6.4.2 Methods of Analysis

Liraglutide

Liraglutide concentrations in plasma or serum were quantified using a competitive binding RIA assay based on displacement of [^{125}I]liraglutide bound to a polyclonal anti-GLP-1 antibody (HER 5 serum from [redacted], specific for N-terminal amino acids 7+8) or a colorimetric sandwich ELISA using 2 monoclonal antibodies directed at different liraglutide b(4)

epitopes (liraglutide epitopes not identified) using an immobilized N-terminal antibody to capture liraglutide and a biotin-labeled C-terminal antibody to label it. The RIA assay was developed first, but since the assay detected both liraglutide and endogenous GLP-1, the ELISA was developed to include a procedure to eliminate endogenous GLP-1 by incubating plasma for 4 hours at 37C. Because assay standards used for assay calibration were diluted in human plasma, plasma samples from nonclinical species were also diluted in human plasma (5-fold dilution of plasma samples from mice, rabbits, monkeys, and pigs, and 25-fold dilution for rat plasma), and this increased the lower limit of quantification for nonclinical samples (Table 2).

Table 2 Lower limit of quantification (LLOQ) of the ELISA in preclinical species and human

Animal	Lower Limit of Quantification (LLOQ)
Mouse	90 pM
Rat	450 pM
Rabbit	90 pM
Monkey	90 pM
Pig	90 pM
Human [1]	18 pM

1) In 2001 the LLOQ for human plasma samples were updated from 50 pM til 18 pM.

[PK Written Summary, P10]

Several conditions and compounds interfere with the ELISA. Anti-liraglutide antibodies decrease the response, hemolysis of the sample increases the response, and several liraglutide-related compounds interfere with the assay including the metabolite liraglutide (9-37), liraglutide isomer, major proglucagon fragment, GLP-1(15-37), and GLP-1(16-37). GLP-1(1-36)amide and GLP-1(7-36)amide do not interfere with the assay, but the effects of GLP-1(9-36) or GLP-1(9-36)amide are unknown. Liraglutide exposures determined using RIA and ELISA methods performed on the same plasma samples from mice, rats, rabbits, and monkeys showed exposures were comparable at low doses and there was < 2 fold difference between exposures at high doses. The ELISA method was used to determine liraglutide concentrations in all human studies and in nonclinical chronic repeat dose toxicity studies and carcinogenicity studies. Table 4 shows an overview of liraglutide assays used to measure plasma drug concentrations in nonclinical studies.

Table 4 Overview of the bioanalytical methods used in all TK/PK reports of liraglutide

Animal	NN Study No.	Study Duration or Type of Study	Bioassay	ELISA / RIA comparison
Mouse	990267	Single dose; plasma concentration (no TK)	RIA	x
	200031	ob/ob, db/db	ELISA	
	203261	4 weeks toxicology (+day 1)	ELISA	
	204082	13 weeks toxicology (+day 1)	ELISA	
	204229	104 weeks toxicology (+26 and 52 weeks)	ELISA	
	205050	3 days, Exenatide/Liraglutide	ELISA	
	205106	Single dose, calcitonin, KO/CD-1 mice	ELISA	
Rat	970355	Single dose	RIA	
	990268	Single dose, Plasma Concentration (no TK)	RIA	
	980180	7d Dose Range Finding	RIA	
	980183	4 weeks toxicology	RIA	x
	980186	3½ weeks toxicology, Reprotox	RIA	
	980189	13 weeks toxicology (+ day 1)	RIA	x
	200239	26 weeks toxicology (+day 1)	ELISA	
	200240	104 weeks toxicology (+ day 1 and 53 weeks)	ELISA	
	205092	4 weeks, Bridging Formulations	ELISA	
Monkey	980149	3 days	RIA	
	980181	2 weeks, MTD	RIA	
	980182	Single dose, i.v. / s.c., cross-over	RIA	x
	980184	4 weeks toxicology (+ day 1)	RIA	
	990191	13 weeks toxicology	RIA	
	200241	52 weeks toxicology (+ day 1)	ELISA	
	201105	Single dose, i.v., pulmonary	ELISA	
	203262	87 weeks toxicology (+ 72 weeks)	ELISA	
Rabbit	980187 / 980188	Dose range finding and Reprotoxicology	RIA	x
Pig	970219	Single dose, iv/sc	RIA	
	970445	Multiple dose	RIA	

[PK Written Summary, P12]

Anti-Liraglutide Antibodies

Anti-liraglutide antibodies were measured using a RIA based on precipitating protein-bound radioactivity from plasma incubated with [^{125}I]liraglutide. [^{125}I]Liraglutide was added to plasma, incubated overnight, then plasma proteins were precipitated with polyethylene glycol. Samples were considered positive if precipitated radioactivity exceeded the level of radioactivity in control samples + 4 standard deviations and confirmed using the same assay with the same criteria, except subtracting out non-specifically protein bound [^{125}I]liraglutide determined by performing the assay in the presence of a large molar excess of unlabeled liraglutide. To determine if anti-liraglutide antibodies cross-reacted with GLP-1, the same assay was performed, except [^{125}I]GLP-1 was added instead of [^{125}I]liraglutide. The sensitivity of the assay was decreased by unlabeled liraglutide in plasma samples. Using a known monoclonal anti-GLP-1 antibody diluted in plasma determined assay sensitivity was ≥ 25 - 50 ng/mL antibody in the absence of liraglutide, but sensitivity rapidly decreased in the presence of nanomolar concentrations of liraglutide (Table 4).

Table 4 Interference from liraglutide

Liraglutide Conc.	Assay sensitivity
0	25-50 ng/ml
3 nM	100 ng/ml
30 nM	1500 ng/ml
300 nM	25000 ng/ml

[206414 P10]

Anti-liraglutide antibody sensitivity is decreased to > 1 µg/mL at plasma liraglutide concentrations ≥ 20 nM, so assay results in the presence of > 20 nM liraglutide are uninformative. To get around this problem, blood samples for antibody analysis were taken after stopping treatment in some studies. A functional assay to characterize neutralizing activity of anti-liraglutide antibodies was not developed.

Radiolabeled Liraglutide

Radiolabeled liraglutide was used in studies of liraglutide tissue distribution, metabolism, and excretion. Liraglutide was radiolabeled at 1 of 3 different positions: ^{125}I-Tyr and ^{3}H-Tyr labeling the peptide, ^{14}C-labeling the glutamate linker, and ^{3}H-labeling palmitic acid, but none were double or triple-labeled. Radiolabeled sites are shown in Figure 6.

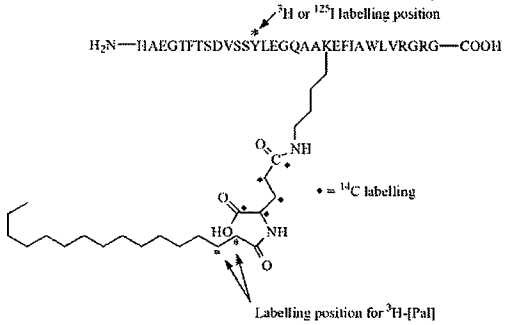


Figure 6 Chemical structure of radiolabelled liraglutide with indication of labelling positions for ^{3}H, ^{125}I and ^{14}C radiolabels.

[PK Written Summary, P21]

2.6.4.3 Absorption

Single dose pharmacokinetic (PK) parameters of liraglutide in plasma from mice, rats, cynomolgus monkeys, and pigs were determined after subcutaneous (mice, rats, monkeys, pigs, and humans), intravenous (monkeys, pigs, and humans), or inhalation (monkeys) dosing. Multiple dose PK/TK parameters were determined in mice, rats, rabbits, monkeys, and pigs after subcutaneous dosing.

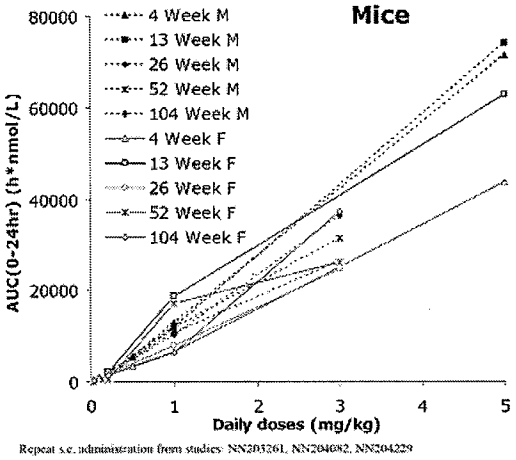
Systemic absorption of liraglutide after sc dosing was similar in monkeys (53%), pigs (76%), and humans (55%). Species comparison after a single dose show plasma liraglutide Cmax occurred within 4 – 9 hours after sc dosing (Table 1) and elimination half-life ranged from 3.5 hours in rats to 15 hours in humans. The shorter half life after iv dosing in pigs and humans suggests delayed absorption after sc dosing contributes to a longer duration of higher plasma levels after sc dosing. Plasma Tmax of radiolabeled drug-related material after subcutaneously dosing with radiolabel liraglutide in mice (1 mg/kg ^{3}H-[Pal]-liraglutide, Tmax 4 – 6 hours), rats (0.1 – 1 mg/kg ^{3}H-[Pal]-liraglutide, ^{125}I-liraglutide, or ^{14}C-liraglutide, Tmax 4 – 8 hours), and monkeys (0.05 or 5 mg/kg ^{3}H-[Pal]-liraglutide, Tmax 8 – 12 hours) were similar to Tmax determined for unlabeled liraglutide.

Table 1 Interspecies comparison of pharmacokinetics parameters for liraglutide based on single doses using non-labelled material (mean males and females)

Species	Mouse	Rat	Rabbit	Monkey		Pig		Human	
Route	s.c	s.c	s.c	i.v.	s.c.	i.v.	s.c	i.v.	s.c
Dose (mg/kg)	0.1	0.1	0.01	0.12	0.05	0.00188	0.00188	0.005	0.005
C_{max}/Dose (nmol/L)/(mg/kg)	750	770	1000	-	600	-	560	-	1780
AUC/Dose (nmol·h/L)/(mg/kg)	11600	8700	14000	49000	9700	18600	14400	43100	26800
t_{max} (h)	6	6	6	-	7.3	-	7	-	9.3
CL (L/h/kg)	-	-	-	0.0055	-	0.016	-	0.006	-
Vz (L/kg)	-	-	-	0.05	-	0.23	-	0.07	-
$t_{½}$ (h)	6.7	3.5	7	6.2	7.1	10	14	8.1	15
Bioanalytical Method	ELISA	ELISA	RIA	ELISA		RIA		ELISA	
Study No. PK	NN203261	NN200239	NN980187	NN201105	NN200241	NN970219		NN2211-1149	
Protein bound (%)	99.5-99.7	95.8-98.2	99.7-99.8	99.3-99.8		99.4-99.7		98.7-99.2	
Study No. Protein binding	NN204284	NN200152	NN200152	NN201222		NN202117		NN200152, NN201224, NN203029, NN204223	

[PK Written Summary P7]

In mice, Tmax occurred 2 – 8 hours after a single sc dose with Cmax and AUC increasing proportionally with dose. The terminal elimination half-life was 4 – 10 hours. Repeat sc dose plasma kinetics were determined in 4 studies up to 104 weeks in both sexes (4, 9, 13, and 104 week studies with sampling in weeks 4, 13, 26, 52, and 104 weeks). Figure 3 shows total 24 hour plasma exposure (AUC_{0-24}) versus dose at different sample times in mice. Exposure increased proportionally with dose with minimal accumulation and no substantive sex differences.

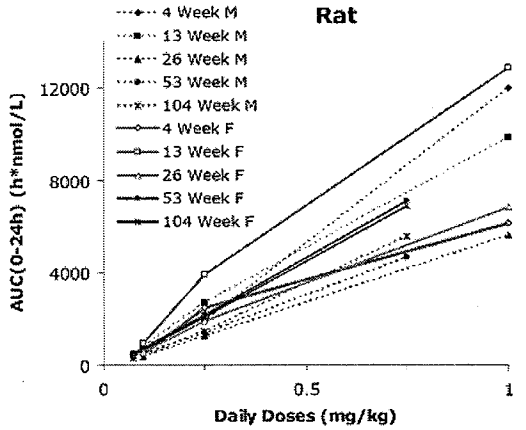


Repeat s.c. administration from studies: NN203261, NN204082, NN204229

Figure 3 Overview of exposure vs. dose level in toxicokinetic studies performed in mice.

[PK Written Summary P18]

In rats, Tmax occurred 4 – 8 hours after a single sc dose with Cmax and AUC increasing proportionally with dose. The terminal elimination half-life was 3 – 8 hours. Repeat sc dose plasma kinetics were determined in 6 studies up to 104 weeks in rats in both sexes (4, 13, 26, 104 with sampling in weeks 4, 13, 26, 53, and 104 weeks). Figure 4 shows total 24 hour plasma exposure (AUC_{0-24}) versus dose at different sample times in rats. Exposure increased proportionally with dose with minimal accumulation and no substantive sex differences.

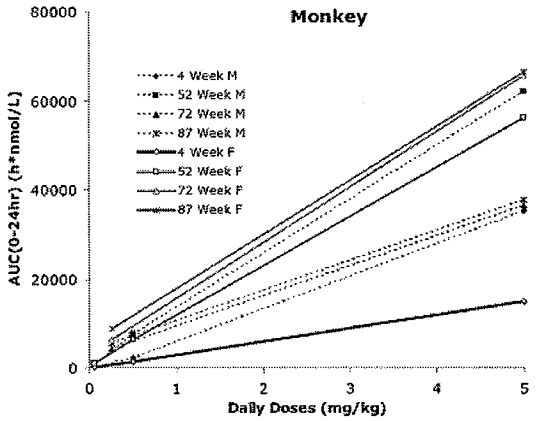


Repeat s.c. administration from studies: NN9901[illegible], NN9901[illegible], NN200239, NN200240; RIA analysis in the 4- and 13-week studies

Figure 4 Overview of exposure vs. dose level in toxicokinetic studies performed in rats.

[PK Written Summary P18]

In cynomolgus monkeys, plasma liraglutide Tmax occurred 4 – 8 hours after a single sc dose. Cmax and AUC increased proportionally with dose. The elimination half-life was 7.1 hours after sc dosing and 6.2 hours after iv dosing. The volume of distribution (Vz) after iv dosing was 0.05 L/kg for a 0.12 mg/kg dose and varied from 0.055 – 0.15 L/kg after a 5 mg/kg dose. Clearance (CL) was 0.0055 L/h/kg after a single iv dose of 0.12 mg/kg liraglutide. Repeat sc dose plasma liraglutide kinetics were determined in 7 studies in monkeys up to 87 weeks in duration. Figure 5 shows total 24 hour exposure (AUC_{0-24}) versus liraglutide dose after 4, 52, 72, and 87 weeks of dosing in both males and females. Exposure increased proportionally with dose with no substantive sex difference or accumulation with repeat dosing.



Repeat s.c. administration from studies: NN9901[illegible], NN200241, NN203262; RIA analysis in the 4 week study)

Figure 5 Overview of exposure vs. dose level in toxicokinetic studies performed in monkey.

[PK Written Summary P19]

In pigs, plasma liraglutide Tmax occurred 7 hours after a single sc dose with 76% bioavailability. The elimination half-life was 14 hours after sc dosing and 10 hours after iv dosing. After a single iv dose, the clearance was 0.016 L/h/kg and the volume of distribution (Vz) was 0.21 L/kg. In a 5-day repeat sc dose study in female pigs administered 0.0002 mg/kg/day liraglutide (0.5 nmol/kg), pharmacokinetic parameters were determined on days 1 and 5 (summary table below).

	C_{max} (pmol/l)	t_{max} (h)	C_{ss} (pmol/l)	$t_{½}$ (h)
Day 1	1285 ± 121	9 ± 1	-	-
Day 5	1820 ± 62	4 ± 2	1363 ± 42	27*

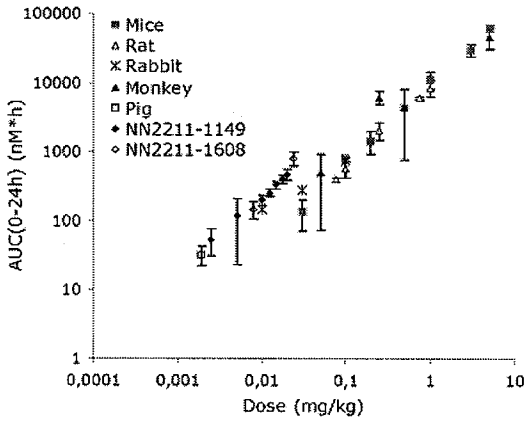
*) Harmonic mean

[970445 P5]

Day 5 PK parameters showed Cmax increased < 2 fold, Tmax was substantially shorter (4 hours after dosing on day 5 compared to 9 hours on day 1), and the elimination half-life was > 24 hours.

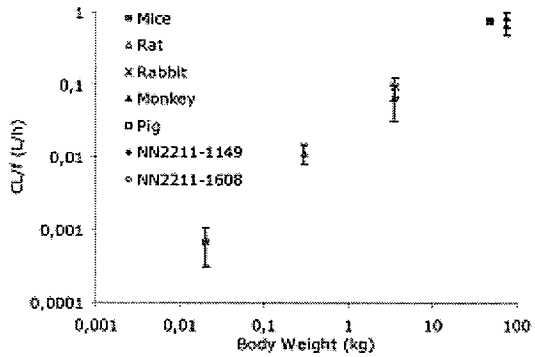
Plasma liraglutide Tmax after a single sc dose in unmated female rabbits occurred 6 – 8 hours after dosing and the elimination half-life was 7 hours. Peak and 24 hour total drug exposure increased proportionally with dose.

An overview of total 24 hour plasma exposure to liraglutide (AUC_{0-24h}) versus dose in mice, rats, rabbits, monkeys, pigs, and humans (clinical studies NN2211-1149 & -1608) is shown in Figure 1 (below). Exposure increased proportionally with dose in all species. A graph of liraglutide plasma clearance (CL/f) versus body weight (Figure 2, below) is consistent with allometric scaling.



Repeat s.c. administration preclinical studies: Mice: NN2202361, NN2204082, NN2204229; Rat: NN9980183, NN9801689, NN2000239, NN2001240; Rabbit: NN9800187, Monkey: NN9980184, NN2002241, NN2002362; Pig: NN9978445. Humans: clinical trials NN2211-1149 (single dose), NN2211-1608 (multiple dose).

Figure 1 Overview of exposure versus administered doses



Humans: NN2211-1149 (single s.c. dose), NN2211-1608 and repeat s.c. administration preclinical studies: Pig NN9978445, Rabbit: NN9980187 (single dose); Monkey: NN2002241, NN2002362; Rat: NN9980183, NN9801689, NN2002239, NN2006269; Mice: NN2202361, NN2204082, NN2204229).

Figure 2 Correlation between body weight and clearance.

[PK Written Summary P17]

Anti-liraglutide Antibodies

In repeat sc dose toxicity or mechanistic studies of liraglutide in mice, rats, and monkeys, anti-liraglutide antibodies did not occur in mice treated for up to 104 weeks or rats treated for up to 69 weeks. In cynomolgus monkeys, anti-liraglutide antibodies occurred in 3/12 high dose monkeys (5 mg/kg/day liraglutide) in a 52 week repeat dose toxicity study (one monkey was positive in week 52 (decreased plasma exposure compared to day 1) and 2 monkeys were positive after the 4 week recovery period (increased plasma exposure in week 52 compared to day 1). In an 87-week mechanistic study, 5 monkeys were antibody positive; 1/10 in the 0.25 mg/kg/day liraglutide group and 4/10 in the 5 mg/kg/day high dose group after 72 or 87 weeks of treatment. Although antibodies in the 5 mg/kg group did not affect plasma exposure (compared to monkeys that were antibody negative), the antibody positive monkey in the 0.25 mg/kg group had a 4 fold increased liraglutide plasma exposure compared to antibody negative monkeys in the same group. Neutralizing activity of anti-liraglutide antibodies was not determined.

Bioavailability of Inhaled Liraglutide

In study 201105, bioavailability and local tolerance of inhaled or intravenously administered NNC 90-1170 was assessed in cynomolgus monkeys (2/sex/phase) in 3 phases: phase A (inhalation of vehicle), phase B (inhalation of 5 mg/mL liraglutide yielding a dose of 0.133 mg/kg) and phase C (intravenous 0.12 mg/kg liraglutide). There was a 7 day washout between phase B and phase C. Composition of the vehicle was not stated, but it was a colorless liquid that included 4.9 mg/mL phenol and its pH was 7.44. The mean analytical concentration of liraglutide in the dosing solution was 0.053 mg/L on day 2, during phase B. The table below shows estimated pulmonary doses of liraglutide during phases A and B. The estimated inhaled dose was 0.133 mg/kg liraglutide. Liraglutide solution droplet diameter was [redacted] µm and it was considered respirable by monkeys. b(4)

Appendix C Estimated Pulmonary Doses: Individual and Mean Values

Phase No./ Treatment	Animal No./Sex	Body Weight (kg)	NNC 90-1170 Mean Analytical Concentration ($mg.l^{-1}$)	Dose Duration ($min.day^{-1}$)	NNC 90-1170 Estimated Achieved Dose ($mg.kg^{-1}$)	Group Mean of NNC 90-1170 Achieved Dose ($mg.kg^{-1}$)
A (Vehicle)	1M	2.3	-	51.12	-	-
	2M	2.4		53.33	-	
	3F	2.1		46.67	-	
	4F	2.3		51.12	-	
B (NNC 90-1170)	1M	2.3	0.053	57.50	0.133	0.133
	2M	2.4		60.00	0.133	
	3F	2.1		52.50	0.133	
	4F	2.3		57.50	0.133	

- = Calculation not possible due to non-quantifiable levels of NNC 90-1170
M = Male
F = Female

[P25]

There were no liraglutide-related changes in clinical signs, body weight, food consumption, or respiratory parameters (minute volume, tidal volume, rate). Toxicokinetic parameters after iv and inhalation dosing are shown in the summary tables below.

TK-parameter values following iv doses:

Gender	Monkey	Body Weight (kg)	AUC (h·nmol/l)	$AUC_{\%ext}$ (%)	λ_z (1/h)	$t_{1/2}$ (h)	CL/f (ml/min/kg)	V_z/f (l/kg)
Female	3	2.2	5640	0.7	0.1041	6.7	0.0945	0.0544
	4	2.5	6870	0.6	0.1080	6.4	0.0777	0.0432
	Mean		6260	0.7	0.1061	6.5 a)	0.0851	0.0488
Male	1	2.5	5270	0.4	0.1163	6.0	0.101	0.0522
	2	2.5	5760	0.4	0.1172	5.9	0.0927	0.0474
	Mean		5510	0.4	0.1168	5.9 a)	0.0969	0.0498

a) Harmonic Mean

[P25]

TK-parameter values following inhaled doses:

Gender	Monkey	Body Weight (kg)	C_{max} (pmol/l)	t_{max} (h)	AUC (h·nmol/l)	$AUC_{\%extrap}$ (%)	λ_z (1/h)	$t_{1/2}$ (h)	CL/f (ml/min/kg)	V_z/f (l/kg)	f (%)
Female	3	2.1	6317	8.00	106	8.0	0.0555	12.5	5.57	6.02	1.7
	4	2.3	2972	6.00	46.1	4.9	0.0667	10.4	12.8	11.5	0.6
	Mean		4645	7.00	76.1	6.4	0.0611	11.4a)	9.20	8.79	1.2
Male	1	2.3	4704	8.00	64.8	5.7	0.0628	11.0	9.13	8.72	1.1
	2	2.4	8459	4.00	106	2.4	0.0780	8.9	5.57	4.29	1.7
	Mean		6582	6.00	85.5	4.0	0.0704	10.0a)	7.35	6.50	1.4

a) Harmonic Mean

[P25]

There were no gender differences in liraglutide exposure after inhalation or iv dosing., but plasma levels from inhaled doses were much lower (~70 fold) compared to iv doses.

2.6.4.4 Distribution

Tissue distribution of radiolabeled liraglutide-related material was determined in male and female albino rats, pregnant albino rats, and pigmented rats (Table 6). Liraglutide was radiolabeled on the K34R hGLP-1(7-37) peptide (^{125}I or ^{3}H-tyrosine 19), glutamate linker (^{14}C), or palmitic acid (^{3}H-[Pal]) (see Figure 6 in section 2.6.4.2) substructures. Dissociation of ^{125}I from ^{125}I-[Tyr]-liraglutide may lead to accumulation of radioactivity in thyroid. ^{3}H incorporated into liraglutide's palmitic acid may be metabolized to ^{3}H-water. ^{14}C incorporated into the glutamate linker bridging palmitic acid to the K34R hGLP1(7-37) peptide in liraglutide may be fully metabolized to $^{14}CO_2$. ^{3}H-[Tyr19]-liraglutide may give the best approximation of the metabolic fate of liraglutide's peptide moiety because it's metabolism is not confounded by dissociation of ^{125}I and tissue selective uptake of free iodine in thyroid.

Table 6 Overview of tissue distribution studies

Technique	Reference	Isotope	Dose (mg/kg)	Male rat (Albino)	Male rat (Pigmented)	Female rat (Albino)	Female rat (Pregnant albino)
QTD[a]	NN200159	^{125}I	0.1	×	n.d.	×	n.d.
QTD	NN200159	^{125}I	1	×	n.d.	×	n.d.
QTD	NN200159	^{14}C	1	×	n.d.	×	n.d.
QTD	NN201201	^{125}I	1	×	n.d.	×	n.d.
QTD	NN201201	^{14}C	1	×	n.d.	×	n.d.
QTD	NN980268	^{125}I	0.1	n.d.	n.d.	n.d.	×
QTD	NN980268	^{125}I	1	n.d.	n.d.	n.d.	×
QTD	NN980268	^{14}C	1	n.d.	n.d.	n.d.	×
QWBA[b]	NN204582	^{3}H-[Tyr]	1	×	n.d.		n.d.
QWBA	NN205477	^{3}H-[Pal]	0.15	×	×	×	n.d.

[a]: QTD, Quantitative tissue distribution; [b]QWBA, Quantitative whole-body autoradiography
n.d = not determined

[PK Written Summary P21]

Whole body autoradiography of albino Sprague Dawley rats (7/sex, 7 – 8 weeks old) sacrificed up to 168 hours after administering a single sc dose of 0.15 mg/kg ^{3}H-[Pal]-liraglutide (tritiated palmitate) showed a high concentration of radiolabeled material at the dorsal surface injection site with poor tissue distribution at early time points (< 24 hours), peak tissue concentrations occurring ~ 24 hours after dosing, tissue concentrations higher than blood at later time points (> 24 hours), and poor CNS penetration throughout the study. Excluding the dorsal subcutis, the highest levels of radioactivity in males and females occurred in lung (1 - 4 hours), kidney medulla (1 hour), liver (4 – 24 hours), adrenals (8 hours), brown fat (8 – 168 hours), preputial gland (24 – 48 hours), and pancreas (168 hours). In females, substantial levels also occurred in ovaries (8 – 24 hours), pineal body (168 hours), and pituitary (168 hours). The highest concentration of radioactivity occurred in the preputial gland 48 hours after dosing (585 ng equiv/g in males, 609 ng equiv/g in females). Figure 12 shows an autoradiogram of a longitudinal section from a female rat 48 hours after dosing.

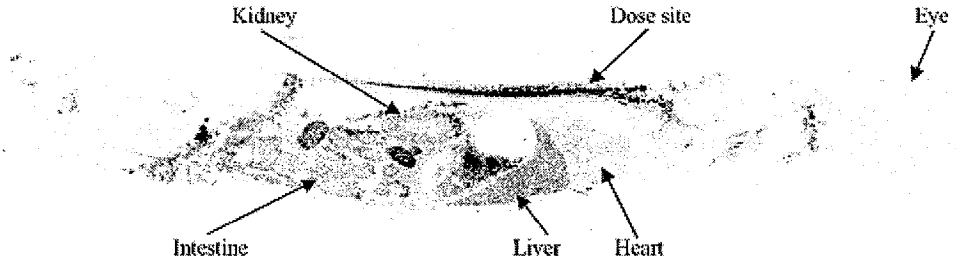


Figure 12 Whole Body Autoradiograms of Sections Through a Female Albino Rat (012F) 24 h Following Subcutaneous Administration of [^{3}H]-NNC 90-1170
Target Dose Level: 0.15 mg/kg

[205477 P37]

To determine if liraglutide distribution was affected by melanin, tissue distribution up to 672 hours after a single sc dose of 0.15 mg/kg ^{3}H-[Pal]-liraglutide was determined in pigmented male Lister Hooded rats (9 rats, 1/time point). A high concentration of radiolabeled material occurred at the dorsal surface injection site with poor tissue distribution at early time points (< 48 hours), peak tissue concentrations occurring ~ 48 hours after dosing, tissue concentrations higher than blood at later time points (> 48 hours), and poor CNS penetration throughout the study. Excluding the dorsal subcutis, the highest levels of radioactivity occurred in lungs (1 – 12 hours), liver (1 - 24 hours), preputial gland (12 hours), adrenals (48 hours), brown fat (48 – 168 hours), pineal body (168 hours), bone marrow (336 – 672 hours), pituitary (672 hours), and spinal cord (672 hours). There was no evidence radioactivity accumulated in pigmented tissues including uveal tract, eyes, or pigmented skin.

Tissue Distribution of ^{3}H-[Pal]-Liraglutide in Albino Sprague Dawley Rats

Liraglutide Dose (mg/kg), [Radionuclide]	Sex / Sample Time (h)	Males 1	Males 4	Males 24	Males 48	Males 168	Females 1	Females 4	Females 24	Females 48	Females 168
0.15, [^{3}H-Pal] (sc dosing)	Plasma, dry (ng equiv / g)	42	125	87	21	1	50	192	76	13	1
	Plasma Radioactivity Concentration Ratio										
	Adrenal	0.4	0.4	1.2	-	30.0	0.3	-	1.2	6.3	-
	Fat (brown)	-	0.1	1.4	5.8	42.0	-	0.2	2.0	6.3	16.0
	Kidney	0.4	0.3	1.2	3.2	22.0	0.4	0.3	1.4	4.8	13.0
	Liver	0.4	0.7	2.1	4.0	11.0	0.4	0.7	2.0	5.2	8.0
	Ovary	-	-	-	-	-	0.2	0.2	4.5	-	-
	Preputial gland	-	-	2.0	27.9	-	-	-	4.4	46.8	-
	Small Intestine wall	-	0.1	1.8	3.2	-	-	0.2	1.6	4.4	9.0
	Thyroid	-	0.2	1.0	2.3	23.0	-	0.1	0.9	5.2	14.0

Whole body autoradiography after a single iv dose of 1 mg/kg ^{3}H-[Tyr19]-liraglutide to male Sprague Dawley rats (7 – 8 weeks old) showed levels of radioactivity in all tissues were lower than blood levels (8 mcg equiv/g) at 0.25 and 1 hour after dosing and in all tissues except lung at 4 hours. An autoradiogram of a longitudinal sections from a rat sacrificed 4 hours after injection is shown in Figure 3. The highest levels of radioactivity occurred in liver, kidney, and adrenal

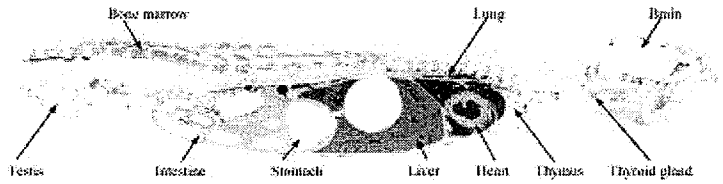


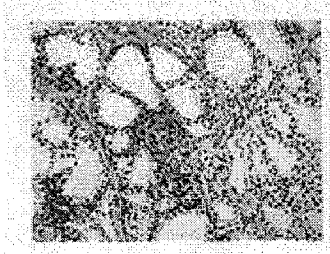
Figure 3 Whole Body Autoradiograms of Sections Through a Male Albino Rat (002M) 4 h Following Intravenous Administration of ^{3}H-[Tyr]-NNC 90-1170

[204382 P25]

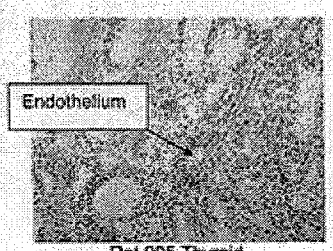
Distribution of liraglutide into pancreas and thyroid was evaluated because pancreas is a target organ for therapeutic effects of liraglutide and thyroid is a target of toxicity in rodents. In 2 year carcinogenicity studies, liraglutide caused thyroid C-cell tumors in rats and mice. Cellular localization of ^{3}H-[Tyr19]-liraglutide in pancreas and thyroid was evaluated by microhistoautoradiography. Calcitonin immunoreactivity was used to identify C-cells in thyroid sections and serial sections of thyroid and pancreas were developed with photographic emulsion to determine cellular localization of ^{3}H-liraglutide-associated radioactivity. Liraglutide-associated radioactivity occurred in pancreatic islet cells from 0.25 to 4 hours after dosing, but no drug-related radioactivity occurred in thyroid C-cells at any time point up to 4 hours after dosing.

4 Hours Post-dose

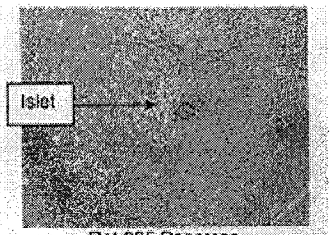
Thyroid: Silver grains were visible within blood vessels (lumina and walls), graded moderate, and throughout thyroid and parathyroid as a non-specific wash, graded mild.



Rat 005 Thyroid.
Calcitonin stain. X 20 objective
Specific staining of C-cells
(adjacent section of thyroid)



Rat 005 Thyroid.
Silver grain stain. X 20 objective
Silver grain distribution within vascular endothelium (specific) and thyroid epithelium (non-specific wash)



Rat 005 Pancreas.
Silver grain stain. X 20 objective
Silver grain distribution within vascular endothelium and islet cells

[204382 P48]

Liraglutide Dose (mg/kg), [Radionuclide]	Sex / Sample Time (h)	Males 1	Males 4	Males 24	Males 48	Males 168	Females 1	Females 4	Females 24	Females 48	Females 168
	Plasma, dry (ng equiv / g)	17,308	13,393	-	-	-	-	-	-	-	-
	Plasma Radioactivity Concentration Ratio										
1, [^{3}H-Tyr] (iv dosing)	Fat (brown)	0.03	0.04	-	-	-	-	-	-	-	-
	Pancreas	0.08	0.13	-	-	-	-	-	-	-	-
	Skin	0.01	0.02	-	-	-	-	-	-	-	-
	Thyroid	0.09	0.12	-	-	-	-	-	-	-	-

Tissue distribution after a single subcutaneous dose of 0.1 or 1 mg/kg ^{125}I-[Tyr]-liraglutide (labeled on R34K hGLP-1(7-37) peptide) or 1 mg/kg ^{14}C-liraglutide (labeled on glutamate linking palmitic acid to R34K hGLP-1 (7-37)) was determined in male and female Sprague Dawley rats by liquid scintillation counting (LSC) of ^{14}C after sample oxidation or by gamma scintillation of tissue samples and trichloroacetic acid (TCA) precipitated ^{125}I from plasma. Radioactivity at the sc injection site was absorbed with $\leq$ 12% remaining 24 hours after dosing (Figure 1 below).

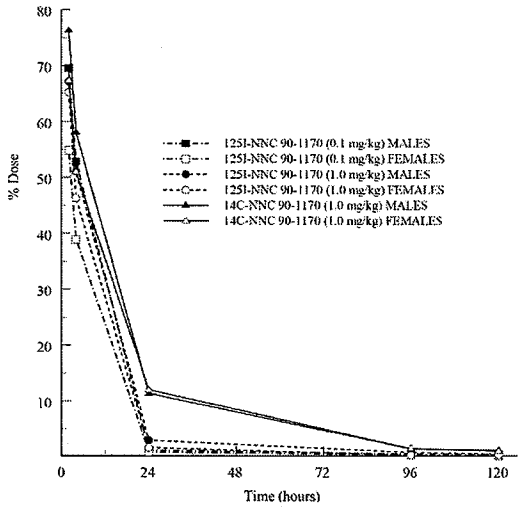


Figure 1 Mean (n=3) proportions of administered radioactivity (% dose) remaining at the site of injection after subcutaneous administration of radiolabelled NNC 90-1170 to rats (Study Phases 1.1 – 1.3)

[200159 P50]

Plasma levels of radioactivity after sc dosing with 0.1 or 1 mg/kg ^{125}I-liraglutide or 1 mg/kg ^{14}C-liraglutide peak 4 hours after dosing. By 96 – 120 hours post-dose, plasma levels of radioactivity were 10-fold lower than peak levels. The summary table below shows plasma levels of radioactivity as ng equivalents of liraglutide per gram of tissue, and for tissues, the plasma radioactivity concentration ratio (liraglutide ng equivalents in tissue / liraglutide ng equivalents in plasma). After dosing with either ^{125}I or ^{14}C-liraglutide, tissue levels exceeded plasma levels at the dose site (from 2 to 120 hours after dosing), in thyroid (from 2 to 120 hours after dosing with ^{125}I-liraglutide and from 96 – 120 hours post-dose with ^{14}C-liraglutide), and in brown fat (2 – 4 hours after dosing with ^{125}I-liaglutide and 2 – 120 hours after dosing with ^{14}C-liraglutide). ^{125}I-Liragltuide associated radioactivity in skin exceeded plasma levels from 24 to 120 hours after dosing in males and from 96 to 120 hours after dosing in females. ^{14}C-Liraglutide-associated radioactivity exceeded plasma levels in kidney (24 to 120 hours after dosing), contents of large intestine (24 hours after dosing, due to excretion), liver (24 to 120 hours after dosing), adrenals (24 to 120 hours after dosing), and Harderian gland (24 to 120 hours after dosing).

Liraglutide Dose (mg/kg), [Radionuclide]	Sex	Males					Females				
	Sample Time (h)	2	4	24	96	120	2	4	24	96	120
0.1, [^{125}I]	Plasma (ng equiv / g)	90	147	60	5.6	4.8	105	168	45	4.9	3.8
	Plasma Radioactivity Concentration Ratio										
	Dose Site	46	21	1.3	6.2	4.4	36	12	1.2	3.6	2.3
	Fat (brown)	11	4.6	0.3	0.33	0.39	22	4.8	0.3	0.6	0.5
	Skin	0.3	0.4	1.4	2.5	1.8	0.3	0.4	0.9	1.3	1.5
	Thyroid	33	62	2653	7675	11790	29	94	2488	14370	18480
1, [^{125}I]	Plasma (ng equiv / g)	1,216	1,801	1,037	112	84	1,451	1,872	923	75	65
	Plasma Radioactivity Concentration Ratio										
	Dose Site	35	17	1.9	3.9	3.2	25	17	1.1	2.9	2.9
	Fat (brown)	4.7	1.6	0.24	0.4	0.3	5.2	2.5	0.3	0.4	0.4
	Skin	0.2	0.4	1.7	2.6	2.1	0.2	0.3	0.9	1.6	2
	Thyroid	26	85	1876	6435	8930	15	35	1893	13370	10270
1, [^{14}C]	Plasma (ng equiv / g)	1,664	2,376	536	30	22	1,702	2,068	422	32	20
	Plasma Radioactivity Concentration Ratio										
	Dose Site	35	16	18	24	27	28	19	19	25	28
	Adrenals	0.2	0.3	2	6.2	5.7	0.3	0.4	2.1	6.9	8
	Fat (brown)	9.8	3.8	13	27	12	16	13	12	15	14
	Harderian	0	0.1	1.9	4.1	3.5	0.1	0.1	2.1	4.3	4
	Kidney	0.1	0.2	5.8	9.2	7.7	0.1	0.2	8.5	12	9.9
	Large Intestine (contents)	0	0	3.2	0.6	0.4	0	0	4.2	0.7	0.6
	Liver	0.3	0.5	4.2	5.9	5.5	0.3	0.4	4	5.9	6.4
	Thyroid	0.1	0.1	0.5	1.7	23	0.1	0.1	0.6	1.6	1.9

In a repeat dose tissue distribution study, tissue and plasma accumulation of radioactivity after 7 days of subcutaneous injections with 1 mg/kg/day ^{125}I-liraglutide (radiolabeled on peptide) or ^{14}C-liraglutide (radiolabeled on glutamate linker) once a day in male and female albino Sprague Dawley rats with monitoring up to 168 hours after the 7th dose showed < 2 fold accumulation of radioactivity (^{125}I or ^{14}C) in plasma. Results from the study are summarized in the table below. Tmax for plasma and blood radioactivity occurred 4 hours after dosing. After the last dose, levels of radioactivity exceeded plasma levels in aorta and skin (24 & 168 hours after dosing in males, 168 hours after dosing in females) and thyroid (4, 24, & 168 hours after dosing in males and females). ^{125}I levels decreased in most tissues between 24 and 168 hours after dosing, but the decline was slower in thyroid, and poorly perfused tissue including bone and perirenal fat. High levels of persistent radioactivity in thyroid were consistent with uptake of free ^{125}I. Approximately 80 – 90% of ^{125}I radioactivity in plasma remained attached to protein (precipitated by TCA) indicating circulating ^{125}I in blood remained attached to liraglutide. Approximately 81% of the total cumulative dose of ^{125}I radioactivity was excreted in urine within 7 days after dosing stopped.

Parameter	^{125}I-NNC 90-1170	^{14}C-NNC 90-1170
% Cumulative dose present at the dose site at: 4 hours 24 hours 168 hours	 8.977 (males), 7.376 (females) 1.477 (males), 1.120 (females) 0.7397 (males), 0.5224 (females)	 14.48 (males), 13.40 (females) 3.896 (males), 3.966 (females) 1.322 (males), 1.338 (females)
Plasma and blood T_{max} (hours after 7th dose)	4 (both sexes)	4 (both sexes)
Plasma C_{max} (µg equivalents/g)	2.585 (males), 2.885 (females)	2.866 (males), 3.080 (females)
Blood C_{max} (µg equivalents/g)	1.668 (males), 1.807 (females)	1.811 (males), 1.922 (females)
Tissue T_{max} (hours after 7th dose)	4 or 24 (aorta and skin in males, thyroid in females)	4 or 24 (urinary bladder, pituitary gland, thyroid, bone marrow (both sexes), aorta, vena cava, bone, Harderian glands, prostate, brown fat, perirenal fat, stomach wall, large intestine wall (males only), lacrimal glands, lymph nodes, salivary glands, uterus, large intestine contents (females only)
Greatest tissue radioactivity concentrations after 7th dose (µg equivalents/g; excludes plasma, blood, gut contents and dose site)	thyroid (5170 males, 5278 females), small intestine wall (1.418 males, 2.252 females), stomach wall (1.863 males, 2.109 females), skin (1.530 males, 0.826 females), aorta (1.446 males, 1.387 females), vena cava (0.961 males, 1.112 females), ovaries (1.031), liver (0.789 males, 0.928 females)	liver (3.002 males, 2.700 females), kidneys (2.286 males, 2.427 females), Harderian glands (2.115 males, 2.247 females), bone marrow (2.055 males, 2.068 females), adrenal glands (1.883 males, 2.120 females), pituitary gland (1.853 males, 1.752 females), thyroid (1.836 males, 1.944 females), vena cava (1.818 males, 3.841 females), ovaries (1.520)
Smallest tissue radioactivity concentrations (µg equivalents/g) at T_{max} (excludes gut contents)	Brain (0.126 males, 0.129 females), skeletal muscle (0.163 males, 0.159 females), spinal cord (0.235 males, 0.207 females), eyes (0.244 males, 0.248 females), bone (0.277 males, 0.232 females)	Brain (0.212 males, 0.241 females), eyes (0.234 males, 0.237 females), spinal cord (0.361 males, 0.362 females), skeletal muscle (0.435 males, 0.376 females)

[201201 P12]

Table 3 Mean (± sd)* tissue : plasma radioactivity concentration ratios after repeated daily subcutaneous administration of ^{125}I-NNC 90-1170 (1.0 mg/kg/day) to male rats for 7 days

Tissue	4 hours**	24 hours**	168 hours**
	Mean ± sd	Mean ± sd	Mean ± sd
Aorta	0.34 ± 0.06	1.45 ± 1.40	5.81
Thyroid	2006 ± 634.9	5204 ± 1078	15760 ± 822.8
Skin	0.58 ± 0.19	1.59 ± 0.46	2.30 ± 0.54

sd Standard deviation
* n=3 (except for pituitary gland and salivary glands at 4 hours, for which n=2)
** After the administration of dose 7
NC Not calculable (radioactivity below the limit of accurate quantification in at least two animals)

[201201 P79]

Table 5 Mean (± sd)* tissue : plasma radioactivity concentration ratios after repeated daily subcutaneous administration of ^{125}I-NNC 90-1170 (1.0 mg/kg/day) to female rats for 7 days

Tissue	4 hours**	24 hours**	168 hours**
	Mean ± sd	Mean ± sd	Mean ± sd
Aorta	0.49 ± 0.33	0.59 ± 0.21	8.76
Thyroid	1660 ± 783.5	7312 ± 986.2	24870 ± 7170
Skin	0.29 ± 0.04	0.60 ± 0.18	1.53 ± 0.66

sd Standard deviation
* n=3 (except for pituitary gland and salivary glands at 4 hours, for which n=2)
** After the administration of dose 7
NC Not calculable (radioactivity below the limit of accurate quantification in at least two animals)

[201201 P81]

After the last dose of ^{14}C-liraglutide, tissue levels of radioactivity exceeded plasma levels in many tissues within 24 hours after dosing and in nearly all tissues 168 hours after dosing. Within 24 hours after the last dose of ^{14}C-liraglutide, the highest levels of radioactivity occurred in kidney,

Harderian gland, liver, bone marrow, thyroid, and adrenal in both sexes (Tables 9 & 11, below). At 168 hours after the last dose, the highest tissue levels of radioactivity occurred in perirenal fat, brown fat, kidneys, skin, and vena cava in both sexes, lymph nodes in males, and in females, adrenal and urinary bladder. Tissue levels of radioactivity decreased between 24 and 168 hours after dosing. Approximately 72% of total administered ^{14}C was excreted in expired air within 7 days after the last dose.

Table 9 Mean (± sd)* tissue : plasma radioactivity concentration ratios after repeated daily subcutaneous administration of ^{14}C-NNC 90-1170 (1.0 mg/kg/day) to male rats for 7 days

Tissue	4 hours**	24 hours**	168 hours**
	Mean ± sd	Mean ± sd	Mean ± sd
Whole-blood	0.64 ± 0.02	0.96 ± 0.07	3.79 ± 0.34
Aorta	0.33 ± 0.03	1.33 ± 0.06	7.81 ± 1.26
Vena cava	0.38 ± 0.05	2.48 ± 0.82	8.97 ± 1.29
Kidneys	0.81 ± 0.08	2.86 ± 0.48	6.74 ± 0.33
Urinary bladder	0.20 ± 0.02	0.86 ± 0.09	6.50 ± 0.97
Liver	1.05 ± 0.03	2.81 ± 0.51	5.42 ± 0.53
Pancreas	0.44 ± 0.02	1.35 ± 0.14	6.63 ± 0.38
Adrenal glands	0.66 ± 0.02	2.51 ± 0.40	7.71 ± 0.40
Harderian glands	0.70 ± 0.03	2.84 ± 0.58	3.94 ± 0.25
Lymph nodes (mesenteric)	0.41 ± 0.04	1.56 ± 0.26	8.43 ± 2.79
Thyroid	0.31 ± 0.04	2.57 ± 1.90	7.05 ± 0.66
Bone (femur)	0.21 ± 0.02	0.90 ± 0.20	7.80 ± 1.31
Bone marrow	0.68 ± 0.04	2.77 ± 0.55	4.44 ± 1.86
Fat (brown)	0.51 ± 0.05	2.02 ± 0.12	11.15 ± 4.90
Fat (perirenal)	0.51 ± 0.14	2.10 ± 0.18	22.46 ± 1.77
Skin	0.30 ± 0.02	1.08 ± 0.10	8.73 ± 0.67

sd Standard deviation
* n=3
** After the administration of dose 7

[201201 P85]

Table 11 Mean (± sd)* tissue : plasma radioactivity concentration ratios after repeated daily subcutaneous administration of ^{14}C-NNC 90-1170 (1.0 mg/kg/day) to female rats for 7 days

Tissue	4 hours**	24 hours**	168 hours**
	Mean ± sd	Mean ± sd	Mean ± sd
Aorta	0.36 ± 0.04	1.40 ± 0.31	5.59 ± 1.29
Vena cava	1.21 ± 1.53	2.47 ± 0.44	6.27 ± 1.08
Kidneys	0.79 ± 0.02	3.29 ± 0.39	6.11 ± 0.79
Urinary bladder	0.20 ± 0.04	0.98 ± 0.23	5.26 ± 0.64
Liver	0.88 ± 0.07	2.64 ± 0.19	4.34 ± 0.33
Pancreas	0.39 ± 0.03	1.36 ± 0.11	5.91 ± 0.64
Adrenal glands	0.69 ± 0.09	2.87 ± 0.16	8.01 ± 2.18
Harderian glands	0.74 ± 0.08	2.92 ± 0.04	4.77 ± 2.05
Lymph nodes (mesenteric)	0.39 ± 0.04	1.71 ± 0.09	4.95 ± 0.25
Thyroid	0.24 ± 0.07	2.77 ± 1.34	4.34 ± 0.87
Bone (femur)	0.18 ± 0.03	0.67 ± 0.09	5.73 ± 1.34
Bone marrow	0.54 ± 0.07	2.91 ± 0.31	3.70 ± 0.84
Fat (brown)	0.45 ± 0.09	1.79 ± 0.40	6.47 ± 2.63
Fat (perirenal)	0.44 ± 0.12	1.86 ± 0.40	16.62 ± 5.19
Skin	0.22 ± 0.03	0.87 ± 0.04	6.78 ± 1.21

sd Standard deviation
* n=3
** After the administration of dose 7

[201201 P87]

Distribution of radioactivity in pregnant rats was determined for up to 120 hours after a single subcutaneous injection of 0.1 or 1 mg/kg ^{125}I-[Tyr19]-liraglutide (peptide labeled) or 1 mg/kg ^{14}C-liraglutide (glutamate linker labeled) administered on gestation day 15. Results are summarized in the table below. Plasma radioactivity increased with liraglutide dose with Tmax occurring 4 hours after dosing with $\geq$ 90% of the dose absorbed from the injection site within 24 hours after dosing. Tmax occurred within 4 hours for most tissues including fetuses, heart, kidneys and ovaries in rats injected with 1 mg/kg ^{125}I-liraglutide and in heart and ovaries after injection with ^{14}C-liraglutide (Table 6 and Table 10). Levels of radioactivity peaked 24 after

dosing in liver, lungs, mammary tissue, uterus, placenta, and amniotic fluid after administering 1 mg/kg ^{125}I-liraglutide and in liver, kidneys, lungs, mammary tissue, uterus, placenta, amniotic fluid and fetuses after dosing with 1 mg/kg ^{14}C-liraglutide. Tissue levels of radioactivity measured within 4 hours after dosing with either ^{125}I or ^{14}C-liraglutide probably better represent liraglutide distribution than tissue radioactivity 24 hours after dosing because lipid and amino acid components of liraglutide was extensively metabolized with ^{14}C-glutamate labeled material entering the general metabolic pool and free ^{125}I or ^{125}I-tyrosine labeled material being excreted in urine. Within 4 hours after dosing, levels of ^{125}I and ^{14}C were similar in blood, plasma, heart, kidneys, lungs, ovaries, placenta, dose site, and uterus. Tissue concentration of radioactivity at the dose site exceeded the concentration in plasma after dosing with either ^{125}I-or ^{14}C-labeled liraglutide. In other tissues, the highest concentrations occurred in mammary tissue, ovaries, uterus, placenta, kidneys, and lungs after dosing with 1 mg/kg ^{125}I-liraglutide and in liver after dosing with ^{14}C- liraglutide. At Tmax, radioactivity in fetuses was much lower than maternal plasma levels and most of it was not protein associated because only 43% of radioactivity in fetal homogenates was precipitated by TCA (Table 4). Radioactivity in amniotic fluid was not protein associated (not precipitated by trichloroacetic acid). At Tmax, the lowest levels of radioactivity occurred in amniotic fluid and fetuses after dosing with either ^{125}I- or ^{14}C-liraglutide. HPLC of extracts from injection sites of pregnant rats 24 hours after dosing with ^{125}I-[Tyr]-liraglutide suggests liraglutide was completely metabolized because remaining radioactivity was either free ^{125}I or ^{125}I-tyrosine (the identity of radioactive peaks from HPLC were not definitively established, but none of the peaks coeluted with the ^{127}I-liraglutide reference standard). Approximately 62 – 67% of a 1 mg/kg ^{14}C-liraglutide dose was excreted in expired air from male or female rats within 48 hours of dosing indicating the glutamate linker of ^{14}C-liraglutide is completely metabolized to $^{14}CO_2$. At 120 hours after dosing, radioactivity in ovaries, amniotic fluid, and fetuses were below the level of quantitation (≤ 1.19 ng equiv/g).

	^{125}I-NNC 90-1170		^{14}C-NNC 90-1170
	0.1 mg/kg	1 mg/kg	1 mg/kg
% Dose present at the dose site at:			
2 hours	73.1	72.4	77.5
4 hours	46.7	47.7	65.4
24 hours	1.05	3.02	10.6
Plasma T_{max} (hours)	4	4	4
Plasma C_{max} (ng equivalents/g)	148.0	1521	1550
Blood T_{max} (hours)	4	4	4
Blood C_{max} (ng equivalents/g)	98.47	1039	908.4
Tissue T_{max} (hours)	Generally 4 hours (with the exception of dose site and mammary tissue)	4 hours (foetuses, heart, kidneys, ovaries) or 24 hours (amniotic fluid, liver, lungs, mammary tissue, placenta and uterus)	4 hours (heart, ovaries) or 24 hours (liver, kidneys, placenta, uterus, lungs, mammary tissue, foetuses and amniotic fluid)
Greatest tissue radioactivity concentrations (ng equivalents/g; excludes blood plasma and dose site)	Ovaries, lungs, kidneys, placenta and liver (41 – 52)	Mammary tissue, ovaries, kidneys, uterus, lungs and placenta (415 – 1395)	Liver (3333 and 1542 at 4 and 24 hours, respectively)
Smallest tissue radioactivity concentrations (ng equivalents/g) at T_{max}	Amniotic fluid (4.04) and foetuses (6.75)	Amniotic fluid (47.43) and foetuses (61.88)	Amniotic fluid (25.14), foetuses (230.6), heart (230.5)

[980268 P11]

Table 6 Mean (± SD)* tissue : plasma radioactivity concentration ratios after subcutaneous administration of ^{125}I-NNC 90-1170 (1.0 mg/kg) to pregnant rats

Tissues	2 Hours	4 Hours	24 Hours	96 Hours	120 Hours
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Blood	0.666 ± 0.017	0.685 ± 0.030	0.757 ± 0.019	0.815 ± 0.031	0.847 ± 0.112
Plasma	1.000 ± <0.001	1.000 ± <0.001	1.000 ± <0.001	1.000 ± <0.001	1.000 ± <0.001
Amniotic fluid	0.019 ± 0.002	0.025 ± 0.003	0.046 ± 0.011	0.227 ± 0.200	0.281 ± 0.255
Foetus	0.047 ± 0.004	0.041 ± 0.003	0.041 ± 0.009	0.217 ± 0.036	0.344 ± 0.101
Heart	0.175 ± 0.015	0.152 ± 0.005	0.209 ± 0.022	0.476 ± 0.029	0.504 ± 0.051
Kidney	0.279 ± 0.010	0.307 ± 0.034	0.427 ± 0.021	0.980 ± 0.087	1.135 ± 0.076
Liver	0.221 ± 0.020	0.227 ± 0.028	0.330 ± 0.028	0.761 ± 0.091	0.956 ± 0.175
Lung	0.302 ± 0.008	0.293 ± 0.024	0.425 ± 0.015	0.656 ± 0.093	0.729 ± 0.039
Mammary gland	0.125 ± 0.007	0.170 ± 0.022	1.321 ± 0.409	3.502 ± 2.428	1.569 ± 0.742
Ovary	0.586 ± 0.115	0.409 ± 0.036	0.391 ± 0.003	0.618 ± 0.090	0.517 ± 0.476
Placenta	0.209 ± 0.005	0.243 ± 0.024	0.392 ± 0.012	1.376 ± 0.377	1.450 ± 0.186
Dose site	50.36 ± 3.185	20.98 ± 6.164	1.932 ± 0.260	6.908 ± 1.614	9.165 ± 3.949
Uterus	0.186 ± 0.010	0.216 ± 0.019	0.430 ± 0.040	0.831 ± 0.171	1.140 ± 0.212

SD Standard deviation

* n=3 females

[980268 P83]

Table 10 Mean (± SD)* tissue : plasma radioactivity concentration ratios after subcutaneous administration of ^{14}C-NNC 90-1170 (1.0 mg/kg) to pregnant rats

Tissues	2 Hours	4 Hours	24 Hours	96 Hours	120 Hours
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Blood	0.565 ± 0.019	0.586 ± 0.010	0.687 ± 0.044	2.029 ± 0.287	2.635 ± 0.235
Plasma	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
Amniotic fluid	0.001 ± 0.001	0.005 ± 0.001	0.036 ± 0.006	0.231 ± 0.035	0.328 ± 0.025
Foetus	0.004 ± 0.002	0.012 ± 0.001	0.340 ± 0.013	3.172 ± 0.536	3.054 ± 0.446
Heart	0.133 ± 0.002	0.149 ± 0.004	0.301 ± 0.038	1.684 ± 0.204	2.675 ± 0.417
Kidney	0.156 ± 0.029	0.193 ± 0.006	1.244 ± 0.102	5.896 ± 1.142	8.014 ± 1.871
Liver	0.401 ± 0.051	0.864 ± 0.189	2.306 ± 0.269	7.346 ± 0.440	7.466 ± 2.459
Lung	0.205 ± 0.013	0.233 ± 0.013	0.600 ± 0.070	2.875 ± 0.585	3.947 ± 0.890
Mammary gland	0.053 ± 0.005	0.070 ± 0.012	0.506 ± 0.179	4.138 ± 1.142	6.879 ± 2.396
Ovary	0.230 ± 0.005	0.443 ± 0.073	1.005 ± 0.071	4.647 ± 0.863	6.114 ± 1.385
Placenta	0.148 ± 0.005	0.218 ± 0.009	1.017 ± 0.097	4.716 ± 0.902	5.525 ± 1.041
Dose site	40.30 ± 9.272	24.86 ± 0.479	12.51 ± 4.039	18.58 ± 4.529	32.10 ± 8.800
Uterus	0.099 ± 0.003	0.144 ± 0.011	0.721 ± 0.087	3.986 ± 0.866	4.879 ± 0.877

SD Standard deviation

* n=3 females

[980268 P87]

Table 4 Mean (± SD)* proportions (% of total sample radioactivity) of radioactivity in selected tissues precipitable by trichloroacetic acid after subcutaneous administration of ^{125}I-NNC 90-1170 (0.1 mg/kg) to pregnant rats

Tissues	2 Hours	4 Hours	24 Hours
	Mean ± SD	Mean ± SD	Mean ± SD
Plasma	74 ± 2	76 ± 1	63 ± 3
Amniotic fluid	ND	ND	NC
Foetus	44 ± 4	43 ± 1	35 ± 4
Mammary gland	63 ± 6	74 ± 11	78 ± 4
Placenta	49 ± 2	51 ± 1	51 ± 3

SD Standard deviation

* n=3 females

ND No radioactivity detected (below the limit of accurate quantification in all three animals)

NC Not calculable (below the limit of accurate quantification in two animals)

[980268 P81]

The concentration of unlabeled liraglutide in plasma of pregnant albino Sprague Dawley rats (9 – 10 weeks old at mating, 3/time point) subcutaneously injected with 1 mg/kg/day liraglutide once a day for 5 days on gestation days 12 – 15 was determined by ELISA. Liraglutide plasma levels were 340.8 nM 8 hours after the last injection and 201.7 nM 16 hours after dosing. Liraglutide levels in amniotic fluid were ~ 1.2 – 1.3% of plasma levels; 4.1 nM 8 hours after and

2.7 nM 16 hours after the last dose. These results suggest liraglutide crossed the placenta into fetuses and fetuses excreted liraglutide into amniotic fluid.

Liraglutide concentration was determined in maternal plasma, amniotic fluid, and fetal plasma from pregnant New Zealand White rabbits (4 months at mating, 3 / time point) treated with 0.05 mg/kg/day liraglutide for 5 days (gestation days 25 – 29). Liraglutide levels were determined in samples taken 8 or 16 hours after the last dose using an ELISA. Results are summarized in the table below. Liraglutide crossed the placental barrier into the fetus, and it was excreted in the amniotic fluid. Eight or 16 hours after the last dose, liraglutide levels in fetal plasma were ~ 1.5 – 4.2% of the levels in maternal plasma and levels in amniotic fluid were 0.6 – 6.2% of the levels in maternal plasma.

Sample Source	Parameter	Hours After Last 8	16
Maternal plasma	[liraglutide], nM	34.18	11.27
Fetal plasma	[liraglutide], nM	0.50	0.47
	Ratio (fetal plasma / maternal plasma)	0.015	0.042
Amnitotic fluid	[liraglutide], nM	0.22	0.70
	Ratio (amniotic fluid / maternal plasma)	0.006	0.062

In vitro plasma protein binding determined by equilibrium dialysis at 37C using plasma from EDTA-treated blood showed liraglutide is highly protein bound in mice, rats, rabbits, monkeys, and humans. The table below summarizes results from in vitro protein binding studies. Liraglutide is highly bound to human albumin and human alpha-1 glycoprotein. The biologic significance of higher protein binding in plasma from mice compared to Sprague Dawley rats or humans is unknown.

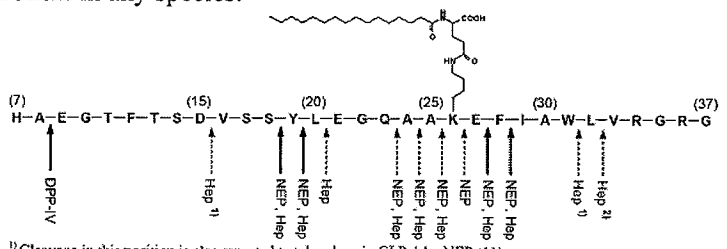
Plasma	Liraglutide, % Bound
mice	99.3 - 99.8
rat, SD	95.8 - 98.2
rat, ZDF	97.9 - 99.8
rabbit	99.8
monkey	99.3 - 99.8
human	98.8 - 99.2
Human Plasma Proteins	**Liraglutide, % Bound**
serum albumin	99.4
alpha-1 glycoprotein	99.3

2.6.4.5 Metabolism

Unlike GLP-1(7-36)amide or GLP-1(7-37) which are largely metabolized by dipeptidyl peptidase IV (DPP-4)cleaving off the N-terminal dipeptide with or without subsequent further metabolism by neutral endopeptidases (NEPs) prior to excretion in urine as GLP-1(9-36)amide, GLP-1(9-37), or their metabolites, complete metabolism of liraglutide is expected to result in peptides, amino acids, and lipids that enter the general pool of amino acids and lipids for further metabolized to CO_2 and H_2O. Liraglutide(9-37) or delipidated liraglutide(9-37) were not major metabolites in plasma or urine from rats, monkeys, or humans indicating liraglutide is a poor

substrate for DPP-4 or that if liraglutide(9-37) metabolites are formed, they're rapidly and extensively metabolized.

Liraglutide metabolism was determined *in vitro* in plasma from CD-1 mice, SD rats, cynomolgus monkeys, and humans, in hepatocytes from CD-1 mice, SD rats, cynomolgus monkeys, and humans, using perfused liver and kidney or liver and kidney slices from rats, or using recombinant human DPP-4 or NEP. *In vivo* metabolism studies characterizing metabolites in plasma, urine, or feces were performed using radiolabeled liraglutide including ^{125}I-[Tyr19]-liraglutide, ^{3}H-[palmitic acid]-liraglutide, or ^{14}C-[glutamate linker]-liraglutide in rats, dogs, and monkeys. Metabolites were characterized by their HPLC retention times with free ^{125}I resulting from dissociation from ^{125}I-liraglutide and $^{3}H_20$ resulting from complete metabolism of ^{3}H-[Pal]-liraglutide eluting near the solvent front. The DPP-4 cleavage product liraglutide(9-37) was used as a reference compound with a relative retention time (Rt_R = t_R metabolite / t_R liraglutide) of 1.05. Figure 7 summarizes liraglutide peptide cleavage sites for DPP-4, neutral endopeptidase (NEP), and peptidases in hepatocytes (Hep). There were no substantive sex differences in liraglutide metabolism in any species.



[1] Cleavage in this position is also reported to take place in GLP-1 by NEP (11)
[2] Cleavage in this position takes place very slowly and is only observed together with a cleavage in position 19-20

Figure 7 Cleavage positions in liraglutide following incubations with rat and human hepatocytes, DPP-IV and NEP (NN206665, NN207147)

[PK Written Summary P29]

Human Metabolites

In adult male human volunteers administered a single sc dose of 0.75 mg ^{3}H-[Pal]-liraglutide, the major plasma drug-related radiolabeled material was liraglutide (Figure 9). The dose of liraglutide used in this study was 42% of the MRHD of 1.8 mg/day liraglutide. Two minor metabolites occurred; the first metabolite had a HPLC relative retention time (Rt_R , relative to liraglutide) of 1.05 minutes and the Rt_R of a second metabolite was 1.15. The $AUC_{1\text{-}24h}$ from pooled plasma was $\leq$ 9% of the total $AUC_{2\text{-}24h}$ for the first metabolite and $\leq$ 5% for the second.

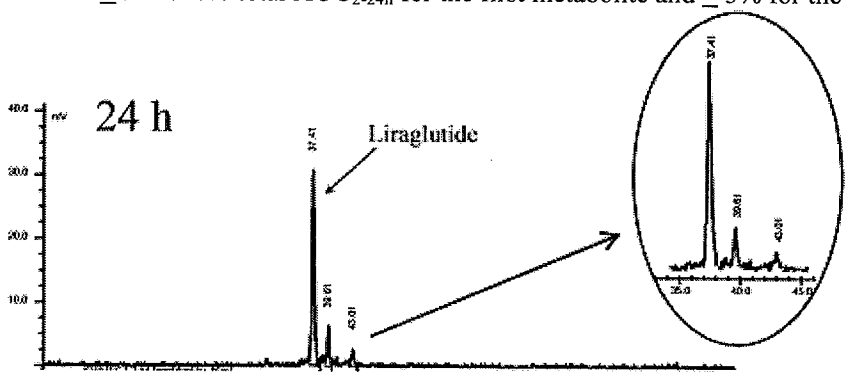


Figure 9 *In vivo* plasma metabolite profile of plasma 6, 12, 24 and 48 h post-dose s.c. administration of 0.75 mg ^{3}H-[Pal]-liraglutide to human volunteers (Subject 2)

[PK Written Summary, modified Figure 9, P34]

Characterization of radiolabeled metabolites after sc dosing with ^{3}H-[Pal]-liraglutide showed the first human metabolite (Rt_R 1.05) also occurred in mice (1 mg/kg), rats (0.1 or 1 mg/kg), and monkeys (0.05 or 5 mg/kg) and the second metabolite (Rt_R 1.15) occurred in rats (Figure 10). These results show there were no major or unique human radiolabeled metabolites of ^{3}H-[Pal]-liraglutide.

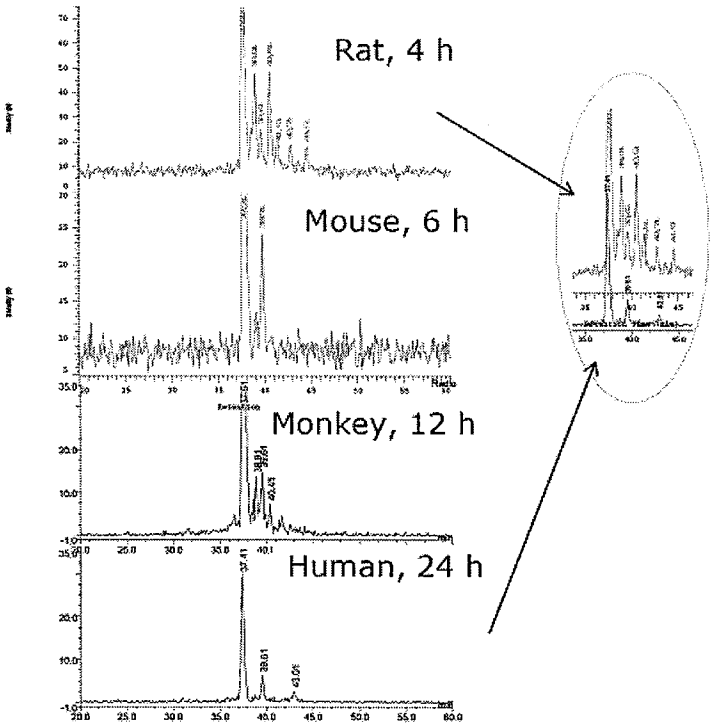


Figure 10 ***In vivo*** **metabolite profiles from rat, mouse, monkey and human following subcutaneous administration with ^{3}H-[Pal]-liraglutide**

[PK Written Summary P35]

Metabolite profiles in urine and feces were determined after sc dosing ^{3}H-[Pal]-liraglutide in humans (0.75 mg) and male and female cynomolgus monkeys(0.05 or 5 mg/kg). In humans, ~20% of total administered radioactivity was recovered in urine and 6% in feces. Three very hydrophilic components were detected in urine, but most of the radioactivity in urine was tritiated water. Six different metabolites, all more hydrophobic than liraglutide, were detected in feces with 2 of them occurring in the majority of subjects. In monkeys, ~ 20% of the total administered radioactivity was recovered in urine and ~8% was recovered in feces. Of the radioactivity recovered in urine, 47% was tritiated water and the remaining radiolabeled material considered hydrophilic non-volatile compounds. Two metabolites identified in feces were more lipophilic than liraglutide, but neither of the fecal metabolites were detected in urine. Metabolite profiling in urine of male and female Sprague Dawley rats administered 0.1 or 1 mg/kg ^{125}I-liraglutide showed ~ 60% of total administered radioactivity was excreted in urine within 24 hours of dosing (0.1 mg/kg) with most of the radioactivity in urine considered free ^{125}I. Unmodified liraglutide was not excreted in urine of feces from any species, including humans.

In Vitro Metabolites

Study 205145 compared the metabolism of 0.01 or 1 µM ^{3}H-[Tyr19]-liraglutide and ^{3}H-[Pal]-liraglutide in hepatocytes from male Sprague Dawley rats, male CD-1 mice, male and

female cynomolgus monkeys, and human donors (pooled, cryopreserved). The *in vitro* human liver metabolite profile of peptide radiolabeled and lipid radiolabeled liraglutide are different. Figure 27 shows an HPLC chromatogram of radiolabeled metabolites formed by incubating ^{3}H-[Tyr19]-liraglutide with human hepatocytes and Figure 28 shows metabolites formed from ^{3}H-[Pal]-liraglutide. ^{3}H-[Tyr19]-liraglutide metabolites are more hydrophilic than ^{3}H-[Pal]-liraglutide metabolites.

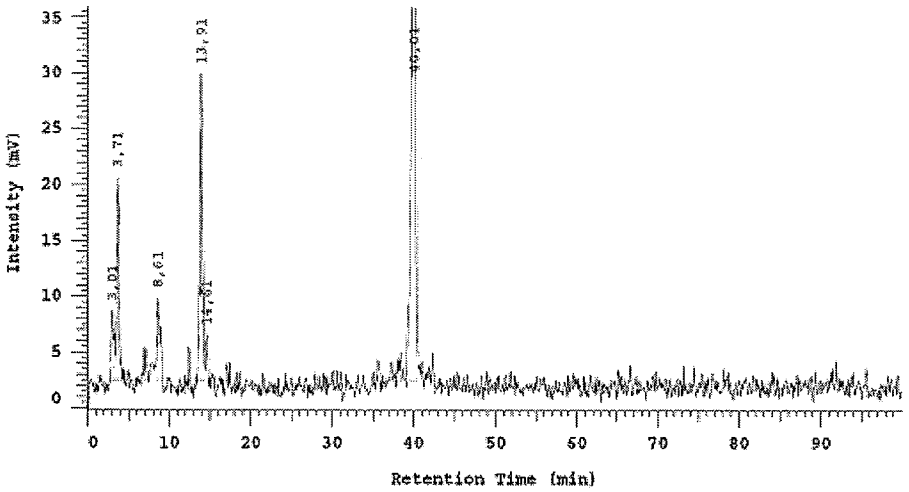


Figure 27 Human hepatocytes: ^{3}H-[Tyr]-liraglutide metabolite profile (1 µM, 4 hr) -enlarged chromatogram

[205145 P44]

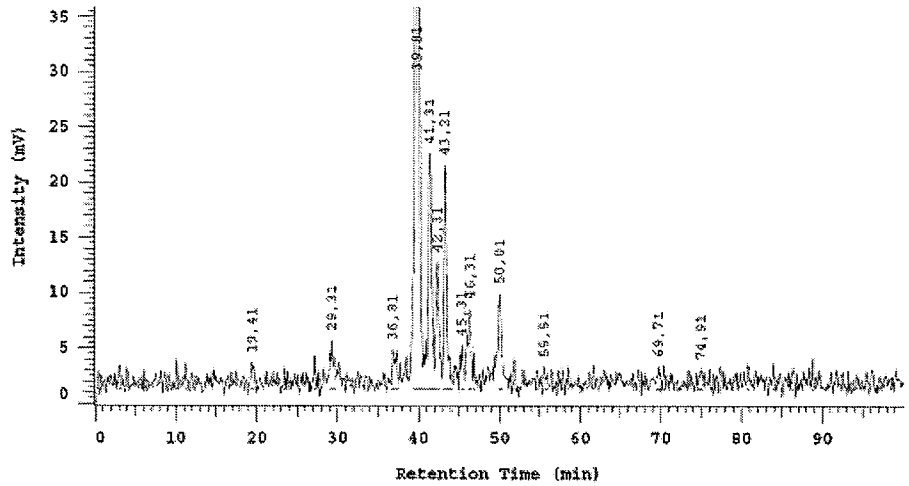


Figure 28 Human hepatocytes: ^{3}H-[Pal]-liraglutide (1 µM, 4 hr) metabolite profile – enlarged chromatogram

[205145 P44]

Species comparison of hepatocyte metabolites shows there are no unique human metabolites (Table 1 and Table 2). The results are consistent with liraglutide initially being cleaved between positions 19 (tyrosine) and 25 (alanine) on K34R hGLP-1(7-37).

Table 1 Cross species comparison of relative retention times (Rt_R) for radioactive peaks formed after incubation with ^{3}H-[pal]-liraglutide in hepatocytes

Species	Rt_R					
	1.04	1.06	1.09	1.1	1.16	1.26
Mouse	✓ (t_R~41.6 min)	✓ (t_R~42.2 min)	✓ (t_R~43.2 min)	✓ (t_R~43.5 min)	✓ (t_R~46.2 min)	✓ (1.25) (t_R~50.0 min
Rat	✓ (t_R~41.4 min)	✓ (t_R~42.4 min)	✓ (1.08) (t_R~43.3 min)	✓ (t_R~43.7 min)	✓ (t_R~46.1min)	✓ (t_R~49.9 min)
Monkey	✓ (t_R~41.5 min)	✓ (t_R~42.2 min)	✓ (t_R~43.3 min)	✓ (t_R~43.8 min)	✓ (t_R~46.3 min)	✓ (t_R~50.1 min)
Human	✓ (t_R~41.4 min)	✓ (t_R~42.3 min)	✓ (t_R~43.3 min)	-	✓ (t_R~46.3 min)	✓ (t_R~50.0 min)

✓ = metabolite present
- = metabolite not observed

Table 2 Cross species comparison of relative retention times (Rt_R) for radioactive peaks formed after incubation with ^{3}H-[tyr]-liraglutide in hepatocytes

Species	Rt_R			
	0.08	0.09	0.21	0.34
Mouse	✓ (t_R~3.1 min)	✓ (t_R~3.6 min)	✓ (t_R~8.5 min)	✓ (t_R~13.8 min)
Rat	✓ (t_R~3.1 min)	✓ (t_R~3.7 min)	✓ (t_R~8.4 min)	✓ (0.35) (t_R~13.9 min)
Monkey	✓ (0.07) (t_R~3.0 min)	✓ (t_R~3.8 min)	✓ (0.20) (t_R~8.2 min)	✓ (t_R~13.9 min)
Human	✓ (0.07) (t_R~3.0 min)	✓ (t_R~3.7 min)	✓ (t_R~8.5 min)	✓ (0.35) (t_R~14.0 min)

✓ = metabolite present
- = metabolite not observed

[205145 P49]

Species comparison of ^{3}H-[tyr19]-GLP-1(7-37) metabolites (Table 3) formed in primary hepatocyte cultures showed there were qualitative species differences in GLP-1(7-37) metabolism and one of the metabolites, t_R 8.5 min, occurs in both GLP-1 and liraglutide metabolite profiles (metabolite at Rt_R 0.31 for GLP-1(7-37) and at Rt_R 0.21 for liraglutide).

Table 3 Cross species comparison of relative retention times (Rt_R) for radioactive peaks formed after hepatocytes incubation with ^{3}H-[tyr]-GLP-1 (7-37)

Species	Rt_R					
	0.11	0.12	0.23	0.31	0.33	1.03
Mouse	✓ (t_R~2.9 min)	✓ (t_R~3.4 min)	✓ (t_R~6.3 min)	✓ (t_R~8.5 min)	✓ (t_R~8.9 min)	✓ (t_R~28.2 min)
Rat	✓ (t_R~2.9 min)	✓ (t_R~3.3 min)	✓ (t_R~6.3 min)	✓ (t_R~8.5 min)	✓ (t_R~9.1 min)	✓ (1.02) (t_R~28.1 min)
Monkey	✓ (t_R~2.9 min)	✓ (t_R~3.3 min)	✓ (0.22) (t_R~6.1 min)	✓ (t_R~8.5 min)	✓ (t_R~9.0 min)	✓ (t_R~28.2 min)
Human	✓ (t_R~2.9 min)	✓ (t_R~3.3 min)	✓ (0.25) (t_R~6.9 min)	✓ (t_R~8.5 min)	✓ (0.32) (t_R~8.9 min)	✓ (t_R~28.2 min)

✓ = metabolite present
- = metabolite not observed

[205145 P50]

Metabolites of ^{3}H-[Pal]-liraglutide in rat and human hepatocytes were identified by HPLC with fractions analyzed by electrospray LC-MS and MS/MS. Hepatocytes were incubated with 0.025 (rat, human), 1 (rat), or 10 µM ^{3}H-[Pal]-liraglutide (human) for 4 hours at 37C. Lipid-radiolabeled liraglutide metabolites from rat and human hepatocytes were nearly identical with most of the metabolites only occurring in small amounts. Liraglutide was extensively metabolized with the initial cleavage occurring at amino acids 18 – 19, 19 – 20, 27 – 28, and 28 – 29 resulting in shorter peptides that all include lysine 26 (because ^{3}H-palmictic acid is attached to lysine 26 via a glutamic acid linker). Palmitic acid was not metabolized. Table 7 summarizes liraglutide metabolites from DPP-4, NEP, and human hepatocytes.

Table 7 Identified liraglutide metabolites from incubations with DPP-IV, NEP and rat and human hepatocytes (NN206665, NN207147)

NAME	Amino acids in the peptide part of liraglutide (7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37)	Relative HPLC retention time (RRT)	*In vitro* system
NNC 90-1170 (7-37)	H A E G T F T S D V S S Y L E G Q A A K E F I A W L V R G R G	1,000	
NNC 90-1170 (7-27)	H A E G T F T S D V S S Y L E G Q A A K E	0.95	Hep[1)]
NNC 90-1170 (9-37)	E G T F T S D V S S Y L E G Q A A K E F I A W L V R G R G	1.05-1.06	DPP-IV
NNC 90-1170 (20-27)	L E G Q A A K E	1.03	NEP, Hep
NNC 90-1170 (20-37)	L E G Q A A K E F I A W L V R G R G	1.03-1.04	NEP, Hep
NNC 90-1170 (19-27)	Y L E G Q A A K E	1.04	NEP
NNC 90-1170 (19-37)	Y L E G Q A A K E F I A W L V R G R G	1.05-1.07	NEP, Hep
NNC 90-1170 (24-27)	A A K E	1.07	NEP
NNC 90-1170 (16-28)	V S S Y L E G Q A A K E F	1.08	Hep
NNC 90-1170 (21-28)	E G Q A A K E F	1,080	Hep
NNC 90-1170 (25-27)	A K E	1.09	NEP
NNC 90-1170 (26-27)	K E	1.11	NEP
NNC 90-1170 (20-28)	L E G Q A A K E F	1.11	NEP, Hep
NNC 90-1170 (25-26)	A K	1.13	NEP
NNC 90-1170 (24-28)	A A K E F	1.14-1.15	NEP, Hep[2)]
NNC 90-1170 (25-28)	A K E F	1.17	NEP, Hep
NNC 90-1170 (20-31)	L E G Q A A K E F I A W	1.19	Hep
NNC 90-1170 (26-28)	K E F	1.19	Hep
NNC 90-1170 (20-32)	L E G Q A A K E F I A W L	1.28	Hep

Hep = rat and human hepatocytes; NEP = neutral endopeptidase; DPP-IV = dipeptidyl peptidase IV.
[1)]This peak was hardly detectable (NN207147) and was not ascribed as a metabolite in another study investigating cross species metabolite profiling in hepatocytes as it was also observed in the control incubations without hepatocytes (NN205145). [2)] Not identified in rat hepatocytes. As many of the metabolites were present in very low amounts, these minor differences were likely due a difference in concentration of the individual metabolites, and therefore some minor ones escaped isolation and identification, than due to a difference in the metabolism in the two species.

[PK Written Summary P30]

Endogenous GLP-1 is metabolized by DPP-4 and NEPs. Metabolism of up to 10 µM ^{3}H-[pal]-liraglutide by DPP-4 and NEP for up to 25 hours was evaluated using recombinant human enzymes in the presence of 1% human serum albumin. Metabolites were analyzed by HPLC and HPLC fractions were collected for analysis by LC-MS and MS/MS. Table 2 (below) and Table 7 (above) show liraglutide metabolites resulting from DPP-4 or NEP hydrolysis. Liraglutide was extensively metabolized by NEP with initial cleavage at amino acids 18-19 and 19-20 and up to 11 metabolites identified. All metabolites included lysine 26, the amino acid serving as the site of attachment for ^{3}H-palmitate via a glutamic acid linker. DPP-4 cleaved off the amino acid dipeptide (at position 8 – 9) yielding liraglutide(9-37), but the reaction was slow.

Table 2 Overview of identified liraglutide metabolites and relative HPLC retention time

Metabolite	NEP	DPP-IV	Molecular weight (mono isotopic mass)	Relative HPLC retention time (to NNC 90-1170)
NNC 90-1170 (7-37)	✓	✓	3749.0	1.000
NNC 90-1170 (20-27)	✓		1211.7	1.026
NNC 90-1170 (20-37)	✓		2367.3	1.039
NNC 90-1170 (19-27)	✓		1374.8	1.039
NNC 90-1170 (19-37)	✓		2530.4	1.066
NNC 90-1170 (24-27)	✓		784.5	1.066
NNC 90-1170 (25-27)	✓		713.5	1.086
NNC 90-1170 (26-27)	✓		642.4	1.105
NNC 90-1170 (20-28)	✓		1358.7	1.105
NNC 90-1170 (25-26)	✓		584.4	1.125
NNC 90-1170 (24-28)	✓		931.6	1.151
NNC 90-1170 (25-28)	✓		860.5	1.171
NNC 90-1170 (9-37)		✓	3540.8	1.060

[206665 P50]

In a second study determining the effect of human serum albumin on the rate DPP-4 or NEP mediated metabolism of liraglutide, NEP mediated metabolism was 9-fold slower and DPP-4 metabolism was ~ 2-fold slower in the presence of 5% albumin (Table 1). The rate of liraglutide metabolism by NEP or DPP-4 was much slower than the rate of GLP-1(7-37) metabolism with or without 5% albumin. Liraglutide binding to human serum albumin substantially decreases metabolism by NEP and it also decreases metabolism by DPP-4, but to a much lesser extent.

Table 1 Comparison of formation rates of liraglutide and GLP-1 metabolites following incubations with DPP-IV and NEP in presence of 5% HSA and without HSA

Enzyme	5 % HSA			0.01% Tween		
	Liraglutide (% metabolite formed/ (min*µg/ml)	GLP-1 (% metabolite formed/ (min*µg/ml)	Fold difference	Liraglutide (% metabolite formed/ (min*µg/ml)	GLP-1 (% metabolite formed/ (min*µg/ml)	Fold difference
NEP	0.00245	0.799	326	0.0221	1.217	55
DPP-IV	0.00413	1.13	274	0.0077	1.310	170

Samples were incubated (n=2) and pooled before analysis. Values (% metabolite formed / min * µg/ml enzyme) are calculated from 8 h incubations with DPP-IV (4 µg/ml) and NEP (3.2 µg/ml) for liraglutide. Values for GLP-1 are calculated from 15 min incubations (5%HSA: DPP-IV (4 µg/ml) and NEP (3.2 µg/ml); 0.01% Tween: DPP-IV (2 µg/ml) and NEP (1.6 µg/ml).

[207312 P20]

Metabolites of 0.01 or 1 µM ^{3}H-[Tyr19]-liraglutide, 0.01 or 1 µM ^{3}H-[Pal]-liraglutide, and 0.05 or 1 µM ^{3}H-[Tyr]-GLP-1(7-37) were evaluated in liver and kidney slices from male Sprague Dawley rats after 4 and 24 hour incubations at 37C. Table 2 (below) summarizes liraglutide metabolites using lipid-radiolabeled (^{3}H-[Pal]-) and peptide-radiolabeled (^{3}H-[Tyr19]-) liraglutide. In general, liraglutide was more extensively metabolized in liver compared to kidneys, there was no overlap between lipid-radiolabeled and peptide-radiolabeled metabolites from liver or kidney slices, and lipid-radiolabeled liraglutide metabolites were more hydrophobic than liraglutide, but peptide-radiolabeled liraglutide metabolites were less hydrophobic than the parent compound. In both liver and kidney slices, GLP-1 metabolism was more rapid and extensive than liraglutide.

Table 2 Overview of ^{3}H-liraglutide metabolites (area>5%) from incubations with rat liver and kidney slices

	^{3}H-[pal]-liraglutide		^{3}H-[tyr]-liraglutide	
	Liver	Kidney	Liver	Kidney
Metabolite $Rt_{R=0.07-0.08}$ (t_R 3.0-3.3 min)	-	-	x[1]	x[1]
Metabolite $Rt_{R=0.09}$ (t_R 3.4-3.5 min)	-	-	x	x
Metabolite $Rt_{R=0.16}$ (t_R 5.2-6.3 min)	-	-	x	-
Metabolite $Rt_{R=0.20-0.21}$ (t_R 7.3-8.2 min)	-	-	x	x
Metabolite $Rt_{R=0.41}$ (t_R 16.01 min)	-	-	x	-
Metabolite $Rt_{R=0.73}$ (t_R 29.1 min)	x[1]	x[1]	x[1]	x[1]
Liraglutide $Rt_{R=1.0}$ (t_R 38.7-40.1 min)	x	x	x	x
Metabolite $Rt_{R=1.02}$ (t_R 39.6-39.7min)	x	-	-	-
Metabolite $Rt_{R=1.04}$ (t_R 40.3-41.6 min)	x	x	-	-
Metabolite $Rt_{R=1.05}$ (t_R 40.8)	x	-	-	-
Metabolite $Rt_{R=1.06}$ (t_R 40.8-42.3 min)	x	x	-	-
Metabolite $Rt_{R=1.07-1.08}$ (t_R 41.7-42.7 min)	x	x	-	-
Metabolite $Rt_{R=1.09}$ (t_R 41.7-43.2 min)	x	-	-	-
Metabolite $Rt_{R=1.10}$ (t_R 42.6-43.6 min)	x	x	-	-
Metabolite $Rt_{R=1.13-1.14}$ (t_R 44.1-44.9 min)	x	x	-	-
Metabolite $Rt_{R=1.16-1.17}$ (t_R 45.3-46.0min)	x	x	-	-
Metabolite $Rt_{R=1.19}$ (t_R 46.2 min)	x	-	-	-
Metabolite $Rt_{R=1.27}$ (t_R 49.3-49.4 min)	x	-	-	-

[1] Also observed in 24 hours control incubation (area) >5%)
x = metabolite observed
- = metabolite not observed or area <5%

[205145 P31]

Metabolism and excretion of ^{3}H-[Pal]-liraglutide was determined using perfused kidney or liver in albino male Sprague Dawley rats. Due to low levels of radioactivity in urine and bile over the 3 hour perfusion period, there was no evidence kidney or liver significantly contributed to elimination of liraglutide. Radiolabeled material in the perfusate (containing 6% human serum albumin and bovine red blood cells) from either kidney or liver was primarily the unmodified parent drug, ^{3}H-[Pal]-liraglutide.

^{3}H-[Pal]-liraglutide was stable in plasma from CD-1 mice, Sprague Dawley rats, cynomolgus monkeys, and humans. There were no metabolites after incubating for up to 24 hours in plasma from monkeys and humans and a small amount of a single metabolite (Rt_R 1.06) occurred after incubating $\geq$ 4 hours in plasma from rats and mice. GLP-1(7-37) was rapidly metabolized in plasma from mice, rats, monkeys, and humans.

Enzyme Inhibition / Induction

The IC_{50} of liraglutide to inhibit CYP450s in human liver microsomes was > 100 µM for CYPs 1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4.

Liraglutide increased CYP activity < 1.6 fold in male and female rats treated with up to 1 mg/kg/day for up to 4 weeks. At 1 mg/kg/day, liraglutide caused a statistically significant 2-fold decrease in CYP2A1 in male rats.

2.6.4.6 Excretion

Excretion of radiolabeled liraglutide-related material was determined after single sc dosing in rats (^{125}I-, ^{14}C-labeling), monkeys (^{3}H-[Pal]-labeled), and humans (^{3}H-[Pal]-labeled) and after 7-day repeat sc dosing in rats (^{125}I-, ^{14}C-labeling). Excretion routes depended on the labeling site with ^{125}I-[Tyr19]-peptide labeled liraglutide-related material mainly excreted in urine, ^{14}C-[glutamate]-linker labeled liraglutide-related material mainly excreted in expired air, and ^{3}H-[palmitate]-lipid liraglutide-related material mainly excreted in urine. Less than 10% of total administered radioactivity was excreted in feces, regardless of the liraglutide-labeling site or species. These results suggest liraglutide is extensively metabolized into its constituent components, amino acids and lipids, that are further metabolized through general pathways prior to excretion.

Excretion of radiolabeled material from Sprague Dawley rats (3/sex/dose) administered a single sc injection of 0.1 or 1 mg/kg ^{125}I-[tyr]-liraglutide or 1 mg/kg ^{14}C-[glutamate linker]-liraglutide was monitored for up to 168 hours after dosing. Since there were no substantive sex differences in excretion, combined results are shown in Tables 8 & 9. Recovery of ^{125}I in excreta was nearly quantitative with 93 - 101% of the administered dose (0.1 or 1 mg/kg) recovered in males (77.0 - 89.1% in urine, 2.7 – 4.7 % in feces, 7.3 – 10.1% in the carcass) and females (78.1 - 89.7% in urine, 3.4 – 5.3% in feces, 6.7 – 9.9 % in the carcass) within 168 hours after dosing. Most of the radioactivity in urine could not be precipitated with TCA indicating it was free ^{125}I, not incorporated into protein. Approximately 91 – 92% of administered ^{14}C was recovered within 168 hours after dosing males (68.4% in expired air, 4.3% in urine, 10.2% in feces, 9.4% in the carcass) and females (74.2% in expired air, 4.4% in urine, 5.8% in feces, 6.8% in the carcass). Recovery and excretion of radioactivity after 7 days of repeat dosing with 1 mg/kg ^{125}I or ^{14}C-liraglutide yielded similar results (Tables 8 & 9). In the 7-day repeat dose study, ^{125}I in urine was first detected after the 4th dose and $^{14}CO_2$ in expired air was first detected after the 2nd dose.

Table 8 Excretion of radioactivity following single and multiple dose administration of 125I-liraglutide

Species	Dose (mg/kg)	Excretion of ^{125}I-related radioactivity (% of dose)			
		Urine	Faeces	Carcass	Total[b]
Rat[a]	0.1	89.4	3.1	7.0	100.6[b]
Rat[a]	1	77.5	5.1	10.0	93.9[b]
Rat[a]	1 (MD, 7 days)	82.1	7.4	-	96.4[c]

[a] Mean of male and female
[b] Includes cage wash. Collection completed 168 h post dosing
[c] Includes cage wash, dose site and residual carcass. Collection completed 168 h post dosing (after last MD)
- = Not measured

[PK Written Summary P38]

Table 9 Excretion of radioactivity following single and multiple dose administration of 14C-liraglutide

Species	Dose (mg/kg)	Excretion of ^{14}C-related radioactivity (% of dose)				
		Urine	Faeces	Expired air	Carcass	Total
Rat	1	4.4	8.0	71.3	8.1	91.7[a]
Rat	1 (MD, 7 days)	6.9	8.7	72.2	-	93.2[b]

[a] Includes cage wash and carcass. Collection completed 168 h post last dosing.
[b] Includes cage wash, dose site and residual carcass. Collection completed 168 h post dosing (after last MD)

[PK Written Summary P38]

Excretion of radioactivity from cynomolgus monkeys and humans administered a single sc injection of ^{3}H-[pal]-liraglutide (0.05 or 5 mg/kg in monkeys, 75 mg in humans) was monitored for up to 168 hours (7 days) in monkeys and up to 312 hours (13 days) in humans. Results are summarized in Table 10. Elimination of radioactivity was somewhat slower in humans compared to monkeys. Recovery of radioactivity from monkeys and humans was low. Approximately 20% of administered ^{3}H in monkeys and humans was recovered in urine with most of the radioactivity considered to be water because it was a volatile. The volatile component of urine ^{3}H increased over time (25 – 48% within 6 hours of dosing in monkeys, then 94 – 97% 168 hours after dosing). Less than 10% of the total dose was recovered in feces and a substantial portion of ^{3}H in feces was also volatile (68% at 168 hours in monkeys) indicating it was probably $^{3}H_2O$. The elimination half life of ^{3}H in monkeys was 144 – 168 hours, which is consistent with the reported half-life of tritiated water in other organisms. A significant portion of ^{3}H may be excreted as water in expired air or sweat, and this would account for the low recovery.

Table 10 Excretion of radioactivity following single dose administration of 3H-[Pal]-liraglutide

Species	Dose (mg/kg)	Post-dose collection period (h)	Excretion of ^{3}H-[Pal]-related radioactivity (% of dose)			
			Urine	Faeces	Other[a]	Total
Monkey	0.05	0-168	21.7	8.8	0.8	31.2[a]
	5	0-168	19.6	7.8	1.1	28.5[a]
Human[c]	0.01[b]	0-168	13.5	5.4	-	18.9
	0.01[b]	0-312	20.1	6.2	-	26.3

[a]Includes cage wash and cage debris. Collection completed 168 h post last dosing.
[b]Human dose has been calculated based on given dose (0.75 mg) and the mean weight of the 7 volunteers (74.5 kg)

[PK Written Summary P39]

Secretion in Milk

Secretion of radiolabeled liraglutide and related material in milk of lactating rats was determined after a single sc 1 mg/kg dose of ^{125}I- or ^{14}C-labeled liraglutide or after a single sc dose of 1 mg/kg ^{3}H-[pal]-liraglutide. The milk to plasma ratio increased over time for all 3 isotopes. Metabolites in milk were profiled after dosing with ^{3}H-[pal]-liraglutide.

Figure 1 (left, from study 201200) shows concentrations of radioactivity (expressed as mcg equivalents of liraglutide / g of fluid) in plasma and milk after a single sc dose of ^{125}I- or ^{14}C-liraglutide. Figure 1 (right, study 206544) shows concentrations after dosing with ^{3}H-[pal]-liraglutide. Liraglutide-related radioactivity in milk peaked 12 hours after dosing and exceeded plasma levels from 6 hours after dosing with ^{125}I-liraglutide, 18 hours after dosing with ^{14}C-liraglutide, and 4, 12, and 24 hours after dosing with ^{3}H-liraglutide. Between 82 – 90% of ^{125}I radioactivity in milk was precipitated by TCA suggesting it's incorporated in protein. HPLC separation of ^{3}H-[pal]-liraglutide radiolabeled material in milk showed most of the radioactivity in milk was the parent drug.

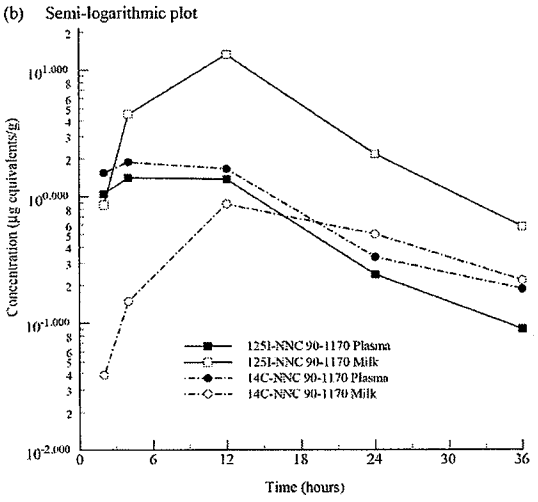


Figure 1 Mean (n=3) concentrations of radioactivity in plasma and milk after subcutaneous administration of radiolabelled NNC 90-1170 (1.0 mg/kg)

[201200 P30]

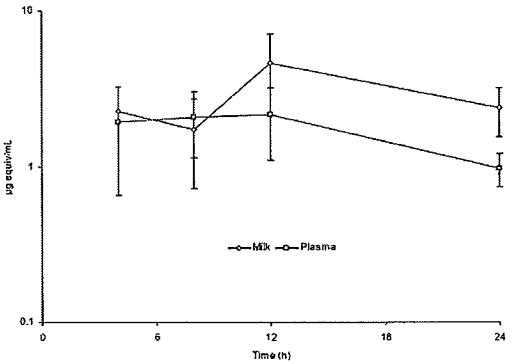


Figure 1 Mean Concentration of Total Radioactivity in Milk and Plasma Following a Single Subcutaneous Administration of 1 mg/kg [^{3}H]-NNC 90-1170 to Lactating Rats

[206544 P20]

Exposure in milk and plasma after dosing with ^{125}I- or ^{14}C-liraglutide is shown in Table 3. Peak and total exposure to ^{125}I labeled material in milk was much higher than ^{14}C-labeled material.

Table 3 Pharmacokinetic parameters for total radioactivity following subcutaneous administration of ^{125}I-NNC 90-1170 or ^{14}C-NNC 90-1170 (1.0 mg/kg) to lactating female rats

(a) ^{125}I-NNC 90-1170

Matrix	C_{max} (µg equivalents/g)	T_{max} (hours)	$AUC_{0-36\,h}$ (µg equivalent.h/g)	k (hours^{-1})	$t_{½}$ (hours)
Plasma	1.409	4	26.4	0.1136	6.1
Milk	13.31	12	186.6	0.1306	5.3

(b) ^{14}C-NNC 90-1170

Matrix	C_{max} (µg equivalents/g)	T_{max} (hours)	$AUC_{0-36\,h}$ (µg equivalent.h/g)	k (hours^{-1})	$t_{½}$ (hours)
Plasma	1.879	4	34.2	0.0801	8.7
Milk	0.876	12	16.9	0.0580	12.0

[201200 P34]

Based on radioactivity in milk, the sponsor estimated the maximum amount of radioactivity in milk consumed by a pup would be 3% of the total daily ^{125}I-liraglutide dose and 0.3% of the total ^{14}C-liraglutide dose.

Table 1 (below) shows the level of total ^{3}H-liraglutide-related material was similar in milk and plasma of lactating rats. These results indicate the concentration of liraglutide-related material in milk is similar to the concentration in plasma in lactating rats.

Table 1 Mean Concentration of Total Radioactivity in Milk and Plasma Following a Single Subcutaneous Administration of [^{3}H]-NNC 90-1170 to Lactating Rats
Target Dose Level: 1 mg/kg

Results expressed as µg equiv/mL

Sample	Time							
	4 h		8 h		12 h		24 h	
Sample	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Milk	2.258	N.A.	1.725	1.006	4.592	2.470	2.376	0.826
Plasma	1.936	1.284	2.086	0.949	2.149	1.059	0.973	0.231
Ratio	0.8	N.A.	0.9	0.7	2.3	1.4	2.4	0.3

Ratio = Ratio of total radioactivity in milk to plasma
N.A. = Not applicable

[206544 P27]

2.6.4.7 Pharmacokinetic drug interactions

In Vitro Plasma Protein Displacement Interactions

Because liraglutide is highly protein bound, displacement of liraglutide by other highly protein bound drugs and fatty acids (myristic and palmitic acids) was determined by equilibrium dialysis. Pioglitazone (1.5 mcg/mL), rosiglitazone (0.6 mcg/mL), warfarin (10 mcg/mL), furosemide (0.2 mcg/mL), tolbutamide (100 mcg/mL), diazepam (1 mcg/mL), glibenclamide (1 mcg/mL), nicardipine (0.35 mcg/mL), repaglinide (1 mcg/mL), acetylsalicylic acid (0.5 mcg/mL), valproic acid (0.2 mcg/mL), metformin (10 mcg/mL), myristic acid (0.4 mM), palmitic acid (0.4 mM), acenocoumarol (10 mcg/mL), and phenprocoumon (10 mcg/mL) did not affect liraglutide (10 nM) protein binding of 99.7% in human plasma from male and female volunteers.

Delayed Gastric Emptying

The effect of liraglutide on gastric emptying, which could potentially alter the absorption of other drugs, was evaluated in pigs coadministered liraglutide and paracetamol. Gastric emptying was delayed in male Gottingen minipigs subcutaneously injected with 0.0033 mg/kg liraglutide once a day for 2 or 4 weeks. Coadministering 500 mg paracetamol and liraglutide

reduced the plasma paracetamol $AUC_{0\text{-}120\ min}$ to 80% of control at weeks 2 and 51% at week 4. In clinical studies, liraglutide delayed paracetamol Tmax 15 minutes compared to placebo in adult type 2 diabetics, but without affecting total absorption of paracetamol.

2.6.4.10Tables and figures to include comparative TK summary

Species	Liraglutide Dose (mg/kg)	Sampe Time	Study Duration	Study #	Sex	Liraglutide Plasma Toxicokinetic Parameters			Liraglutide Plasma Toxicokinetic Parameters, Average			Human Exposure Multiple#	
						Cmax (nM)	AUC (nM.hr)	Tmax (hr)	Cmax (nM)	AUC (nM.hr)	Tmax (hr)	Cmax	AUC
CD-1 mice	0.03	day 1	single dose	205106	M, F	17	206	6.0	15	153	7.5	0.3	0.2
		week 26	104 weeks	204229	M, F	17	128	9.0					
		week 52	104 weeks	204229	M, F	7	91	9.0					
		week 104	104 weeks	204229	M, F	20	185	6.0					
	0.06	day 1	3 days	205050	M	43	576	3.0	47	578	4.5	1.1	0.7
		day 3	3 days	205050	M	51	580	6.0					
	0.1	day 1	28 days	203261	M, F	75	1073	6.0	71	943	6.0	1.6	1.2
		day 28	28 days	203261	M, F	66	812	6.0					
	0.2	day 1	single dose	205106	M, F	136	1451	4.5	136	1,538	5.8	3.1	1.9
			13 weeks	204082	M, F	160	1,932	5.0					
		week 13	13 weeks	204082	M, F	196	1,959	6.0					
		week 26	104 weeks	204229	M, F	125	1,661	5.0					
		week 52	104 weeks	204229	M, F	60	723	6.0					
		week 104	104 weeks	204229	M, F	141	1,501	8.0					
	0.25	day 1	3 days	205050	M	156	2920	6.0	212	2,980	4.5	4.8	3.7
		day 3	3 days	205050	M	268	3040	3.0					
	0.5	day 1	28 days	203261	M, F	595	5,889	5.0	477	5,163	6.5	10.8	6.4
		day 28	28 days	203261	M, F	359	4,436	8.0					
	1	day 1	single dose	205106	M, F	656	7,756	4.5	1,069	11,341	4.9	24.3	14.1
			28 days	203261	M, F	1,189	13,950	4.0					
			13 weeks	204082	M, F	996	12,424	4.0					
		day 28	28 days	203261	M, F	930	9,634	7.0					
		week 13	13 weeks	204082	M, F	1,166	15,223	3.5					
		week 26	104 weeks	204229	M, F	882	9,232	5.0					
		week 52	104 weeks	204229	M, F	1,638	14,355	6.0					
		week 104	104 weeks	204229	M, F	1,093	8,153	5.0					
	3	day 1	single dose	205106	M, F	2,012	23,545	4.5	2,454	28,641	6.9	55.8	35.5
		week 26	104 weeks	204229	M, F	2,113	25,385	9.0					
		week 52	104 weeks	204229	M, F	2,167	28,805	8.0					
		week 104	104 weeks	204229	M, F	3,523	36,830	6.0					
	5	day 1	28 days	203261	M, F	5,826	82,252	5.0	5,715	70,612	4.8	129.9	87.6
			13 weeks	204082	M, F	5,899	74,051	6.0					
		day 28	28 days	203261	M, F	5,026	57,609	4.0					
		week 13	13 weeks	204082	M, F	6,108	68,534	4.0					
NMRI mice	0.02	day 1	single dose	990267	M	10	ND	8.0	10	ND	8.0	0.2	-
	0.2	day 1	single dose	990267	M	58	ND	8.0	58	ND	8.0	1.3	-
	2	day1	single dose	990267	M	592	ND	8.0	592	ND	8.0	13.5	-
ob/ob mice	0.2	day 1	single dose	200031	F	181	2,132	6.0	181	2,132	6.0	4.1	2.6
db/db mice	0.2	day 1	single dose	200031	F	227	2,428	4.0	227	2,428	4.0	5.2	3.0

#Human exposure multiples were calculated based on steady state plasma exposures of Cmax 44 nM and $AUC_{0\text{-}24h}$ 809 nM.hr liraglutide at the MRHD of 1.8 mg/day.

Species	Liraglutide Dose (mg/kg)	Sampe Time	Study Duration	Study #	Sex	Liraglutide Plasma Toxicokinetic Parameters			Liraglutide Plasma Toxicokinetic Parameters, Average			Human Exposure Multiple#	
						Cmax (nM)	AUC (nM.hr)	Tmax (hr)	Cmax (nM)	AUC (nM.hr)	Tmax (hr)	Cmax	AUC
SD rats	0.00188	day 1	single dose	970355	F	0.5	4	4.0	0.5	4.0	4.0	0.0	0.0
	0.02	day 1	single dose	990268	M	6.0	ND	8.0	6.0	ND	8.0	0.1	-
	0.075	day 1	104 weeks	200240	M, F	32	339	6.0	32	411	7.3	0.7	0.5
		week 53	104 weeks	200240	M, F	33	470	8.0					
		week 104	104 weeks	200240	M, F	31	423	8.0					
	0.1	day 1	28 days	980183	M, F	38	482	6.0	51	651	5.5	1.1	0.8
			13 weeks	980189	M, F	69	810	5.0					
			26 weeks	200239	M, F	77	872	6.0					
		day 28	28 days	980183	M, F	38	505	6.0					
		week 13	13 weeks	980189	M, F	46	754	3.0					
		week 26	26 weeks	200239	M, F	35	481	7.0					
	0.125	day 1	7 days	980180	M, F	85	1,185	6.0	89	1,103	6.0	2.0	1.4
		day 7	7 days	980180	M, F	92	1,020	6.0					
	0.2	day 1	single dose	990268	M	74	ND	4.0	74	ND	4.0	1.7	-
	0.25	day 1	7 days	980180	M, F	220	2,567	6.0	174	2,235	7.0	4.0	2.8
			28 days	980183	M, F	115	1,813	8.0					
			13 weeks	980189	M, F	267	2,652	7.0					
			26 weeks	200239	M, F	188	2,130	5.0					
			104 weeks	200240	M, F	112	1,320	8.0					
		day 7	7 days	980180	M, F	208	2,603	4.0					
		day 28	28 days	980183	M, F	164	2,200	8.0					
		week 13	13 weeks	980189	M, F	256	3,088	6.0					
		week 26	26 weeks	200239	M, F	107	1,585	9.0					
		week 53	104 weeks	200240	M, F	129	2,305	8.0					
		week 104	104 weeks	200240	M, F	151	2,320	8.0					
	0.75	day 1	104 weeks	200240	M, F	350	5,030	7.0	373	6,182	7.0	8.5	7.7
		week 53	104 weeks	200240	M, F	370	5,895	6.0					
		week 104	104 weeks	200240	M, F	398	7,620	8.0					
	1	day 1	7 days	980180	M, F	597	7,750	5.0	565	7,399	6.6	12.8	9.2
			28 days	980183	M, F	804	8,198	4.0					
			4 weeks (grp 2)	205092	M, F	365	4,927	7.0					
			4 weeks (grp 3)	205092	M, F	344	4,939	8.0					
			13 weeks	980189	M, F	1,040	12,742	4.0					
			26 weeks	200239	M, F	618	8,375	7.0					
		day 7	7 days	980180	M, F	448	5,959	5.0					
		day 28	28 days	980183	M, F	577	9,074	10.0					
		day 30	4 weeks (grp 2)	205092	M, F	463	5,100	6.0					
		day 30	4 weeks (grp 3)	205092	M, F	397	4,784	7.0					
		week 13	13 weeks	980189	M, F	563	10,698	9.0					
		week 26	26 weeks	200239	M, F	568	6,240	7.0					
	2	day 1	single dose	990268	M	1,045	ND	4.0	1,045	ND	4.0	23.8	-
SD rats (unmated and mated)	0.1	day 1 (unmated)	Seg I/II	980186	F	60	680	8.0	60	680	8.0	1.4	0.8
		GD 17	Seg I/II	980186	F	75	691	4.0	75	691	4.0	1.7	0.9
	0.25	day 1 (unmated)	Seg I/II	980186	F	158	1,980	8.0	158	1980	8.0	3.6	2.5
		GD 17	Seg I/II	980186	F	214	2,693	8.0	214	2693	8.0	4.9	3.3
	1	day 1 (unmated)	Seg I/II	980186	F	612	9,148	8.0	612	9148	8.0	13.9	11.3
		GD 17	Seg I/II	980186	F	1,241	9,211	4.0	1241	9211	4.0	28.2	11.4

#Human exposure multiples were calculated based on steady state plasma exposures of Cmax 44 nM and $AUC_{0\text{-}24h}$ 809 nM.hr liraglutide at the MRHD of 1.8 mg/day.

Species	Liraglutide Dose (mg/kg)	Sampe Time	Study Duration	Study #	Sex	Liraglutide Plasma Toxicokinetic Parameters			Liraglutide Plasma Toxicokinetic Parameters, Average			Human Exposure Multiple#	
						Cmax (nM)	AUC (nM.hr)	Tmax (hr)	Cmax (nM)	AUC (nM.hr)	Tmax (hr)	Cmax	AUC
NZW rabbits (unmated and mated)	0.01	day 1 (unmated)	Seg II	980187/8	F	10	140	6.0	10	140	6.0	0.2	0.2
		GD 6	Seg II	980187/8	F	9	125	6.0	9	125	6.0	0.2	0.2
		GD 16	Seg II	980187/8	F	11	148	6.0	11	148	6.0	0.3	0.2
	0.02	day 1 (unmated)	Seg II	980187/8	F	17	245	8.0	17	245	8.0	0.4	0.3
	0.03	GD 6	Seg II	980187/8	F	19	288	6.0	19	288	6.0	0.4	0.4
		GD 16	Seg II	980187/8	F	17	280	5.0	17	280	5.0	0.4	0.3
	0.1	GD 6	Seg II	980187/8	F	36	571	9.0	36	571	9.0	0.8	0.7
		GD 16	Seg II	980187/8	F	51	766	13.0	51	766	13.0	1.2	1.0
Cynomolgus monkeys	0.05	day 1	28 days	980184	M, F	15	234	11.5	26	434	8.8	0.6	0.5
			52 weeks	200241	M, F	30	484	7.3					
		day 28	28 days	980184	M, F	13	202	8.5					
		week 52	52 weeks	200241	M, F	46	817	8.0					
	0.1	day 3	3 days	970455	M, F	185	2,627	4.0	185	2,627	4.0	4.2	3.3
	0.25	week 72	87 weeks	203262	M, F	342	5,352	8.0	383	6,258	8.0	8.7	7.8
		week 87	87 weeks	203262	M, F	423	7,163	8.0					
	0.5	day 1	28 days	980184	M, F	187	2,684	8.0	292	4,693	7.3	6.6	5.8
			52 weeks	200241	M, F	417	7,210	7.3					
		day 28	28 days	980184	M, F	142	1,858	6.5					
		week 52	52 weeks	200241	M, F	423	7,020	7.3					
	2.5	day 3	3 days	970455	M, F	1,899	30,933	7.0	1,899	30,933	7.0	43.2	38.4
	4	day 1	14 days	980181	M, F	3,331	56,425	7.5	4,127	58,834	7.0	93.8	73.0
		day 14	14 days	980181	M, F	4,923	61,242	6.5					
	5	day 1	single dose	980182	M, F	4,489	83,153	8.0	3,451	59,403	7.0	78.4	73.7
			28 days	980184	M, F	2,098	32,430	9.5					
			52 weeks	200241	M, F	5,030	102,900	8.5					
		day 3	3 days	970455	M, F	3,725	69,158	8.0					
		day 28	28 days	980184	M, F	2,486	25,160	7.0					
		week 52	52 weeks	200241	M, F	3,525	59,200	5.2					
		week 72	87 weeks	203262	M, F	2,942	51,100	6.0					
		week 87	87 weeks	203262	M, F	3,314	52,120	3.8					

#Human exposure multiples were calculated based on steady state plasma exposures of Cmax 44 nM and $AUC_{0\text{-}24h}$ 809 nM.hr liraglutide at the MRHD of 1.8 mg/day.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Toxicity of subcutaneously injected liraglutide (NNC 90-1170), a long-acting lipidated GLP-1 analog intended for subcutaneous (sc) dosing in humans, was determined in CD-1 mice, Sprague Dawley rats (including pregnant rats), New Zealand White rabbits (unmated, mated, and pregnant females), and cynomolgus monkeys. Local toxicity after single sc injection of liraglutide was evaluated in female pigs. Liraglutide activates cloned GLP-1Rs from all 5 species, and it was pharmacologically active in mouse, rat, pig, and monkey models of type 2 diabetes and/or obesity. Liraglutide was dissolved in an aqueous vehicle and in most toxicity studies, the vehicle contained sodium phosphate (——, phenol (————, and (———— propylene glycol (————. b(4)

In general, liraglutide exposure increased with dose with no substantive sex differences in exposure in any species. Anti-liraglutide antibodies didn't occur in mice or rats, but did occur in monkeys in 52- and 87-week repeat dose studies. Nanomolar concentrations of liraglutide in plasma decreased the sensitivity of the anti-liraglutide antibody assay confounding interpretation of assay results from liraglutide-treated animals. This may be the reason 2 monkeys in the 5 mg/kg/day liraglutide high dose group were antibody negative at the end of the 52-week treatment period, but antibody positive after a 4-week recovery period.

Local toxicity after repeat sc dosing with liraglutide was assessed using nonclinical dosing formulations that were more dilute than the 6 mg/mL liraglutide concentration in the clinical formulation. For example, the concentration of liraglutide in high dose group dosing solutions was 0.75 mg/mL in the rat carcinogenicity study (8-fold lower than the clinical formulation), 0.6 mg/mL in the mouse carcinogenicity study (10-fold lower), and 2 mg/mL in the 52-week chronic toxicity study in monkeys (3-fold lower). Injection site reactions occurred in monkeys, and in mice, treatment-related skin and subcutis fibrosarcomas in high dose males were attributed to high local concentration of drug. Single dose local tolerance studies performed in pigs using the clinical formulation showed injection site reactions occurred in vehicle-treated and liraglutide-treated groups, but inflammation was more severe in drug-treated animals.

Multiples of human exposure were calculated based on a maximum recommended human dose (MRHD) of 1.8 mg/day liraglutide yielding steady state pharmacokinetic parameters of Cmax 44 nM and $AUC_{0\text{-}24h}$ of 809 nM.hr in type 2 diabetic patients. Unless otherwise stated, human exposure multiples were calculated as the ratio of plasma liraglutide $AUC_{0\text{-}24h}$ in animals divided by $AUC_{0\text{-}24h}$ in humans at the MRHD. Higher doses up to 3 mg/day are being evaluated for the treatment of obesity, and human exposure multiples at higher doses will be lower.

General toxicology:

Liraglutide toxicity was evaluated in single sc and intravenous (iv) dose studies in mice and rats, an rising 3-day repeat sc dose study in monkeys, and repeat sc dose studies of 1-, 4- and 13-weeks in mice, 4-, 13-, and 26-weeks in rats, and 2-, 4-, 13-, and 52-weeks in monkeys. Liraglutide was well-tolerated in acute and general toxicity studies.

Transiently decreased food consumption and weight loss in rats and monkeys were considered pharmacologic effects, but in rats, the severity of decreased food consumption and body weight loss was dose limiting. Although decreased food consumption and body weight gain were transient in rats and monkeys and resolved after a few weeks of treatment, group mean body weight lower than controls persisted in liraglutide treated groups throughout most repeat dose studies. Although high dose liraglutide transiently decreased food consumption and body weight gain in mice, the effect did not persist and liraglutide did not lower body weight in mice treated for $\geq$ 4 weeks.

A dose-limiting toxicity was not identified in repeat sc dose toxicity studies in mice treated for up to 13 weeks with doses up to 5 mg/kg/day liraglutide (a dose yielding HEM $\leq$ 88X) or in monkeys treated with up to 5 mg/kg/day (HEM $\leq$ 74X) for up to 52 weeks in a chronic toxicity study or up to 87 weeks in a mechanistic study. In rats, the maximum tolerated dose (MTD) in repeat dose studies was 1 mg/kg/day liraglutide based on clinical signs of toxicity (piloerection, rolling / high stepping gait, hunched posture, dark extremities, and thin appearance), inappetence and decreased body weight, and moribund condition leading to euthanasia at 2 and 10 mg/kg/day in a 7-day repeat dose study. Although clinical signs of toxicity occurred at lower doses in repeat dose studies in rats, the severity was diminished even for rats treated for up to 26 weeks at doses up to 1 mg/kg/day (HEM $\leq$ 9X).

Thyroid was the only target organs identified in repeat dose toxicity studies, and only in mice. In thyroid of mice, liraglutide caused ultimobranchial cysts and C-cell focal hyperplasia in 4 and 13 week studies. Because C-cell proliferative lesions are rare in mice, a Pathology Working Group consisting of expert verterinary pathologists was convened to reach a consensus on C-cell pathology in liraglutide-treated mice. The results of this group are discussed in more detail in sections reviewing mouse carcinogenicity and mechanistic studies of liraglutide-induced C-cell tumors. Carcinogenicity and mechanistic studies of liraglutide, along with data from other GLP-1R agonists in development indicate drug-induced C-cell focal hyperplasia and tumors in mice and rats are a pharmacological class effect. Although liraglutide caused C-cell focal hyperplasia and tumors in a rat carcinogenicity study, it had no effect on C-cells in rats treated up to 26 weeks. Compared to mice, C-cells in rats < 8 months are insensitive to liraglutide, even after adjusting for differences in drug exposure. Liraglutide did not affect thyroid C-cells in monkeys.

Mild anemia characterized by decreased RBC count, hemoglobin, and hematocrit occurred in mice, rats, and monkeys. Although a cause for decreased RBC parameters was not established, it could be due to hemolysis (mice) and/or bone marrow toxicity (rats).

In the absence of correlative histopathology, the toxicological significance of organ weight changes in heart (decreased in rats, increased in monkeys), pancreas (increased in monkeys), and male reproductive organs (decreased seminal vesicle, prostate, and epididymis in rats) is uncertain.

Mice

In single sc or iv dose toxicity studies of 0 or 10 mg/kg liraglutide in CD-1 mice (5/sex/dose), liraglutide transiently decreased food consumption and body weight with recovery within 3 days of dosing. There were no treatment-related mortalities or macroscopic necropsy findings in mice sacrificed 14 days after dosing.

In an exploratory 7-day repeat sc dose study of 5 mg/kg/day liraglutide in mice (3/sex/dose), the MTD was $\geq$ 5 mg/kg/day. Food consumption, body weight gain, and in some mice, body weight , were transiently decreased, but without clinical signs of toxicity.

In a 4-week repeat sc dose study of 0, 0.1, 0.5, 1, or 5 mg/kg/day liraglutide in mice (10/sex/dose) with a satellite toxicokinetic group, multiples of human exposure were 1, 6, 12, and 71, respectively. The NOAEL was 5 mg/kg/day liraglutide, the highest dose tested. No treatment related mortalities occurred and no target organs were identified. Transiently decreased food consumption, decreased body weight gain, and diuresis in all liraglutide groups were considered pharmacologic effects. A mild anemia occurred in males at 1 mg/kg/day and in females at $\geq$ 0.1 mg/kg/day. Thyroid C-cells findings characterized as treatment-related focal hyperplasia by the original study pathologist occurred in 1/10 males in the 1 mg/kg group and in 2/10 females in the 5 mg/kg/day group were later reclassified as unilateral focal perithyroidal C-cells unrelated to treatment by a Pathology Working Group examining liraglutide-associated C-cell findings in mice. However, considering liraglutide caused focal C-cell hyperplasia in studies $\geq$ 9 weeks, it's likely focal hyperplasia occurred in the 4 week study, particularly in females.

In a 13-week repeat sc dose toxicity study of 0, 0.2, 1, or 5 mg/kg/day liraglutide in mice (10/sex/dose main study) with satellite toxicokinetic (28/sex/dose) and antibody study (15/sex/dose) groups, multiples of human exposure were 2, 19, and 85, respectively. No treatment-related unscheduled deaths occurred. The NOAEL was < 0.2 mg/kg/day based on thyroid C-cell hyperplasia and ultimobranchial cysts at ≥ 0.2 mg/kg/day. Thyroid was the only target organ. The description of C-cell hyperplasia noted on hemotoxylin-eosin staining was consistent with focal hyperplasia (slightly enlarged, pale eosinophilic-stained cells with granular cytoplasm and round to oval nuclei). A mild anemia occurred in all liraglutide-treated groups. Transiently decreased food consumption at ≥ 1 mg/kg/day, transiently decreased body weight gain at ≥ 0.2 mg/kg/day in males and at 5 mg/kg/day in females, and diuresis on day 1 at 5 mg/kg/day (increased urine volume, increased excretion of sodium, chloride, and phosphate, and decreased excretion, specific gravity, magnesium, potassium, and calcium) were considered pharmacological effects of liraglutide. Anti-liraglutide antibodies were not detected in this study.

Rats

In single sc or iv dose toxicity studies of 0 or 10 mg/kg liraglutide in Sprague Dawley rats (5/sex/dose) sacrificed 15 days after dosing, there were no liraglutide-related mortalities or any macroscopic necropsy findings. Liraglutide transiently decreased food consumption and body weight gain with recovery within 4 days of dosing. Hunched posture occurred in some treated rats on study day 2. Rats administered 10 mg/kg liraglutide intravenously showed clinical signs of tremors, increased breathing, piloerection, red discharge from eyes and/or nose, and stained perigenital area, but increased breathing and tremor also occurred in the control group.

In 7-day repeat sc dose studies in rats, the MTD was 1 mg/kg/day liraglutide and the NOAEL was 0.25 mg/kg/day. In the first 7-day study (5 rats /sex/dose) using doses of 0, 0.4, 2, or 10 mg/kg/day liraglutide, the MTD was 0.4 mg/kg/day based on moribund sacrifice of rats in 2 and 10 mg/kg/day groups with clinical signs of toxicity (piloerection, rolling / high stepping gait, hunched posture, dark extremities, and thin appearance), decreased appetence and fecal output, and severe body weight loss. In a second 7-day study using doses of 0, 0.125, 0.25, or 1 mg/kg/day in larger Sprague Dawley rats, the MTD was 1 mg/kg/day. Decreased food consumption, body weight gain and body weight, and decreased fecal output occurred in all dose groups, but with reduced severity at doses ≤ 1 mg/kg/day. Clinical signs of toxicity were hunched posture, piloerection, rolling/high stepping gait, and thin appearance at ≥ 0.25 mg/kg/day liraglutide, but with reduced severity compared to doses ≥ 2 mg/kg/day. Relative heart weight (normalized to body weight) decreased up to 11.1% in males and up to 15.7% in females, but the changes were not dose-related. Anti-liraglutide antibodies were not detected.

In a 28-day repeat sc dose study of 0, 0.1, 0.25, or 1 mg/kg/day liraglutide in rats (10/sex/dose) with a toxicokinetic satellite group, human exposure multiples were 0.6, 3, and 11, respectively. There were no treatment-related unscheduled deaths. The NOAEL was 0.25 mg/kg/day liraglutide based on clinical signs of toxicity (hunched posture, piloerection, and rolling or high stepping gait) at 1 mg/kg/day that dissipated after the first week of dosing. Liraglutide reduced food consumption, fecal output, and body weight gain during the first week of treatment at ≥ 0.25 mg/kg/day in males and at 1 mg/kg/day in females and decreased body weight persisted to the end of treatment in high dose males. A mild anemia characterized by decreased RBC count, hematocrit, and hemoglobin occurred at all doses in males and females. Absolute heart weight significantly decreased 10.6 - 15.6% in males and 8.2 – 9.2% in females at ≥ 0.25 mg/kg/day, but relative heart weight was not significantly decreased and decreased heart weight lacked correlative histopathology. However, CPK was significantly elevated in the same dose groups (at ≥ 0.25 mg/kg/day liraglutide in both males and females). Decreased absolute weight of thyroid up to 31 % and decreased relative weight up to 30% lacked correlative microscopic pathology. Injection site reactions of hemorrhage and panniclulits occurred at a slightly higher incidence in the 1 mg/kg/day high dose groups compared to controls. Liraglutide

did not increase the incidence of micronucleated erythrocytes in peripheral blood and bone marrow from rats treated for 4 weeks and it had no substantive effect on liver CYP450 content. Anti-liraglutide antibodies were not detected.

A 13-week repeat sc dose study of 0, 0.1, 0.25, or 1 mg/kg/day liraglutide in rats (10/sex/dose) yielding human exposure multiples of 0.9, 3, and 14, respectively, included control and high dose recovery groups (5/sex/dose, 4 week recovery) and toxicokinetic satellite groups (5/sex/dose). There were no treatment-related unscheduled deaths. The MTD was 1 mg/kg/day liraglutide, the highest dose tested. The NOAEL was 0.25 mg/kg/day liraglutide based on clinical signs of thin appearance (males only) and hunched posture at 1 mg/kg during the first week of treatment and rolling / high stepping gait and piloerection at 1 mg/kg/day that persisted in high dose recovery group males and females. No target organs were identified. Absolute heart weight dose-dependently decreased 11.4 – 17.0% in males at ≥ 0.1 mg/kg/day, but relative heart weight was not significantly changed (normalized to body weight) and decreased absolute weight lacked correlative histopathology findings. However, CPK was higher than controls in week 13 in all dose groups, but the increase was not statistically significant at any dose. Food consumption was transiently decreased at all doses in males and at ≥ 0.25 mg/kg/day in females. In males, dose dependent decreased body weight gain and body weight occurred at ≥ 0.25 mg/kg/day, but body weight gain was not affected in females. During recovery, body weight gain was 132% higher in high dose recovery group males compared to controls. Compared to concurrent controls at the end of treatment, the incidence of abnormal sperm was similar in high dose males.

In a 26-week chronic sc dose toxicity study of 0, 0.1, 0.25, or 1 mg/kg/day liraglutide in rats (15/sex/dose) yielding human exposure multiples of 0.6, 2, and 8, respectively, there were no unscheduled deaths considered treatment-related. The MTD was 1 mg/kg/day liraglutide and the NOAEL was 0.25 mg/kg/day based on clinical signs of toxicity at 1 mg/kg/day. Liraglutide dose-dependently decreased body weight gain up to 19.1 % and body weight compared to controls was up to 13.6% lower in males at all doses. Food consumption transiently decreased for up to 14 days at all doses in males and at ≥ 0.25 mg/kg/day in females. Decreased food consumption and decreased body weight gain were considered pharmacologic effects of liraglutide. Compared to concurrent controls, absolute heart weight was 15.2 – 19.9% lower in males and 9.2 – 14.6% lower in females at ≥ 0.1 mg/kg, but relative heart weight changes were < 10% (normalized to body weight) and decreased heart weight lacked correlative histopathology. However, LDH was elevated at ≥ 0.25 mg/kg in females in week 13, but not week 25, and CPK was elevated in one high dose male in week 25 with correlative minimal focal myocarditis. In the exocrine pancreas, the incidence of up to mild acinar cell atrophy was increased at 1 mg/kg in both males and females and the incidence of minimal focal inflammation was increased in high dose females. Liraglutide did not cause cell proliferation in the pancreas or thyroid C-cells assessed by staining for proliferating cell nuclear antigen (PCNA). No proliferative thyroid C-cell lesions were noted. Injection site reactions consisting of macroscopic reddening and microscopic focal dermatitis, focal panniculitis, and subcutaneous fibrosis and hemorrhage were attributed to the vehicle.

Monkeys

In a rising sc dose tolerability study of 0.1, 0.5, 2.5, and 5 mg/kg/day liraglutide in cynomolgus monkeys (2/sex) treated for 3 days with a 4 day washout period prior to dose escalation, there were no unscheduled deaths. The MTD was 5 mg/kg/day liraglutide, the highest dose tested. Over the 4 week treatment period, body weight loss of 0.1 kg in both females (5 – 5.6% of starting body weight) was considered a pharmacologic effect. Injection site reaction characterized as enlarged lesion and thickened vein was noted in one male.

In a 14-day tolerability study of 4 mg/kg/day liraglutide sc injected once a day in monkeys (2/sex), the MTD was 4 mg/kg/day, the highest dose tested. There were no unscheduled deaths. Plasma toxicokinetic analysis showed there were no substantive sex differences in exposure. Anti-liraglutide antibodies were not identified in plasma samples. Food consumption

transiently decreased in the first week of treatment, and monkeys lost 4.5 – 10.5% of their body weight by the end of treatment. A mild regenerative anemia was characterized by decreased RBC count, hematocrit, and hemoglobin and increased reticulocytes. CPK increased 4.4 fold in one male. Injection site reaction had clinical signs of thickening from day 4 onward and necropsy findings of subcutaneous reddening.

In a 28 day repeat sc dose study of 0, 0.05, 0.5, or 5 mg/kg/day liraglutide in monkeys (3/sex/dose) yielding human exposure multiples of 0.2, 2, and 31, respectively, the NOAEL was 5 mg/kg/day, the highest dose tested. No unscheduled deaths occurred. Injection site reaction, attributed to the vehicle, was characterized by subcutaneous thickening starting in week 3 with correlative macroscopic pathology of reddening and microscopic pathology findings of subacute or chronic fasciitis, hemorrhage, and pigmented macrophages. Decreased body weight gain at $\geq$ 0.5 mg/kg/day in males and females and ~10% decreased body weight at $\geq$ 0.5 mg/kg in males and at 5 mg/kg in females had correlative decreased food consumption during the first week. Absolute and relative weight of pancreas increased 30 – 32% compared to control in males at $\geq$ 0.05 mg/kg/day, but the increase lacked correlative histopathology.

In a 13 week repeat sc dose study of 0, 0.05, 0.5, or 5 mg/kg/day liraglutide in monkeys (4/sex/dose) with control and high dose 2 week recovery groups (2/sex), liraglutide plasma levels measured in predose and 8 hour post-dose confirmed drug exposure in liraglutide-treated groups on study day 1 and in weeks 6 and 13, but toxicokinetic parameters were not determined. Low levels of liraglutide up to 0.85 nM were detected prior to dosing on day 1 in 4/12 control group monkeys and 1/8 monkeys in the 0.05 mg/kg/day liraglutide group, but the source of drug in plasma from untreated monkeys was not identified. There were no unscheduled deaths. The NOAEL was < 0.05 mg/kg/day based on injection site reactions and increased blood eosinophils at 0.05 mg/kg/day in females. In males, increased eosinophils and injection site reaction occurred at 5 mg/kg/day. Injection site reaction was characterized by clinical signs of subcutaneous thickening with macroscopic pathology findings of reddening and thickening and correlative microscopic chronic active fasciitis. Chronic fasciitis had fibrosis and mononuclear cell infiltrate which occurred in all dose groups including controls, but in affected liraglutide treated monkeys, chronic fasciitis was characterized as active with localized edema and multifocal perivascular infiltration of lymphocytes and eosinophils and increased blood eosinophils. Chronic fasciitis and increased blood eosinophils were not fully reversed in high dose recovery females, and in this group hemorrhage at the injection site occurred at the end of recovery. A mild anemia occurred at 5 mg/kg/day and in high dose males, it persisted in the recovery period accompanied by a regenerative response. Alkaline phosphatase dose-dependently decreased at $\geq$ 0.05 mg/kg/day in males and females and the decrease persisted in high dose recovery groups, but the specific isozyme affected was not identified. Decreased body weight gain and lower body weight compared to controls occurred at 5 mg/kg/day in males and at $\geq$ 0.5 mg/kg/day in females. Food consumption transiently decreased during the first week of treatment in all dose groups. Decreased food consumption and decreased body weight gain were considered pharmacological effects, and decreased body weight was reversed at the end of recovery. No anti-liraglutide antibodies were detected in plasma from monkeys treated for 13 weeks.

In a 52-week repeat sc dose toxicity study of 0, 0.05, 0.5, or 5 mg/kg/day liraglutide in monkeys (4/sex/dose) with control and high dose 4-week recovery groups (2/sex/dose), human exposure multiples were 1, 9, and 73, respectively. There were no unscheduled deaths. The NOAEL was < 0.05 mg/kg/day based on injection site reactions in males and females and inflammatory cell infiltrates in kidney (females) or stomach pylorus (males) at $\geq$ 0.05 mg/kg. The incidence of thickened injection site with correlative subcutaneous inflammatory cell infiltration increased with liraglutide dose at $\geq$ 0.05 mg/kg/day. In the high dose group, subcutaneous sclerosis, foreign material, and foreign body giant cells also occurred at injection sites. Although treatment-related injection site reactions persisted after a 4 week recovery period, the severity was diminished. Dose-dependent increased relative weight of pancreas (normalized to body weight)

occurred at ≥ 0.05 mg/kg/day in males (53 – 111%) and at ≥ 0.5 mg/kg/day in females (46 – 79%) with correlative increased mass of exocrine cells and ducts at 5 mg/kg/day in females. Relative weight of heart increased 23 – 49% in males at ≥ 0.05 mg/kg/day, but the increase lacked correlative histopathology. Given the magnitude of relative organ weights increases in males, the 6 – 24% lower body weight in liraglutide groups compared to control does not account for the much larger change in organ weights. Relative increased weight of heart and pancreas were diminished in high dose recovery groups further indicating that the changes were treatment-related. Decreased absolute and relative weight of thymus (normalized to body weight) had correlative pathology findings of atrophy at ≥ 0.5 mg/kg/day in males. Retrospective analysis of thyroid tissue samples showed liraglutide did not cause C-cell proliferation (measured by PCNA staining) and it did not cause diffuse or focal C-cell hypertrophy (measured by quantitative analysis of calcitonin immunoreactive cells). A reversible mild anemia (decreased RBC count, hemoglobin, and hematocrit) occurred at ≥ 0.5 mg/kg/day from week 26 to the end of treatment, and a regenerative response occurred in high dose groups in week 52. Increased total bilirubin at ≥ 0.5 mg/kg/day in males and at 5 mg/kg/day in females suggests anemia may be hemolytic. In week 52, ALP was decreased in all male liraglutide groups and in females at 5 mg/kg/day, but the isozyme decreased was not identified. Decreased body weight gain and body weight compared to controls in males at ≥ 0.5 mg/kg/day had correlative transiently decreased food consumption, but it only occurred on the first day of treatment. Anti-liraglutide antibodies cross-reacting with GLP-1 were confirmed in 3 high dose group monkeys (2 males and 1 female).

Genetic toxicology:

Liraglutide was negative in a bacterial reverse mutation assay in the absence or presence of rat liver S9 metabolic activation, a chromosomal aberration assay using human peripheral blood lymphocytes from adult female volunteers in the absence or presence of rat liver S9 metabolic activation, a bone marrow erythrocyte micronucleus assay in male rats treated for 4 days with up to 30 mg/kg/day liraglutide, and a bone marrow erythrocyte assay in male and female rats treated for 28 days with up to 1 mg/kg/day liraglutide.

Carcinogenicity:

Carcinogenicity was assessed in 2-year lifetime exposure studies of subcutaneously administered liraglutide injected once a day in CD-1 mice and Sprague Dawley rats. Liraglutide was a nongenotoxic, multisex, multispecies carcinogen causing thyroid C-cell tumors in male and female mice and rats at low multiples of human exposure and fibrosarcomas in male mice on the dorsal skin and subcutis, the body surface used for drug injection. Human exposure multiples based on plasma $AUC_{0\text{-}24h}$ ratio were calculated using exposure from rats or mice in week 104 (average of male and female combined) and steady state exposure at the MRHD of 1.8 mg/day. Fibrosarcomas in the dorsal skin and subcutis are likely related to locally high concentrations of liraglutide and not systemic exposure. Liraglutide concentration in high dose drug formulation was 0.6 mg/mL liraglutide, 10-fold lower than the concentration in the clinical formulation (6 mg/mL).

Rats and mice are different with respect to their propensity to develop proliferative thyroid C-cell lesions and their age of susceptibilty to GLP-1R agonist effects on C-cells. In rats, the incidence of proliferative C-cell lesions increases with age with diffuse hyperplasia progressing to focal hyperplasia, then benign adenomas. Due to increased C-cell mass in rats, plasma calcitonin levels also increases with age. However, malignant C-cell carcinomas are rare in Sprague Dawley rats (incidence < 1%). In mice, the incidence of spontaneous or drug-induced C-cell diffuse hyperplasia, focal hyperplasia, adenomas, or carcinomas is low (< 1%). Unexpectedly, young adult mice were susceptible to liraglutide-induced C-cell focal hyperplasia, but young adult rats were not. High dose liraglutide causes C-cell focal hyperplasia within 4 weeks in 6 – 8 week old mice ('young adults') and 8 month old rats ('middle-age'), but liraglutide

does not cause C-cell focal hyperplasia in rats < 8 months old, even after treating 2 month old rats with high dose liraglutide for 6 months. C-cell tumors appear to be a pharmacologic class effect due to persistent GLP-1R activation. Recent results from repeat dose toxicity studies and preliminary results from carcinogenicity studies show in addition to causing C-cell tumors in rats, persistent GLP-1R activation causes focal hyperplasia and C-cell tumors in mice.

Mice

A 104-week carcinogen bioassay of 0, 0.03, 0.2, 1, or 3 mg/kg/day liraglutide 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). Liraglutide doses of 0.03, 0.2, 1, or 3 mg/kg/day yielded human exposure multiples of 0.2, 2, 10, and 45, respectively. Exposures were not corrected for slightly higher protein binding in mice compared to humans, which would decrease calculated human exposure mulitples by ~3-fold. 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. Tumor analysis combined results from both main study and week 78/104 groups. The NOAEL for C-cell tumors in mice was 0.2 mg/kg/day liraglutide.

Liraglutide treatment-related neoplastic findings occurred in thyroid C-cells (males and females) and dorsal skin and subcutis (males). Liraglutide 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 and carcinomas at $\geq$ 1 mg/kg/day in females. Group mean plasma calcitonin was higher than controls at $\geq$ 0.2 mg/kg/day in males and females, the same dose causing focal C-cell hyperplasia or C-cell tumors in mice. Between weeks 26 and 104, plasma calcitonin levels increased $\geq$ 2 fold at 3 mg/kg/day in females. Fibrosarcomas on the dorsal skin and subcutis occurred at 3 mg/kg/day liraglutide in males. 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 liraglutide in males ($p < 0.001$). There were equivocal finding of dose-related dorsal skin and subcutis rhabdomyosarcoma and injection site fibrosarcoma in males, which exceeded the historical control range for both tumors, but the increased incidence for either finding never reached statistical significance by pair-wise comparison with controls..

Anti-liraglutide antibodies were not detected in mice treated with up to 3 mg/kg/day liraglutide for 26, 52, 78, or 104 weeks. Liraglutide had no sustained effect on body weight or food consumption in CD-1 mice. A mild hemolytic anemia occurred at $\geq$ 0.2 mg/kg/day NNC 90-1770 characterized by decreased RBCs (males and females), increased reticulocytes (males), pigmented liver Kupffer cells (males and females), and hemosiderin in spleen (females). Treatment-related necropsy findings were a low incidence of masses in thyroid of 3 mice in the 3 mg/kg/day liraglutide. Masses occasionally occurred in the bones, heart, intestine, duodenum, and cecum of other mice. Treatment-related non-neoplastic histopathology findings occurred in thyroid (inflammatory cell infiltrate occurred at 0.03 mg/kg/day and focal C-cell hyperplasia, a precursor to tumors, occurred at $\geq$ 0.2 mg/kg/day in males and a females), liver (pigmented Kupffer cells, centrilobular hypertrophy, and diffuse centrilobular hepatocyte vacuolation occurred at $\geq$ 0.03 mg/kg/day in males and pigmented Kupffer cells at $\geq$ 1 mg/kg/day in females), spleen (hemosiderin accumulation at $\geq$ 0.03 mg/kg/day in females), femoro-tibial joint (degenerative disease at $\geq$ 0.03 mg/kg/day in males and at 0.03, 1, and 3 mg/kg/day in females), seminal vesicles (lymphocytic infiltration at $\geq$ 0.03 mg/kg/day and inflammation at 3 mg/kg/day), and thymus (tubular cystic hyperplasia at $\geq$ 0.03 mg/kg/day in males and at $\geq$ 0.2 mg/kg/day in females).

Rats

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, multiples of human exposure of 0.5, 2, and 8, respectively. Survival was unaffected by treatment. Anti-liraglutide antibodies were not assessed in this study, but sustained decreased body weight gain in liraglutide treated group compared to controls indicates if antibodies did occur, they were not neurtalizing. The NOAEL for C-cell tumors in rats was < 0.075 mg/kg/day liraglutide.

Treatment-related neoplastic findings occurred in thyroid C-cells (males and females). Liraglutide caused thyroid C-cell tumors in male and female rats, and tumors were considered a progression of focal hyperplasia to benign adenomas, and adenomas to malignant carcinomas. Liraglutide dose-dependently increased the incidence of thyroid C-cell adenomas at ≥ 0.25 mg/kg/day in males and at ≥ 0.075 mg/kg/day in females, increased the incidence of C-cell carcinomas at 0.75 mg/kg/day in males, and increased total combined C-cell adenomas or carcinomas at ≥ 0.25 mg/kg/day in males and at ≥ 0.075 mg/kg/day in females. 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 concurrent and historical controls at ≥ 0.075 mg/kg/day in males and at ≥ 0.25 mg/kg/day in females. Focal C-cell hyperplasia, considered a precursor to tumors, occurred at ≥ 0.075 mg/kg/day in males and at ≥ 0.25 mg/kg/day in females.

Consistent with its pharmacologic activity, liraglutide decreased food consumption, body weight gain, and body weight. Liraglutide dose-dependently decreased group mean body weight compared to controls up to 19.6% in males and up to 23.9% in females at ≥ 0.075 mg/kg. Despite the relatively large decrease in body weight gain and lower body weight compared to controls at higher doses, survival wasn't affected. Clinical signs of toxicity were hunched posture and piloerection at ≥ 0.25 mg/kg/day in females and staining of fur near the injection site at all doses in both sexes. Treatment-related non-neoplastic histopathology findings only occurred in the thyroid (focal C-cell hyperplasia).

Reproductive and Developmental Toxicology

The effect of subcutaneously injected liraglutide on reproduction was assessed in a 13-week toxicity study evaluating sperm from treated Sprague Dawley rats, combined fertility and embryofetal development studies in Sprague Dawley rats, embryofetal development studies in New Zealand White rabbits, and a multigeneration prenatal and postnatal study in Sprague Dawley rats. Although liraglutide cause inflammation in seminal vesicles in a carcinogenicity study of CD-1 mice and decreased the absolute weight of seminal vesicles, prostate, and epididymis in repeat-dose studies in male rats, it did not affect fertility or the incidence of sperm abnormalities in rats. In rats, liraglutide increased the incidence of early embryonic deaths, decreased fetal weight, and it caused fetal malformations and variations, but in the absence of a dose-response or a larger number of affected litters, the relation to treatment for malformations was equivocal. In an embryofetal development study in rabbits, liraglutide exposures were lower than human exposure at all doses tested. Liraglutide decreased fetal weight of rabbits and it caused fetal malformations and variations. In a multigeneration prenatal and postnatal toxicity study in rats, liraglutide slightly delayed parturition, decreased F_0 maternal body, decreased body weight of the F_1 generation during nursing, but it had no significant effect on body weight in the F_2 generation.

Fertility and Early Embryofetal Development

In a dose range-finding combined fertility and embryofetal development toxicity study of subcutaneously injected 0, 0.1, 0.25, or 1 mg/kg liraglutide in Sprague Dawley rats administered to males 4 weeks prior to mating and during the mating period and administered to females from 2 weeks prior to mating to gestation day 17 (study 980186), human exposure multiples were 0.9, 3, and 11, respectively. The paternal and maternal NOAELs were < 0.1 mg/kg with clinical signs

of toxicity (hunched posture, rolling gate) at ≥ 0.1 mg/kg. Body weight gain and food consumption were transiently decreased in all liraglutide groups compared to controls, but these were considered pharmacological effects. The NOAEL for reproductive toxicity was 1 mg/kg in both males and females. There were no effects on mating performance, fertility, or pregnancy performance or necropsy findings related to treatment. Three fetal abnormalities were noted: a dam in the 0.1 mg/kg group had a fetus with a thread-like tail and absent anus and a second fetus had an absent tail and possible absent anus. A dam in the 0.25 mg/kg group had a fetus with subcutaneous edema, shortened head, and slightly shortened tail. Due to the low incidence and absence of a dose response, the relation to treatment for fetal abnormalities was equivocal. Toxicokinetic parameters showed there were no substantive differences between mated rats and unmated rats from a 13 week study.

In a definitive combined fertility and embryofetal development toxicity in Sprague Dawley rats (study 990284) using the same doses and dosing schedule, multiples of human exposure were 0.9, 3, and 11 for doses of 0.1, 0.25, and 1 mg/kg liraglutide, respectively (based on exposure in study 980186). The NOAEL for reproductive toxicity in males was 1 mg/kg. The NOAEL for reproductive toxicity in females was 0.25 mg/kg based on increased early embryonic deaths at 1 mg/kg. The NOAEL for maternal toxicity was 0.25 mg/kg liraglutide with clinical signs of toxicity (hunched posture, rolling gate) at 1 mg/kg. In parental rats, body weight gain and food consumption were transiently decreased in all liraglutide groups compared to controls, but these were considered pharmacological effects. By gestation day 20, there were no significant treatment-related differences in body weight or body weight gain in pregnant dams. There were no effects on mating performance, fertility, or necropsy findings related to treatment, but the incidence of early embryonic deaths increased in the 1 mg/kg high dose group. Absolute weight of seminal vesicles decreased compared to concurrent controls at ≥ 0.25 mg/kg and absolute weight of prostate and epididymides decreased at 1 mg/kg, but decreased absolute weight of male reproductive organs was due, at least in part, to lower body weight. Sperm count in epididymides or testes and sperm viability was not determined in this study, but in a 13-week repeat sc dose toxicity study in SD rats, 1 mg/kg liraglutide had no effect on the incidence of abnormalities in eosin-stained sperm from the cauda epididymis (one thousand sperm/rat in study 980189). The NOAEL for fetal toxicity was < 0.1 mg/kg liraglutide based on fetal abnormalities of displaced kidneys, azygous vein displaced, and small additional ossified area within the cranial structure or fontanel at ≥ 0.1 mg/kg, and at 1 mg/kg liraglutide. A more complete state of ossification compared to controls occurred in liraglutide treated groups. Fetal malformations of misshaped oropharynx and narrowed opening of the larynx occurred in 3 fetuses in one low dose litter and umbilical hernias occurred in 2 rats in 2 different litters (one at 0.1 mg/kg liraglutide and a second at 0.25 mg/kg), but the relation to treatment for major abnormalities was equivocal because of the low incidence and lack of a dose-response.

In rabbits, maternal toxicity of liraglutide was evaluated in 2 dose range-findings studies; one in unmated female New Zealand White rabbits and a second in mated females. In study 980187, unmated female rabbits (2/dose) were subcutaneously injected with 0.4 mg/kg liraglutide for 3 days (group 1, dose level 1), 0.1 mg/kg for 2 days (group 2), 0.02 mg/kg for 13 days (group 3), 0.01 mg/kg for 13 days (group 4), or 0.1 mg/kg for 7 days (group 1, dose level 5) (study). The dose of minimal toxicity in unmated female rabbits was 0.1 mg/kg liraglutide based on the severity of decreased food consumption and decreased body weight at 0.4 mg/kg.

In a second dose-range findings study, mated females rabbits (6/dose, study 980188) were dosed with 0, 0.01, 0.03, or 0.1 mg/kg liraglutide on gestation days 6 – 18 (day of mating was gestation day 0). Study parameters were clinical signs, body weight, food consumption, toxicokinetics, and on gestation day 22, gross necropsy of adult females, external examination of fetuses for visible anomalies, and recording of litter and fetal weight. There were no unscheduled deaths. The duration and severity of transiently decreased food consumption with decreased fecal output and decreased body weight was liraglutide dose-related at all doses. During treatment, no

body weight gain occurred at 0.1 mg/kg and only slight body weight gain occurred at ≤ 0.03 mg/kg. Mean litter mean fetal weight was 8.0% lower than concurrent controls at 0.1 mg/kg. The dose of minimal toxicity in pregnant rabbits was ≥ 0.1 mg/kg.

In a definitive embryofetal development toxicity study of subcutaneously injected 0, 0.01, 0.025, or 0.05 mg/kg liraglutide administered from gestation days 6 to 18 in New Zealand White rabbits (20/dose) with terminal sacrifice on GD day 29, human exposure multiples were estimated at 0.2, 0.3, and 0.5, respectively, based on plasma exposures from dose-range finding studies (studies 980187 & 980188). The NOAEL for maternal toxicity was 0.05 mg/kg liraglutide, the highest dose tested. In mated females, there were no treatment-related unscheduled deaths, clinical signs of toxicity, or necropsy findings. Decreased food consumption with corresponding transiently decreased body weight, body weight gain, and fecal output at ≥ 0.01 mg/kg were considered pharmacological effects of liraglutide. The NOAEL for fetal toxicity was < 0.01 mg/kg, the lowest dose tested, based on decreased fetal weight compared to controls, increased incidence of total fetal malformations in individual fetuses and litters (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 liraglutide, respectively), malformations (microophthalmia with or without retinal folds, forelimb flexure, right kidney represented by a small area of tissue with an attached cyst, curved scapula) and variations (bilobed or bifurcated gall bladder, intermediate lung lobe absent, jugal fused to maxilla, superior angle of lamina of axis incompletely ossified, , slight downward pelvic shift, slight asymetric alignment of pelvic bones) at 0.01 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.

Prenatal and Postnatal Development

In a multigeneration prenatal and postnatal developmental toxicity study in rats, doses of 0 (vehicle), 0.1, 0.25, or 1 mg/kg liraglutide injected subcutaneously in pregnant Sprague Dawley rats from gestation day 6 to shortly after weaning F_1 litters (~ lactation day 24) yielded estimated multiples of human exposure of 1, 3, and 11, respectively (rat toxicokinetic analysis performed in study 980186). The F_0 generation was injected with liraglutide from gestation day 6 to weaning or lactation day 24, whichever came first. The F_1 generation was exposed to liraglutide *in utero* and while nursing, but since liraglutide is a lipidated peptide with limited oral bioavailability, systemic exposure after oral ingestion was probably negligible. The F_2 generation was not exposed to the drug.

The F_0 maternal NOAEL was < 0.1 mg/kg with clinical signs of toxicity (hunched posture, piloerection, wet coat) at ≥ 0.1 mg/kg liraglutide. Decreased body weight, body weight gain, and food consumption were considered pharmacologic effects of liraglutide in F_0 dams. Between gestation days 6 and 9, liraglutide dose-dependently decreased food consumption and body weight gain at ≥ 0.1 mg/kg and decreased body weight at ≥ 0.25 mg/kg with body weight significantly lower than controls at all doses. Decreased body weight gain was transient, but decreased body weight persisted to gestation day 20 at ≥ 0.25 mg/kg. Maternal post partum body weights (lactation day 1) were significantly lower than controls in all liraglutide groups, but by the end of the 24 day lactation period, only group mean body weight in 1 mg/kg dams was significantly lower. Necropsy revealed a low incidence of scabbing at or near the injection site in 1 mg/kg dams. The NOAEL for F_0 reproductive toxicity was < 0.1 mg/kg liraglutide based on a dose-related increased incidence of continuing gestation to day 22 (33%, 58%, 67%, and 96% of

dams delivering on day 22 at 0, 0.1, 0.25, and 1 mg/kg liraglutide, respectively, had) with increased gestation duration from 21.3 to 22.0 days at 1 mg/kg. F_0 generation reproductive parameters unaffected by treatment included number of implant sites, number of pups/litter born and the number of pups/litter surviving to lactation day 21.

The F_1 generation NOAEL was < 0.1 mg/kg liraglutide based on significantly decreased body weight compared to controls from postnatal day 7 to week 16 in males and from postnatal day 7 to week 10 in females. Liraglutide had no effect on viability of live born pups, lactation, or the overall survival of pups from birth to lactation day 21. Litter weight of all liraglutide groups was lower than controls from lactation days 14 to 21. Exposure to liraglutide during development and prior to weaning (in maternal milk) affected body weight and body weight gain in rats. Although there were no significant differences in body weight between control and treated groups (maternal F_0 treatment) on lactation day 1, body weight was significantly and dose-dependent lower than controls in all liraglutide treated groups from lactation day 7 to day 21. Food consumption was not measured. Prior to weaning, there were no treatment-related effects on postnatal physical development or functional development. In F_1 generation weaned rats, liraglutide had no effect on the time to onset of sexual maturity. Clinical signs of bleeding scabs and agitated behavior occurred in 1 mg/kg group males. Body weight was significantly lower than controls in males at $\geq$ 0.1 mg/kg between postnatal week 4 and week 16 in males and significantly lower than controls in females at 0.1 and 1 mg/kg between weeks 4 and 10. Through most of the gestation period (days 1 – 14, but not day 20) and lactation period (days 1 – 14), body weight of F_1 females was significantly lower than controls at 0.1 and 1 mg/kg, and between lactation days 7 to 14, body weight in all liraglutide dose groups was lower than controls. Liraglutide didn't affect functional development of weaned rats assessed by open field, rota-rod, or multiple Y-maze tests or the mating performance of mated F_1 male and female rats or the reproductive indices of females. Low incidence of scabbing and focus on the uterine horns were found at necropsy.

The F_2 generation was not exposed to liraglutide, and there were no differences in litter survival indices or significant differences in group mean litter weights or body weights of male or female pups. Group mean average pup weights were consistently lower throughout the lactation period (lactation day 1 to day 14) in males and females descended from F_0 rats treated with 1 mg/kg liraglutide, but differences were not statistically significant.

Local Tolerance

In the mouse carcinogenicity study, 3 mg/kg/day liraglutide injected sc into the dorsal surface caused fibrosarcomas on the dorsal skin and subcutis in males using a dosing formulation with a liraglutide concentration that was 10-fold lower than the concentration of liraglutide in the clinical formulation. Irreversible injection site reactions consisiting of inflammation and fibrosis occurred in the 52-week chronic toxicity study in 5 mg/kg treated high dose monkeys using a liraglutide dosing solution that was 3-fold more dilute than the clinical formulation.

Local tolerance after single subcutaneous injections of liraglutide was evaluated in 3 separate studies in female SPF pigs determining injection site reactions 2 and 5 days post-injection. In these studies, 200 µL of test article was injected using a NovoPen injection device with a 28G needle. The injection volume tested in pigs was 100 µL less than the injection volume for the MRHD; 300 µL. Injections were on the dorsal surface with all treatments given to each animal in the study. Study 980185 evaluated tolerability of liraglutide formulation using a vehicle containing _____ that was used in the early phases of clinical development. Study 203294 compared tolerability of 2 different formulations; the clinical phase 2 formulation using a vehicle containing _____ and a phase 3 formulation replacing _____ Study 204291 evaluated phase 3 formulations containing propylene glycol, but adjusted to different pHs; 7.7, 7.9, or 8.15. Inflammation at the injection site, attributed to the vehicle, occurred 2 and 5 days after dosing in all 3 studies. There

b(4)

were no substantive differences in injection site reactions at sites injected with liraglutide or vehicle alone and no substantive differences between vehicles.

In study 980185, 0 (vehicle containing [redacted] 0 ([redacted]), 0 ([redacted] and 5 mg/kg liraglutide were administered to female pigs on study days 1 and 4 and necropsied on day 6, there were no substantive differences in injection site reactions between liraglutide, vehicle, or saline injection sites. Subacute inflammation at injection sites was characterized by minimal to slight cellular infiltration of macrophages with or without granulocytes and minimal necrosis 2 days after dosing. Five days after dosing, cellular infiltrate consisted of macrophages and epitheloid / giant cells with slight formation of collagen tissue and fat necrosis.

Study 203294 compared local tolerability of a phase 2 formulation (containing [redacted] and 5 mg/mL liraglutide, [redacted] and phase 3 formulations (replacing [redacted] and containing 6.25 mg/mL liraglutide, pH [redacted] Pigs were injected with 0 (phase 2 vehicle, pH [redacted], 0 (phase 3 vehicle, pH [redacted], 0 ([redacted] 5 mg/mL liraglutide (in phase 2 vehicle), and 6.25 mg/mL liraglutide (in phase 3 vehicle, pH [redacted]). There were no substantive differences between phase 2 or phase 3 formulations of liraglutide. Injections site inflammation and hemorrhage occurred on day 2, with inflammation characterized by cellular infiltration. By day 5, injection site reaction was characterized by cellular infiltrate, formation of collagen tissue, and fat necrosis. b(4)

Study 204291 compared injection site toxicity of propylene glycol-containing phase 3 formulations at pH 7.7, 7.9, or 8.15. Five pigs were injected with 0 (vehicle, [redacted], 0 ([redacted], and 6.25 mg/mL liraglutide (pH 7.7, 7.9, and 8.15). Vehicle pH had no substantive effect on injection site reactions. Two days after dosing, minimal to slight edema, cellular infiltrate, and necrosis with or without hemorrhage occurred at sites injected with vehicle or liraglutide. Five days after dosing, up to moderate cellular infiltrate, formation of collagen, and fat necrosis with or without hemorrhage occurred at vehicle and liraglutide injection sites.

Special toxicology:

Mechanistic Studies of Liraglutide-Induced Rodent Thyroid C-cell Tumors

The applicant proposed a mode-of-action for liraglutide-induced thyroid C-cell tumors in mice and rats in an effort to determine their human relevance, and then evaluated the mode-of-action in an extensive series of mechanistic studies including *in vivo* studies in mice, rats, and monkeys. A review of mechanistic studies is attached as Appendix C.

To determine human relevance of liraglutide-induced thyroid C-cell tumors in rodents, the applicant proposed a novel GLP-1R-dependent mode of action.

1. GLP-1R agonists activate thyroid C-cell GLP-1Rs.
2. C-cell GLP-1R activation stimulates calcitonin secretion (calcitonin is a 'prehyperplasia' biomarker).
3. C-cell GLP-1R activation increases calcitonin synthesis.
4. Persistent calcitonin secretion and increased calcitonin synthesis causes C-cell hyperplasia.
5. C-cell hyperplasia progresses to C-cell tumors, including progression of benign adenomas to carcinomas.

The proposed mode of action implies diffuse C-cell hyperplasia, a physiologic response, precedes focal C-cell hyperplasia, a preneoplastic lesion, and that liraglutide causes C-cell proliferation. Drug-induced persistent calcitonin secretion leading to thyroid C-cell tumors is a novel mode of action that has not been previously demonstrated for any approved drug causing thyroid C-cell tumors in rats. Cinacalcet, a type II calcium sensing receptor agonist and a calcitonin secretagogue in rats, only transiently increases plasma calcitonin due to the counter-regulatory

effects of calcitonin-induced hypocalcemia. Cinacalcet dose-dependently decreased the incidence of thyroid C-cell adenomas in 2-year carcinogenicity studies in rats and did not cause C-cell tumors in mice. At least for cinacalcet, increased calcitonin secretion by itself is not sufficient to induce tumors.

The mode of action for liraglutide-induced thyroid C-cell tumors requires further study for 3 reasons. First, the proposed mode of action does not account for the persistence of liraglutide-induced calcitonin secretion, when it occurs, or the absence of any counter-regulatory effect of increased calcitonin that would otherwise inhibit persistent calcitonin secretion. Second, other potential modes of action have not been ruled out including either direct GLP-1R mediated transformation of thyroid C-cells, possibly through a RET-dependent mechanism. Activating mutations in RET are the most common molecular pathology in human MTCs. Third, liraglutide caused tumors in more than one tissue because in addition to C-cell tumors, it caused fibrosarcomas in the dorsal skin and subcutis in male mice, so GLP-1R-independent mechanisms of liraglutide-induced thyroid C-cell tumors should be ruled out.

The weight of evidence from mechanistic studies of liraglutide-induced thyroid C-cell tumors does not support the proposed mode of action in rats because:

1. Although published studies demonstrate GLP-1Rs in rat thyroid by autoradiographic tissue binding, GLP-1R agonist increased calcium-dependent calcitonin release from perfused rat thyroid cells, and inactivating the GLP-1R in mice reduces thyroid calcitonin transcript levels, the applicant's immunohistochemical and in situ hybridization studies did not conclusively demonstrate GLP-1Rs localized to C-cells.
2. Calcitonin was not a biomarker for liraglutide-induced thyroid tumors in rats, and there was no consistent, sustained effect of liraglutide on plasma calcitonin.
3. Liraglutide did not consistently increase thyroid calcitonin mRNA.
4. Liraglutide increased the incidence of age-dependent focal C-cell hyperplasia, but without accelerating its onset and without causing diffuse C-cell hyperplasia.
5. The incidence of liraglutide-induced thyroid C-cell tumors in rats increased with treatment duration, but required at least 7 months of treatment in both young and aged male rats. Therefore, its carcinogenic effects were independent on the incidence of focal hyperplasia, which is higher in aged rats than in young rats.

The weight of evidence from mechanistic studies of liraglutide-induced thyroid C-cell tumors do not support the proposed mode of action in mice because:

1. Immunohistochemical localization and in situ hybridization studies of GLP-1Rs in thyroid did not adequately demonstrate the receptor protein or transcript were 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 cell-type.
2. Liraglutide caused focal C-cell hyperplasia, a preneoplastic lesion, without causing proliferation of normal C-cells (diffuse hyperplasia). These results indicate liraglutide transforms normal C-cells into preneoplastic C-cells in mice, a species lacking age-related increases in either plasma calcitonin or proliferative C-cell lesions.

Liraglutide caused thyroid C-cell adenomas (benign) and carcinomas (malignant) in rats and mice and malignant fibrosarcomas in the dorsal skin and subcutis in male mice. Carcinogenicity studies in rats and mice, mechanistic studies of liraglutide-induced proliferative C-cell lesions, and clinical data are insufficient to conclude thyroid C-cell tumor findings in rodents are not relevant to human risk because:

1. Mechanistic studies did not adequately support the applicant's proposed novel mode of action for liraglutide-induced C-cell tumors in rats and mice.
2. After 26 to 28 weeks of treatment, liraglutide dose-dependently increased calcitonin in clinical study subjects, so if the proposed mode of action is correct, it may be operable in humans.

Qualification of Impurities and Degradation Products

Toxicity of liraglutide in an old formulation (drug substance from ——— and new formulation (drug substance from campaign 5A, pH 8.15) was evaluated in a 4-week repeat subcutaneous dose study in Sprague Dawley rats (10/sex/dose) using doses of 0 (vehicle) or 1 mg/kg/day liraglutide (old and new formulations). The new formulation had undergone —— degradation by storing it at ——— months prior to the study. There were no substantive differences in toxicity between old and new formulations of liraglutide. Genetic toxicity of impurities were not adequately qualified in in vitro studies, and a liraglutide dosing formulation 10-times more dilute than the clinical formulaiton caused fibrosarcomas of the skin and subcutis at or near the injection site. b(4)

2.6.6.2 Single-dose toxicity

Acute toxicity of subcutaneously or intravenously administered 0 or 10 mg/kg NNC 90-1170 was evaluated in CD-1 mice and Sprague Dawley rats. Study results are summarized in the table below. Transiently decreased body weight and food consumption in both rats and mice were considered pharmacologic effects due to GLP-1R mediated decreased gastric emptying and appetite suppression.

STUDY NUMBER	SPECIES	NNC 90-1170 DOSE (mg/kg) & ROUTE	OBSERVATIONS
980178	CD-1 Mice (5/sex/dose)	0, 10 s.c.	• MTD ≥ 10 mg/kg. • No mortality or clinical signs up to day 15. •Food consumption ↓ 70 and 81% and body weight ↓ 14 and 13% in treated males and females on day 2, recovered after day 3 (pharmacologic effects). •No necropsy findings.
980179	CD-1 Mice (5/sex/dose)	0, 10 i.v.	• MTD ≥ 10 mg/kg. • No mortality or adverse clinical signs up to day 14. •Food consumption ↓70 and 71% in treated males and females within 24 hours after dosing and body weight ↓14 and 9% in treated males and females on day 2, both recovered after day 3 (pharmacologic effects). •No necropsy findings.
980175	SD Rats (5/sex/dose)	0, 10 s.c.	• No mortality up to day 15. • Hunched appearance in all treated rats on day 2. • Food consumption ↓95 and 91% and body weight ↓ 19 and 11% in 10 mg/kg males and females 24 hours after dosing with reocvery after day 4 (pharmacologic effects). • Necropsy: hair loss on the fore and hind limbs, ventral, dorsal, and sacral regions in 4 treated males, many pale foci on the spleen of one male.
980177	SD Rats (5/sex/dose)	0, 10 i.v.	• No mortality up to day 15. • Tremors and increased breathing (noted at dosing), and piloerection, red discharge from the eyes and/or nose, stained perigenital in 10 mg/kg rats. Increased breathing and tremor in control rats. • Food consumption ↓95% on day 2 and 50% on day 3 at 10 mg/kg with ↓ body weight gain on days 2 to 4 (↓ 21, 15 and 9% in males and ↓ 22, 17 and 10% in females) with recovery after day 4 (pharmacologic effects). • No necropsy findings.

In CD-1 mice, the MTD was ≥ 10 mg/kg NNC 90-1170 administered subcutaneously or intravenously. There were no unscheduled deaths, clinical signs of toxicity, or necropsy findings.

In SD rats subcutaneously administered 10 mg/kg NNC 90-1170, no unscheduled deaths occurred. Hunched posture occurred on day 2 and hair loss occurred on the limbs, and ventral, dorsal, and sacral regions. After iv dosing with 10 mg/kg, no unscheduled deaths occurred. Piloerection, red discharge from eyes and nose, and stained perigenital area occurred in NNC 90-1170-treated rats, but there were no necropsy findings.

2.6.6.3 Repeat-dose toxicity

Mice

Study title: NNC 90-1170: 4-Week toxicity study in mice with subcutaneous administration – In vivo study and calcitonin determinations in mouse plasma

Key study findings:

- There were no treatment-related unscheduled deaths.
- The NOAEL was 5 mg/kg/day liraglutide, the highest dose tested.
- Focal C-cell hyperplasia occurring in 1/10 males in the 1 mg/kg/day NNC 90-1170 group and in 2/10 females in the 5 mg/kg/day group was reclassified as unilateral perithyroid foci of C-cells and attributed to aberrant developmental and unrelated to treatment. However, since longer duration mechanistic and repeat dose toxicity studies of NNC 90-1170 show focal C-cell hyperplasia develops in mice treated with liraglutide for ≥ 9 weeks and C-cell tumors occurred in the 104 week mouse carcinogenicity study, the impact of the distinction on safety assessment is minimal.
- Mild anemia occurred at ≥ 0.1 mg/kg/day liraglutide in females and at 1 mg/kg/day in males.
- Transiently decreased food consumption, decreased body weight, and diuresis were considered pharmacologic effects.
- Decreased urine pH in 0.1, 0.5, and 5 mg/kg/day females occurred in the absence of microscopic changes in kidneys or changes in serum BUN or creatinine.

Study no.: 203261
Module and page #: Module 4.2.3.2.1, pages 1- 428
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 24 October 2003
GLP compliance: Yes (OECD Principles of GLP, [redacted]
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch NLDP005 (6.25 mg/mL), purity 96.8 % (certificate of analysis page 89). The vehicle was 1.42 mg/ml disodium [redacted]phosphate dihydrate, 14 mg/ml propylene glycol and 5.5 mg/ml phenol, pH 7.75. b(4)

Results and Conclusions:

Prior to initiating the 28 day study, toxicity of 5 mg/kg NNC 90-1170 was evaluated in a 7-day repeat sc dose study in CD-1 mice (3/sex) monitoring body weight, food consumption, and clinical signs. Body weight and food consumption were transiently decreased and there were no clinical signs of toxicity, so the MTD was ≥ 5 mg/kg.

In a 28-day repeat sc dose study of 0 (vehicle), 0.1, 0.5, 1 or 5 mg/kg/day NNC 90-1170 (10 mice /sex/dose main study) with a toxicokinetic satellite group (15/sex/dose), study parameters were viability and clinical signs, body weight, food & water consumption, ophthalmoscopy, hematology, serum chemistry, urinalysis, toxicokinetics (day 1 and day 28), organ weight, and macroscopic and microscopic pathology. Blood and urine samples were taken in week 4.

The NOAEL was 5 mg/kg/day NNC 90-1170. No target organs were identified.

There were no NNC 90-1170 related clinical signs or changes in water consumption, opthalmoscopy parameters, or gross pathology.

One main study group (1 mg/kg male in week 4) and 3 TK group mice were sacrificed moribund. Moribund condition of the TK group mice was attributed to retro-orbital blood sampling. The poor condition of the 1 mg/kg main study male was equivocally related to treatment with clinical signs of toxicity including hunched posture, piloerection, weight loss, walking on tip toes, rolling gait, dark skin on dorsal and ventral abdomen, firm and irregularly shaped abdomen, and irregular respiration and necropsy findings of intestines distended with gas and a prolapsed penis.

Body weight was transiently decreased in NNC 90-1170 treated groups compared to controls, but by day 28, there were no significant differences in body weight or body weight gain for any NNC 90-1170 dose in males or females. Compared to controls, food consumption was transiently decreased for up to 7 days in NNC 90-1170 treated groups.

Figure 1 Group Mean Body Weight (g): Main Study – Males

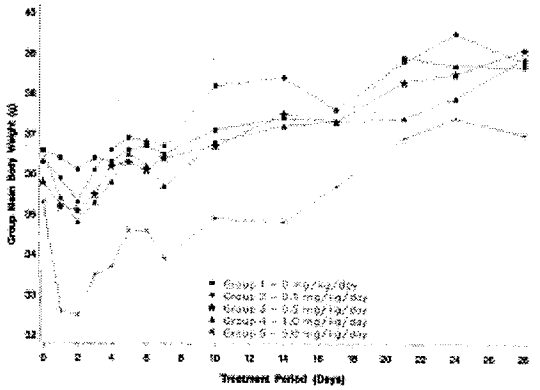
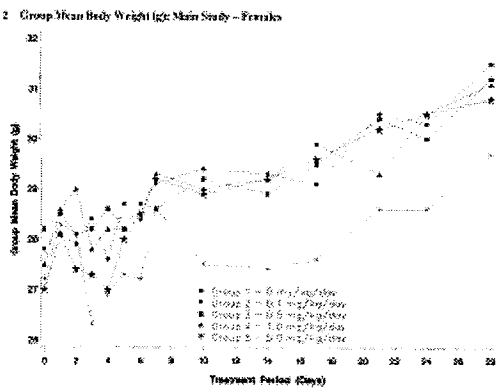


Figure 2 Group Mean Body Weight (g): Main Study – Females



[P87-88]

Liraglutide-related hematology changes were mild anemia characterized by decreased RBC parameters (RBC, Hb, Hct) in all female groups and at 1 mg/kg/day in males. Decreased RBC parameters were dose-related in females, but not in males.

Males Females

Group Dose Level mg/kg/day		Hb	RBC	Hct
1 (0)	Number	9	9	9
	Mean	14.0	9.29	0.471
	SE	0.4	0.24	0.011
2 (0.1)	Number	10	10	10
	Mean	14.4	9.25	0.475
	SE	0.3	0.23	0.011
	Prob.			
3 (0.5)	Number	9	9	9
	Mean	14.3	9.16	0.474
	SE	0.4	0.24	0.011
	Prob.			
4 (1.0)	Number	9	9	9
	Mean	13.3	8.65	0.441
	SE	0.4	0.24	0.011
	Prob.	ac	ac	ac
5 (5.0)	Number	10	10	10
	Mean	14.2	9.28	0.471
	SE	0.3	0.23	0.011
	Prob.			

Group Dose Level mg/kg/day		Hb	RBC	Hct
1 (0)	Number	10	10	10
	Mean	14.9	9.39	0.484
	SE	0.2	0.18	0.007
2 (0.1)	Number	10	10	10
	Mean	14.5	9.21	0.470
	SE	0.2	0.18	0.007
	Prob.			
3 (0.5)	Number	9	9	9
	Mean	13.8	8.60	0.444
	SE	0.2	0.19	0.008
	Prob.			
4 (1.0)	Number	10	10	10
	Mean	14.0	8.84	0.457
	SE	0.2	0.18	0.007
	Prob.	ac	ac	ac
5 (5.0)	Number	10	10	10
	Mean	13.7	8.60	0.448
	SE	0.2	0.18	0.007
	Prob.			

[P52-53]

Serum chemistry parameters changes were significantly decreased cholesterol in males at 0.1, 0.5, and 5 mg/kg NNC 90-1170. Decreased cholesterol was not considered relevant to NNC 90-1170 toxicity.

Serum Chemistry (main study n = 9 or 10/dose)

Sex	Male					Female				
NNC 90-1170 (mg/kg/day)	0	0.1	0.5	1	5	0	0.1	0.5	1	5
Parameter	Absolute Value	% Difference from Control				Absolute Value	% Difference from Control			
cholesterol (mM)	3.3	-21.2	-18.2	-9.1	-21.2	2.2	0.0	0.0	-9.1	-9.1

Statistically signficant differences from control are underlined ($p < 0.05$).

Urinalysis parameter changes were increased urine volume at $\geq$ 0.1 mg/kg/day and decreased pH in females at 0.1, 0.5, and 5 mg/kg, but in the absence of kidney pathology or changes in serum or urine ion concentrations, the relevance of this finding to NNC 90-1170 toxicity is unclear.

Urinalysis (main study n = 9 or 10/dose)

Sex	Male					Female				
NNC 90-1170 (mg/kg/day)	0	0.1	0.5	1	5	0	0.1	0.5	1	5
Volume (mL)	0.3	0.7	0.5	0.7	1.0	0.6	0.8	0.7	0.9	0.6
pH	8.5	7.6	8.4	7.8	7.6	8.5	7.4	7.4	7.7	7.1

Statistically signficant differences from control are underlined ($p < 0.05$).

Absolute spleen weight decreased up to 20% in males at 0.5 and 1 mg/kg, but in the absence of a dose response or correlative histopathology, the finding is not considered relevant to NNC 90-1170 toxicity.

Organ Weights

Sex	Male					Female				
NNC 90-1170 (mg/kg/day)	0	0.1	0.5	1	5	0	0.1	0.5	1	5
N	10	10	10	9	10	10	10	10	10	10
	Value	% Difference from Control				Value	% Difference from Control			
body (g)	37	2.7	2.7	2.7	-2.7	29	3.4	3.4	3.4	0.0
spleen g	0.11	-19.1	-20.0	-16.4	-10.9	0.11	13.6	-2.7	0.9	3.6
spleen % of bw	0.297	-21.2	-22.1	-18.6	-8.4	0.379	9.8	-6.0	-2.5	3.6

Statistically signficant differences from control are underlined ($p < 0.05$).

NNC 90-1170-related microscopic pathology findings occurred in thyroid. The following table summarizes histopathology findings in thyroid from review of the 4-week mouse study submitted to the IND. Focal C-cell hyperplasia was noted in 1 male at 1 mg/kg/day and in 2 females at 5 mg/kg/day.

Sex	Male					Female				
Doses, m/k/d	0	0.1	0.5	1	5	0	0.1	0.5	1	5
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 distension	0	0	0	0	0	0	1	1	1	1

Re-examination of thyroid findings by an expert Pathology Working Group convened by the sponsor to examine thyroid C-cell findings in mice reclassified focal C-cell hyperplasia as unilateral focal perithyroidal C-cells and identified ultimobranchial cysts in males at 1 mg/kg/day and in all dose groups in females.

HISTOLOGICAL FINDINGS	GROUP TOTALS									
	Males					Females				
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 2 0.1 mg/kg /day	Grp 3 0.5 mg/kg /day	Grp 4 1.0 mg/kg /day	Grp 5 5 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 0.1 mg/kg /day	Grp 3 0.5 mg/kg /day	Grp 4 1.0 mg/kg /day	Grp 5 5 mg/kg /day
ENDOCRINE SYSTEM										
THYROID GLAND	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
No abnormality detected	10	10	10	9	10	10	9	9	9	8
C-cells, unilateral, perithyroidal, focal	0	0	0	1	0	0	0	0	0	2
Ultimobranchial cyst	0	0	0	1	0	0	1	1	1	1
Only one examined	0	0	0	0	0	1	0	0	0	1

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

[P62]

The sponsor provided the following statement regarding thyroid C-cell findings in 3 NNC 90-1170 treated mice.

> *One male (Animal 83) from the Intermediate II dose group and two female animals (Animals 239 and 242) in the High Dose group showed a focal change*

involving C-cells as confirmed by immunohistochemical staining for the presence of calcitonin in the perithyroidal medial connective tissue of the thyroid gland. The foci were composed of ectopic C-cells and in two mice (Animals 83 and 242) the structures consisted of dilated ultimobranchial ducts with calcitonin positive cells associated with the cystic structures. In one mouse, a solid focus of calcitonin positive ectopic C-cells was located in the perithyroidal tissue. These findings were considered to represent perithyroidal accumulations of ectopic C-cells adjacent to the ultimobranchial ducts associated with the embryonic migration of these cells into the thyroid gland and therefore of developmental origin and unrelated to treatment.

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing.

Table 1 Summary of TK parameter estimates in mice after single dose of NNC 90-1170

Day	Dose (mg/kg)	Gender	C_{max} (nmol/l)	t_{max} (h)	AUC (h·nmol/l)
1	0.1	Male	75.07	8	1155
		Female	74.74	4	990
		Mean	74.90	6	1073
		SD	0.23	2.8	117
	0.5	Male	522.50	6	6891
		Female	666.50	4	4883
		Mean	594.50	5	5889
		SD	101.82	1.4	1416
	1	Male	1246.00	4	15554
		Female	1132.00	4	12345
		Mean	1189.00	4	13950
		SD	80.61	0	2269
	5	Male	6505.50	4	100125
		Female	5145.50	6	64378
		Mean	5825.50	5	82252
		SD	961.67	1.4	25277

Table 2 Summary of TK parameter estimates of NNC 90-1170 in mice at steady-state

Day	Dose (mg/kg)	Gender	C_{max} (nmol/l)	t_{max} (h)	AUC_τ (h·nmol/l)
28	0.1	Male	72.36	4	992
		Female	58.60	8	632
		Mean	65.48	6	812
		SD	9.73	2.8	255
	0.5	Male	410.40	8	5462
		Female	307.30	8	3411
		Mean	358.85	8	4436
		SD	72.90	0	1460
	1	Male	1308.05	6	12758
		Female	552.73	8	6509
		Mean	930.39	7	9634
		SD	534.10	1.4	4418
	5	Male	5712.50	4	71417
		Female	4340.00	4	43601
		Mean	5026.25	4	57609
		SD	970.50	0	19527

[P243]

Study title: NNC 90-1170: 13 Week toxicity study in mice with subcutaneous administration

Key study findings:

- No treatment-related unscheduled deaths occurred.
- The NOAEL was < 0.2 mg/kg/day NNC 90-1170 in male and female mice based on minimal to mild thyroid C-cell focal hyperplasia and ultimobranchial cysts at ≥ 0.2 mg/kg.
- Thyroid was a target organ. Thyroid tissue sections from main study and 13 week TK group mice were stained for calcitonin to identify C-cells. The description of hematoxylin-eosin stained C-cells were consistent with focal hyperplasia (slightly enlarged, pale eosinophilic-stained cells with granular cytoplasm and round to oval nuclei)
- A mild anemia occurred in all liraglutide groups.
- Injection site reactions were attributed to the vehicle because the incidence and severity of microscopic inflammation was similar between control and high dose groups.
- Transiently decreased body weight and transiently decreased food consumption during the first week of treatment were considered pharmacologic effects of NNC 90-1170 (decreased gastric emptying and appetite suppression).
- Anti-liraglutide antibodies were not detected.

Study no.: 204082
Module and page #: Module 4.2.3.2.1, pages 1- 458
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 10 March 2004
GLP compliance: Yes (OECD Principles of GLP, [redacted]
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch PQ50102 (6.25 mg/mL), purity 97.04 % (certificate of analysis page 89)

Methods

Doses: 0 (vehicle), 0.2, 1, 5 mg/kg/day NNC 90-1170

Rationale for dose selection: Doses were selected based on results from a 4 week repeat dose toxicity study showing decreased body weight gain at 1 and 5 mg/kg in both sexes and transiently decreased food consumption at 5 mg/kg in males and at 0.5, 1, and 5 mg/kg in females.

Species/strain: Crl:CD-1® (ICR) mice
Number/sex/group: The study design is summarized in the table below.
10/sex/dose main study
28/sex/dose satellite toxicokinetic groups
15/sex/dose antibody study

Group	Treatment (mg/kg/day)		Animal Numbers					
			Main Study		Satellite Study		Antibody Study	
			Males	Females	Males	Females	Males	Females
1	Control	0	1-10	183-192	11-38	193-220	39-53	221-235
2	Low	0.2	54-63	236-245	64-91	246-273	92-96	274-278
3	Intermediate I	1	97-106	279-288	107-134	289-316	135-139	317-321
4	High Dose	5	140-149	322-331	150-177	332-359	178-182	360-364

[P18]

Route, formulation, volume: subcutaneous injection (rotating between at 2 sites on the back) once a day, 6.25 mg/mL NNC 90-1170 solution in vehicle (1.42 mg/ml disodium ———— phosphate dihydrate, 14 mg/mL propylene glycol, and 5.5 mg/ml phenol), 5 mL/kg. b(4)
Age: ~5 weeks (on arrival, ~7 weeks at first dose)
Weight: 19.9 – 31.5 g males, 21.1 – 29.4 g females
Sampling times: Orbital sinus blood for toxicokinetic analysis was collected from isoflurane anesthetized TK satellite group mice (2/dose/timepoint) on study day 1 and in week 13 prior to dosing and 1, 2, 4, 6, 8, and 24 hours after dosing.
Unique study design and Protocol deviations
Anti-NNC 90-1170 antibodies were detected using a screening assay precipitating Ig bound [^{125}I]liraglutide.

Results:

Mortality:

No treatment-related unscheduled deaths occurred. A female in the toxicokinetic control group female was found dead from an unknown cause in study week 7.
Clinical signs: *Observed daily with detailed physical examinations once a week for main study mice.*

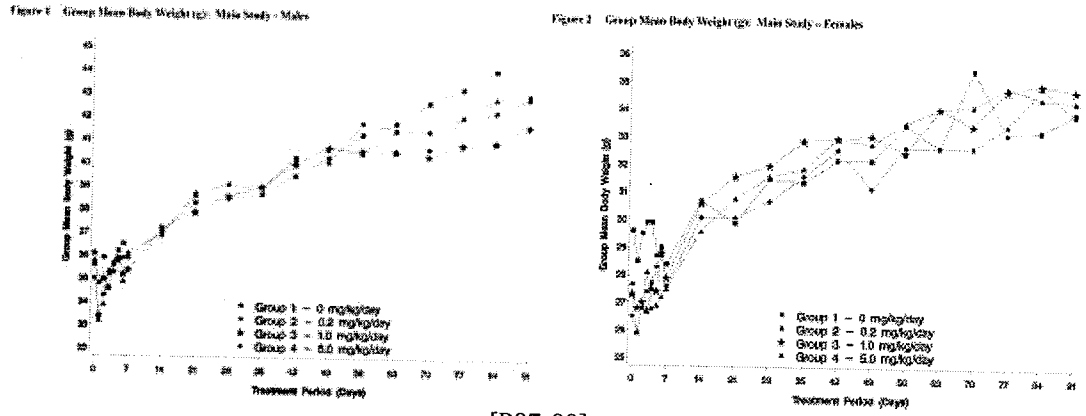
There were no clinical signs considered treatment-related.
Body weights: *Weighed daily, but reported once a week.*

NNC 90-1170 transiently and significantly decreased body weight during the first week of treatment at ≥ 0.2 mg/kg in males and at 5 mg/kg in females. By the end of treatment, there were no significant differences in body weight and body weight gain between control and NNC 90-1170 group males. Body weight was significantly higher than controls in females at 0.2 and 1 mg/kg, but this difference was attributed in part to usually low body weight gain in control group females during the dosing period (2.2 g weight gain for control group females).

Treatment Period

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.2	1	5	0	0.2	1	5
Parameter									
Body weight	N (day 91)	10	10	10	10	10	10	10	10
	g, day 0	36.6	36.0	36.1	36.8	29.6	26.5	27.3	27.7
	g, day 91	43.0	43.5	41.8	42.7	31.8	35.5	35.4	34.5
	% difference from control, day 91	0.0	1.2	-2.8	-0.7	0.0	11.6	11.3	8.5
Body weight gain (day 0 to day 91)	g	6.4	7.5	5.7	5.9	2.2	9.0	8.1	6.8
	% of pretest body weight	17	21	16	16	7	34	30	25
	% difference from control	0.0	17.2	-10.9	-7.8	0.0	309.1	268.2	209.1

Statistically significant differences from control are underlined ($p < 0.05$).



[P87-88]

Food and water consumption: *Measured and recorded once weekly. Water consumption was monitored by visual inspection.*

Food consumption was transiently decreased ~10% at 1 mg/kg and ~18% at 5 mg/kg during the first week of treatment. Food consumption in NNC 90-1170 treated groups was similar to controls from week 2 onward.

Ophthalmoscopy: *Anterior, lenticular, and fundic areas of both eyes were examined by indirect ophthalmoscopy for all mice in week 13 for all main study mice and all control and surviving satellite group high dose group mice. Examinations were performed after instilling a mydriatic (1% tropicamide).*

There were no treatment-related findings.

Anti-NNC 90-1170 antibody levels: *Orbital sinus blood from isoflurane anesthetized mice was collected from 10/sex/control group prior to treatment and from 5/sex/dose 3 days after the last dose in week 13. Anti-NNC 90-1170 antibodies were assessed using a validated method by precipitating Ig-bound radioactivity from plasma incubated overnight with [^{125}I]liraglutide (SOP 878-LP-08006).*

Anti-liraglutide antibodies were not detected in blood samples from control or liraglutide treated mice.

Hematology: *Orbital sinus blood from isoflurane anesthetized main study mice was collected prior to necropsy. Parameters were RBC, Hct, Hb, RDW, MCV, MCH, MCHC, plat, retic, total and differential WBC. Coagulation parameters were PT and APTT.*

A mild anemia occurred in all liraglutide-treated groups. Red cell band distribution width significantly decreased 7% at 0.2 and 5 mg/kg in females. Minimally decreased RBC parameters (RBC count, Hb, Hct, MCV, and RDW) occurred in all dose group and both sexes, but the changes weren't dose-related.

Select Hematology Parameters (week 13, n = 10/group)

Sex	Male				Female			
NNC 90-1170 (mg/kg/day)	0	0.2	1	5	0	0.2	1	5
	Absolute Value	% Difference from Control			Absolute Value	% Difference from Control		
RBC (x 10^12/L)	9.02	-5.9	-12.4	-4.9	8.93	-3.1	-5.9	-5.9
Hb (g/dL)	13.9	-3.6	-9.4	-3.6	14.0	-0.7	-2.9	-2.9
Hct (L/L)	0.464	-5.2	-10.1	-4.5	0.453	-1.5	-3.1	-2.9
Ret (%)	3.4	11.8	23.5	-2.9	4.0	-27.5	-17.5	-20.0
RDW (%)	12.9	2.3	6.2	0.8	14.2	-7.0	-5.6	-7.0

Statistically significant differences from control are underlined ($p < 0.05$)

Coagulation parameters were unaffected by treatment.

Serum chemistry: *Orbital sinus blood from isoflurane anesthetized main study mice was collected prior to necropsy. Parameters were AST, ALT, ALP, GDH, total bilirubin, gluc, urea, creatinine, Ca, Na, K, P, total protein, albumin, globulin, A/G ratio,chol, TG.*

There were no toxicologically relevant treatment-related changes. At the end of treatment, cholesterol (-21.2 to -30.3%) and triglycerides (-40.0 – 41.4%) were significantly decreased at ≥ 1 mg/kg in males and total protein was increased 7.8% at 5 mg/kg in females. Total protein was significantly increased at 5 mg/kg in females. Decreased cholesterol and triglycerides may be a pharmacologic effect of liraglutide, but it's notable that control group levels in males were substantially higher than in females. Although total protein was increased, in the absence of evidence of dehydration, the biological relevance was equivocal.

Serum Chemistry (main study n = 10/dose)

Sex	Male				Female			
NNC 90-1170 (mg/kg/day)	0	0.2	1	5	0	0.2	1	5
Parameter	Absolute Value	% Difference from Control			Absolute Value	% Difference from Control		
Cholesterol (mM)	3.3	-12.1	-30.3	-21.2	2.0	0.0	10.0	5.0
TG (mM)	2.10	-13.3	-41.4	-40.0	0.95	6.3	37.9	-16.8
Total protein (g/L)	52	1.9	-3.8	0.0	51	0.0	0.0	7.8

Statistically signficant differences from control are underlined ($p < 0.05$).

Urinalysis: *Urine was collected from mice in control and high dose groups on day 1 and from all main study mice in week 13. Mice were placed in a metabolism cage with free access to water and deprived of food immediately after dosing for an 8 hour collection period. Parameters were volume, specific gravity, Ca, phosphate, Mg, pH, Na, K, Cl.*

Transient changes in urinalysis parameters occurred after the first dose, but none were evident in week 13. After the first dose, urine volume, sodium, chloride, and phosphate were increased and specific gravity, magnesium, potassium, and calcium were decreased.

Table 10 Urinalysis: Main Study: Day 1
Group Mean Values: Males

Group Dose Level mg/kg/day		S.G.	UVol	U.pH	UMg	UNa	UK	UCl	UCa	UPhos
1 (0)	Number	9	9	9	9	7	7	7	9	9
	Mean	1.027	0.3	6.2	7.78	16.0	117.54	35.4	1.68	10.67
	SE	0.002	0.1	0.2	0.80	7.5	9.86	9.8	0.19	2.81
4 (5.0)	Number	10	10	10	10	10	10	10	10	10
	Mean	1.015	1.1	6.3	4.17	74.8	75.15	57.9	1.13	12.70
	SE	0.002	0.1	0.2	0.76	6.2	6.25	6.2	0.18	2.66
	Prob.	cc	cc		cc	cc	cc	cc	cc	

Sexes tested combined (c) and significantly different from the control: ac P<=0.05, bc P<=0.01, cc P<=0.001.

Table 12 Urinalysis: Main Study: Day 1
Group Mean Values: Females

Group / Dose Level mg/kg/day		S.G.	UVol	U.pH	UMg	UNa	UK	UCl	UCa	UPhos
1 (0)	Number	9	10	6	6	6	6	6	6	6
	Mean	1.027	0.2	7.8	8.08	37.2	100.57	52.7	3.00	13.80
	SE	0.002	0.1	0.4	1.01	6.6	9.58	9.5	0.40	6.59
4 (5.0)	Number	10	10	10	10	10	10	10	10	10
	Mean	1.017	0.8	7.7	4.78	95.4	67.04	88.5	1.10	19.13
	SE	0.002	0.1	0.3	0.79	5.4	7.42	7.4	0.31	4.05
	Prob.	cc	cc		cc	cc	cc	cc	cc	

Sexes tested combined (c) and significantly different from the control: ac P<=0.05, bc P<=0.01, cc P<=0.001.

[P58, 60]

Organ weights: *Paired organs weighed separately, but total organ weight was reported.*

There were no biologically relevant organ weight changes. A small decrease in absolute weight of submaxillary salivary glands lacked correlative histopathology findings.

Organ Weights, Main Study

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.2	1	5	0	0.2	1	5
N		10	10	10	10	10	10	10	10
		Value	ference from Co			Value	ference from Co		
body (g)		42	0.0	-4.8	-2.4	31	0.0	3.2	3.2
brain	g	0.52	-1.9	-1.9	1.9	0.52	0.0	-1.9	-1.9
	% of bw	1.238	-1.9	3.0	4.4	1.677	0.0	-5.0	-5.0
salivary gland	g	0.30	-8.1	-10.1	-10.0	0.16	-5.8	-6.2	-9.3
	% of bw	0.725	-8.1	-5.6	-7.9	0.520	-5.8	-9.1	-12.1

Statistically significant differences from controls are underlined ($p < 0.05$).

Gross Pathology and Histopathology: *Tissues collected for microscopic examination are shown in the histopathology inventory table. Tissues were fixed in 10% neutral buffered formalin. Optic nerve and eyes were fixed in Davidson's fluid. Tissue sections were stained with eosin and hematoxylin, and examined microscopically. Thyroid and trachea from week 13 satellite toxicokinetic group mice were evaluated microscopically. Tissue sections from the left thyroid gland from main study and week 13 TK satellite group mice were immunohistochemically stained for calcitonin to identify C-cells.*

Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X), except microscopic pathology of thyroid was internally peer reviewed by a qualified experienced veterinary pathologist and findings were presented to a Pathology Working Group. The PWG reviewed thyroid gland histopathology from subchronic toxicity studies of liraglutide in mice. Consensus diagnosis presented in the report are in accordance with conclusion from the PWG.

There were no treatment-related gross pathology findings.

The first histopathology table (below) shows the incidence of histopathology findings in main study mice only. The second table shows thyroid histopathology findings in main study and week 13 TK group mice combined.

An increased incidence of minimal to mild thyroid C-cell hyperplasia occurred at ≥ 0.2 mg/kg in males and females for both main study only or main study and week 13 TK groups combined. The following text from the report describes thyroid C-cell hyperplasia.

> "[C-cell hyperplasia] *was characterized by an increase in the distribution of C-cells among the follicles near the centre of the gland, extending towards the outer margins where they occurred in larger numbers than seen in controls. The increased number of C-cells was more obvious in the slides stained for calcitonin as compared to sections stained with haematoxylin and eosin (H & E). Animals with the greatest increase in the number of C-cells had on H & E sections, slightly enlarged pale eosinophilic-staining cells with granular cytoplasm and round to oval nuclei situated within the thyroid follicles between the basement membrane and the basal region of follicular cells, or in the interfollicular region.*"
>
> [P35]

Based on the description of hemotoxylin-eosin stained thyroid, this narrative describes focal C-cell hyperplasia, not diffuse hyperplasia.

In main study mice, the incidence of ultimobranchial cysts was above control group levels at ≥ 0.2 mg/kg in males and at 0.2 and 5 mg/kg in females. When data from main study and week 13 TK groups were combined, the increased incidence occurred at ≥ 0.2 mg/kg in both sexes. Ultimobranchial cysts were coincident with C-cell hyperplasia, and it was not known if the association was due to the ultimobranchial cysts becoming more conspicuous or less involution of ultimobranchial bodies.

HISTOLOGICAL FINDINGS	GROUP TOTALS							
	Males				Females			
GROUP DOSE	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	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
KIDNEY	(10)			(10)	(10)			(10)
Tubular regeneration	1			6	2			3
PANCREAS (EXOCRINE)	(10)			(10)	(10)			(9)
No abnormality detected	9			10	9			8
Acinar cell degranulation	0			0	1			0
Lobular atrophy, with inflammation, chronic active	1			0	0			0
INJECTION/TREATMENT SITE	(10)			(10)	(10)			(10)
No abnormality detected	2			2	3			1
Inflammation	7			7	7			9
Inflammation and necrosis, needle track	1			1	1			0
Squamous cell hyperplasia	2			1	0			1
Pigmented macrophages, localised	0			0	1			0
Fibrosis, dermis, localised	2			0	0			0
THYROID GLAND	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
No abnormality detected	9	5	3*	3*	8	6	8	6
C-cell hyperplasia								
minimal	0	3	3	2	0	2	2	2
mild	0	0	0	2	0	0	0	2
Total incidence	0	3	3	4	0	2	2	4
Ultimobranchial cyst	1	3	7*	5	2	4	0	3

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

[Compiled from Table 18]

Summary of Histological Findings: Main Study and Satellite Study Animals Combined

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		(23)	(24)	(24)	(24)	(23)	(23)	(24)	(24)
No abnormality detected		21	9***	9***	4***	17	10	12	8**
Ultimobranchial cyst		2	10*	12**	17***	6	12	8	11
C-cell hyperplasia									
minimal		0	9**	8**	6*	0	8**	10***	8**
mild		0	0	0	4	0	0	0	5*
Total Incidence		0	9**	8**	10***	0	8**	10***	13***

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

[P36]

Injection site microscopic pathology findings were similar between control and high dose groups indicating any affect of treatment was due to the vehicle.

Toxicokinetics: *Plasma NNC 90-1170 was quantified using a validated ELISA with a LLOQ of 0.09 nM.*

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing. NNC 90-1170 concentrations peaked between 1 to 8 hours after dosing.

Table 1 Summary of TK parameter estimates in mice after single dose of NNC 90-1170

Dose (mg/kg)	Gender	C_{max} (nmol/l)	t_{max} (h)	AUC (h*nmol/l)
0.2	Male	142	4.0	2229
	Female	179	6.0	1636
	Mean	160	5.0	1932
	SD	26	1.4	420
1.0	Male	975	4.0	13549
	Female	1017	4.0	11298
	Mean	996	4.0	12424
	SD	30	0.0	1592
5.0	Male	4670	8.0	73336
	Female	7127	4.0	74765
	Mean	5899	6.0	74051
	SD	1737	2.8	1011

Table 2 Summary of TK parameter estimates of NNC 90-1170 in mice at steady-state (week 13)

Dose (mg/kg)	Gender	C_{max} (nmol/l)	t_{max} (h)	AUCτ (h*nmol/l)
0.20	Male	145	4.0	1876
	Female	247	8.0	2042
	Mean	196	6.0	1959
	SD	72	2.8	117
1.00	Male	1012	1.0	11854
	Female	1320	6.0	18592
	Mean	1166	3.5	15223
	SD	218	3.5	4764
5.00	Male	5927	4.0	74212
	Female	6289	4.0	62857
	Mean	6108	4.0	68534
	SD	256	0.0	8029

[P255]

Summary and Conclusions

In a 13 week repeat subcutaneous dose toxicity study of 0, 0.2, 1, or 5 mg/kg/day NNC 90-1170 in CD-1 mice (10/sex/dose) with satellite anti-NNC 90-1170 antibody groups (15/sex/dose) and a toxicokinetic satellite group (28/sex/dose), there were no mortalities considered treatment-related. Because proliferative thyroid C-cell lesions were noted in NNC 90-1170 toxicity studies in mice, thyroid microscopic pathology was determined for main study and 13 week TK group mice with results peer reviewed by a qualified veterinary pathology and considered by a Pathology Working Group. The NOAEL was < 0.2 mg/kg NNC 90-1170 in male and female mice based on minimal to mild thyroid C-cell hyperplasia and ultimobranchial cysts at ≥ 0.2 mg/kg. Thyroid was a target organ.

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing. Anti-NNC 90-1170 antibodies were not detected in any NNC 90-1170 treated mice.

There were no toxicologically relevant changes in body weight, body weight gain, food or water consumption, ophthalmoscopy parameters, coagulation parameters, serum chemistry parameters, organ weight or macroscopic pathology.

A mild anemia, characterized by decreased RBC, Hb, Hct, and RDW occurred at all liraglutide doses.

The incidence of minimal to mild thyroid C-cell hyperplasia and ultimobranchial cysts generally increased with dose at ≥ 0.2 mg/kg NNC 90-1170 in males and females. The sponsor did not differentiate between diffuse and focal C-cell hyperplasia, but these descriptions suggests it was focal.

Injection site reactions occurred in both control and high dose groups at similar frequencies and severity, so it was attributed to the vehicle.

Rats

Study title: NNC 90-1170: 7 Day subcutaneous dose range findings study in rats (Study 980180)

Key study findings:

- In a 7 day repeat sc dose study, the MTD was 1 mg/kg/day NNC 90-1170 based on moribund sacrifice of rats within 2 days of dosing at ≥ 2 mg/kg with clinical signs of toxicity (piloerection, decreased fecal output, rolling gait/high stepping gait, hunched posture, dark extremities, and thin appearance), severe body weight loss, and inappetence.

- The NOAEL was 0.25 mg/kg based on clinical signs of toxicity, decreased body weight gain, and decreased food consumption throughout the treatment period at ≥ 0.4 mg/kg.
- Decreased fecal output and transiently decreased body weight gain and food consumption were considered pharmacological effects of NNC 90-1170 and not considered adverse at ≤ 0.25 mg/kg.
- NNC 90-1170 toxicity was greater in males than females, but the differences were not due to differences in plasma exposure.
- Anti-NNC 90-1170 antibodies were not detected.

Results and Conclusions

In a 7-day repeat sc dose toxicity study of 0, 0.4, 2, or 10 mg/kg/day NNC 90-1170 in SD rats (5/sex/dose) with toxicokinetic samples taken on days 1 and 7, 2 and 10 mg/kg groups were sacrificed moribund on days 2 or 3 due to clinical signs of toxicity (Table 1, including piloerection, decreased fecal output, rolling gait/high stepping gait, hunched posture, dark extremities, and thin appearance), body weight loss (Figures 1 and 2), and decreased food consumption (10 mg/kg rats didn't eat after dosing). Dosing was continued in 0 and 0.4 mg/kg groups for 7 days. Body weight, body weight gain, and food consumption were transiently decreased at 0.4 mg/kg with return to near control group levels by day 7. There were no treatment related effects on water consumption, organ weight, or necropsy findings in the 0.4 mg/kg group.

Table 1 Incidence of Selected Clinical Observations: Males and Females – Original Study

	Males (Gps) Dose Levels (mg/kg/day)				Females (Gps) Dose Levels (mg/kg/day)			
Sign/Observation	1 (0)	2 (0.4)	3 (2.0)	4 (10.0)	1 (0)	2 (0.4)	3 (2.0)	4 (10.0)
Decreased Faecal Output		5	5	5		5	5	5
Unkempt		1	2	1				
Staining			3	5		1	3	2
Piloerection			2	2		3	5	5
Rolling gait/High Stepping gait							3	1
Subdued				2			1	1
Thin /Hunched			2			1	5	4
Killed in *extremis*			5	5			5	5
Soft faeces			2				2	
Dark feet			1	3			4	3

[P3]

Figure 1 Group Mean Body Weights: Males - Original Study

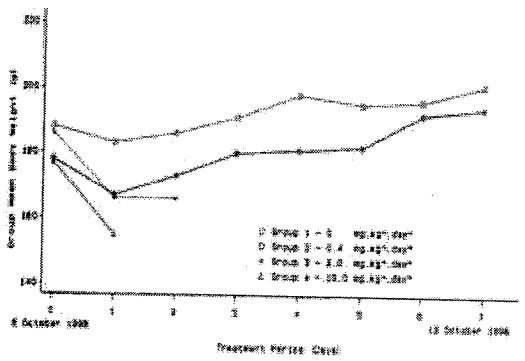
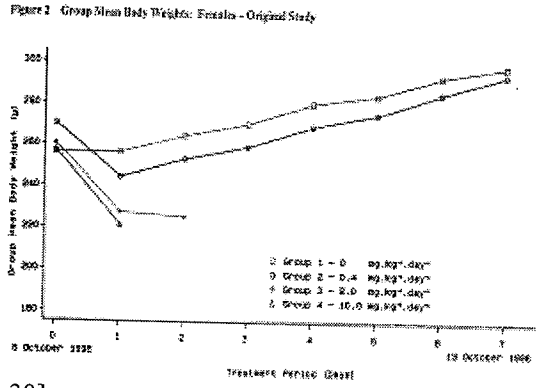


Figure 2 Group Mean Body Weights: Females - Original Study



[P29-30]

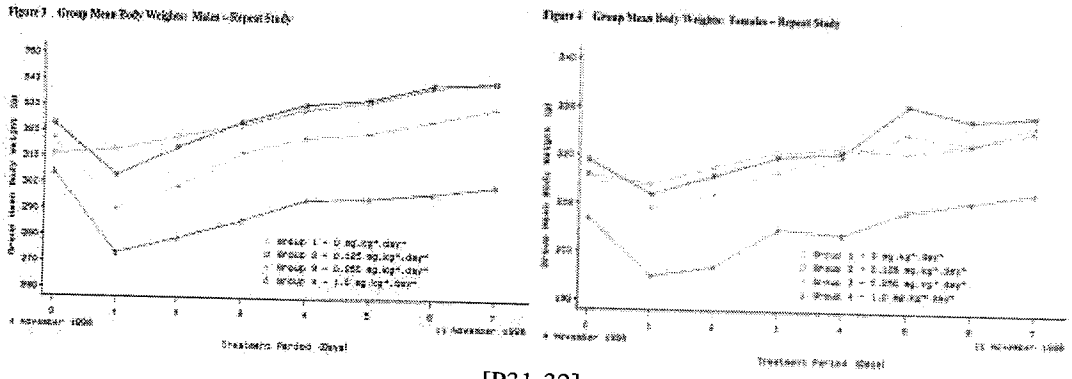
Original Study – Group Mean Food Consumption:

Group/Dose Level	Day 0 Food Cons (g per rat per day)	Day 2 Food Cons (g per rat per day)
2 (0.4 mg/kg/day)	31.34	12.80
3 (2.0 mg/kg/day)	30.30	4.68
4 (10 mg/kg/day)	30.04	0 (a)

(a) animals killed by Day 2 therefore no food consumption measurable.

[P26]

In a second 7-day repeat dose study of 0, 0.125, 0.25 or 1 mg/kg/day NNC 90-1170 in rats (5/sex/dose) using larger rats to avoid deleterious affects of transient weight loss, there were no unscheduled deaths. Decreased fecal output, decreased body weight, and decreased food consumption occurred in all NNC 90-1170 groups, but with reduced severity compared to doses ≥ 2 mg/kg. Hunched posture, piloerection, rolling / high stepping gait, and thin appearance were present at 0.25 and 1 mg/kg, but the severity was reduced compared to higher doses. At 0.125 and 0.25 mg/kg, mildly reduced body weight and food consumption were transient. At 1 mg/kg/day, body weight and food consumption were reduced throughout the treatment period, but the magnitude of the effect was diminished compared to higher doses. Anti-NNC 90-1170 antibodies weren't detected by precipitating Ig-bound radioactivity from plasma samples incubated with [^{125}I]acylated GLP-1.



[P31-32]

At necropsy, body weight was 12.3% lower at 1 mg/kg males compared to controls. Absolute heart was significantly, dose-dependently decreased up to 17.6% in males at ≥ 0.25mg/kg. Relative heart weight was not significantly different from control group males at any dose and the magnitude of the decrease was diminished due to decreased body weight, at least in part.

Organ Weights

Sex	Male				Female			
NNC 90-1170 (mg/kg/day)	0	0.125	0.25	1	0	0.125	0.25	1
N	5	5	5	5	5	5	5	5
	Value	% Difference from Control[b]			Value	% Difference from Control[b]		
body (g)	334	0.6	-4.2	-12.3	278	0.0	-1.3	-9.0
heart g	1.42	-4.2	-14.8	-17.6	1.04	-10.9	-16.8	-6.9
heart % of bw	0.425	-4.8	-11.1	-6.1	0.374	-10.9	-15.7	2.2

Statistically signficant differences from control are underlined ($p < 0.05$).

Day 1 (sc doses of 0.125, 0.25. 0.4, 1, 2, and 10 mg/kg) and day 7(sc doses of 0.125, 0.25, 0.4, and 1 mg/kg) plasma NNC 90-1170 concentration versus time profiles are shown in Figures 1 and 2 below.

Figure 1 Dose levels 0-10 mg/kg. Individual plasma concentration profiles after s.c. administration of 3 different dose levels of NNC 90-1170 /kg to male Sprague Dawley Rats.

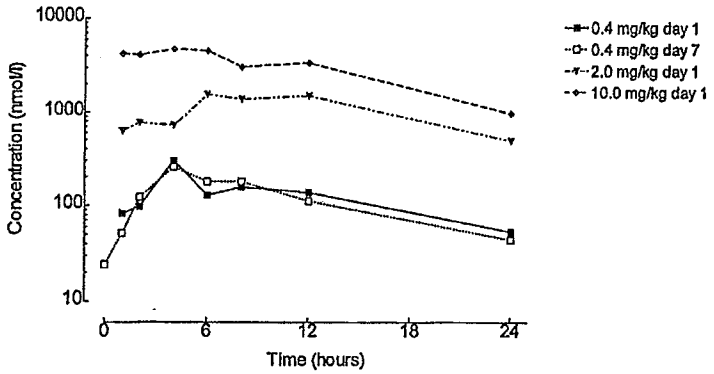
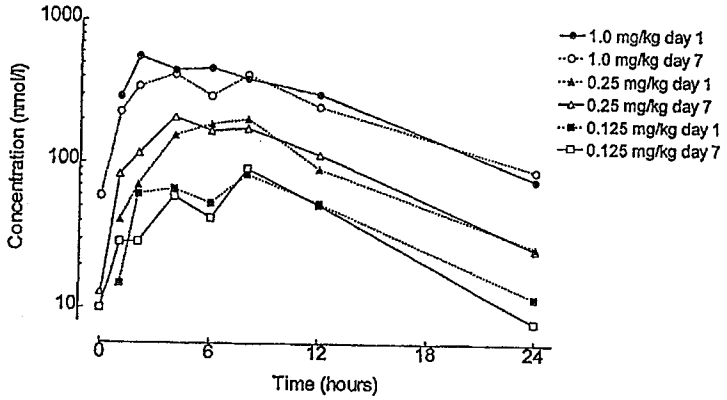


Figure 2: Dose levels 0-1 mg/kg. Individual plasma concentration profiles on day 1 and day 7 after s.c. administration of 3 different dose levels of NNC 90-1170 /kg to Sprague Dawley Rats.



[P128-9]

Study title: NNC 90-1170: 28-day subcutaneous toxicity study in rats

Key study findings:

- There were no treatment-related unscheduled deaths.
- The NOAEL was 0.25 mg/kg based on clinical signs of toxicity in males and females at 1 mg/kg/day NNC 90-1170.
- Decreased body weight, decreased body weight gain and transiently decreased food consumption were considered pharmacologic effects (decreased gastric emptying and appetite suppression).
- A mild anemia occurred at $\geq$ 0.1 mg/kg/day.
- Absolute heart weight was significantly decreased compared to controls at $\geq$ 0.25 mg/kg in males and females. The relevance to NNC 90-1170 toxicity was equivocal because although there was no significant effect on relative heart weight at any dose and no

correlative histopathology findings, CPK was significantly elevated in both males and females at the same doses, ≥ 0.25 mg/kg/day.

- Injection site reactions were attributed to the vehicle because the incidence and severity of panniculitis was similar between control and high dose groups.
- CYP2A1 activity (testosterone 7α-hydroxylase) in liver microsomes from 1 mg/kg/day liraglutide treated males was 2 fold lower than controls.

Study no.: 980183
Module and page #: Module 4.2.3.2.1, pages 1- 313 b(4)
Conducting laboratory and location: [redacted]
Date of study initiation: 20 November 1998
GLP compliance: Yes (OECD Principles of GLP, [redacted]
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch 433-980813-01 (2 mg/mL), purity 97.9 % (certificate of analysis page 84). The vehicle was 0.71 mg/ml disodium monohydrogenphosphate dihydrate, 0.62 mg/ml monosodium hydrogenphosphate dihydrate, 38 mg/ml mannitol and 5 mg/ml phenol.

Results and Conclusions:

In a repeat sc dose toxicity study of 0 (vehicle), 0.1, 0.25, or 1 mg/kg/day NNC 90-1170 (1 mL/kg) in Sprague Dawley rats (10/sex/dose main study) with a toxicokinetic satellite group (10/sex/NNC 90-1170 dose only), study parameters were viability and clinical signs, body weight, food & water consumption, ophthalmoscopy, hematology & coagulation, serum chemistry, urinalysis, toxicokinetics (day 1 and day 28) & anti-NNC 90-1170 antibodies, peripheral and bone marrow erythrocytes for micronucleus assessment, organ weight, analysis of CYP450 activity in liver, and macroscopic and microscopic pathology. Blood and urine samples were taken in week 4. Analysis of micronuclei from peripheral and bone marrow erythrocytes were reported separately in the genetic toxicity section (study 990072). Liver CYP450 activity was reported separately.

The NOAEL was 0.25 mg/kg based on clinical signs of toxicity in males and females at 1 mg/kg/day NNC 90-1170. There were no treatment-related unscheduled deaths or biologically relevant effects on ophthalmoscopy parameters, serum chemistry parameters, urinalysis parameters, organ weight, or gross pathology. Anti-NNC 90-1170 antibodies were not detected in blood samples taken after 4 weeks of treatment.

At 1 mg/kg, clinical signs of toxicity were hunched posture, piloerection, and a rolling or high stepping gait, but these effects dissipated with continued dosing and were no longer present after the 2nd week.

Table 1 NNC 90-1170
28 Day Subcutaneous Toxicity Study in Rats
Incidence of Selected Clinical Signs

Males:

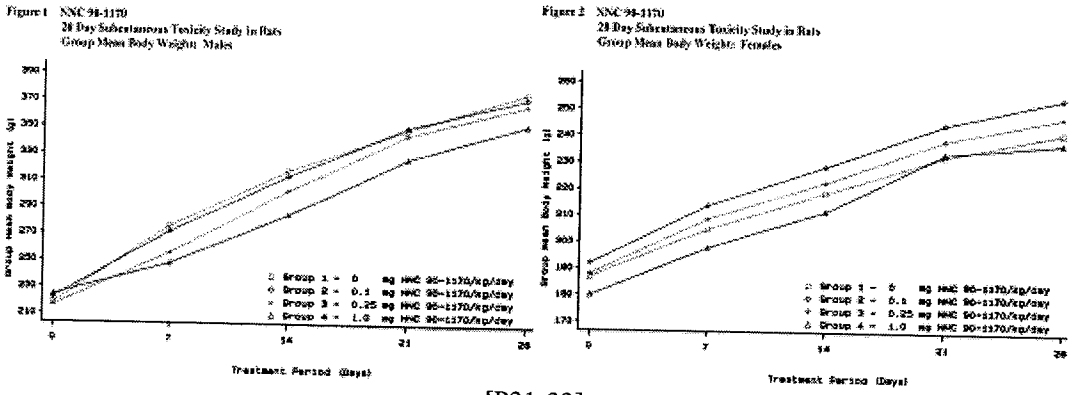
Observation/Finding	Group/Dose Level (mg/kg/day)						
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)	5 (0.1)	6 (0.25)	7 (1.0)
Number of animals in group	10	10	10	10	10	10	10
Decreased faecal output			10	10		10	10
Rolling gait				10			8
Hunched				7			6
Piloerection				6			2
Unkempt				6			3
Coat staining				2			3
High stepping gait				1			
Partial hairloss		1		2			

Females:

Observation/Finding	Group/Dose Level (mg/kg/day)						
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)	5 (0.1)	6 (0.25)	7 (1.0)
Number of animals in group	10	10	10	10	10	10	10
Decreased faecal output			10	10		10	10
Rolling gait				10			9
Hunched				9			6
Piloerection				4			7
Unkempt				2			1
Coat staining		1	1	2		1	5
High stepping gait				3			2
Partial hairloss				3	1		2

[P33]

Reduced fecal output correlated with decreased body weight, decreased body weight gain, and reduced food consumption during the first week of treatment at ≥ 0.25 mg/kg in males and at 1 mg/kg in females. These effects were transient, except decreased body weight compared to concurrent controls persisted to the end of treatment in high dose males. Decreased food consumption, decreased body weight, and decreased body weight gain were considered pharmacologic effects.



[P31-32]

A mild anemia, characterized by decreased RBC parameters (RBC count, Hct, and Hb), occurred at ≥ 0.1 mg/kg in males and females. Coagulation parameters were unaffected by treatment.

Select Hematology Parameters (DAY 28, n = 9 or 10/group)

Sex	Male				Female			
NNC 90-1170 (mg/kg/day)	0	0.1	0.25	1	0	0.1	0.25	1
	Absolute Value	% Difference from Control			Absolute Value	% Difference from Control		
RBC (x 10^12/L)	7.76	-2.8	-3.1	-3.7	7.85	-4.7	-3.7	-4.5
Hb (g/dL)	15.0	-2.7	-2.7	-2.7	14.7	-2.0	-3.4	-4.1
Hct (L/L)	0.439	-3.6	-2.5	-4.3	0.420	-2.6	-3.3	-4.0
Ret (%)	2.4	-20.8	-8.3	4.2	1.6	-6.3	-12.5	18.8
RDW (%)	12.3	0.8	2.4	4.1	11.3	0.9	2.7	5.3

Serum chemistry parameter changes were limited to CPK. CPK was significantly increased in males and females at $\geq$ 0.25 mg/kg, but the increase was not considered toxicologically relevant because of the magnitude of the change (< 2 fold) and the absence of a dose-response.

Serum Chemistry (main study n = 10/dose)

Sex	Male				Female			
NNC 90-1170 (mg/kg/day)	0	0.1	0.25	1	0	0.1	0.25	1
Parameter	Absolute Value	% Difference from Control			Absolute Value	% Difference from Control		
CPK (IU/L)	159	14.5	72.3	32.1	111	27.0	52.3	50.5

Statistically signficant differences from control are underlined ($p < 0.05$).

Absolute heart weight decreased up to 15.6% in males (dose-related) and up to 9.2% in females at $\geq$ 0.25 mg/kg NNC 90-1170. Relative heart weight decreased up to 9.8% in male and up to 10.7% in females. The diminished effect on relative heart weight in males was due to lower body weight compared to controls at $\geq$ 0.25 mg/kg. Absolute weight of thyroid was significantly decreased at 0.1 and 0.25 mg/kg in males, but it lacked correlative histopathology.

Organ Weights

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
N		10	10	10	10	10	10	10	10
		Value	% Difference from Control			Value	% Difference from Control		
body (g)		334	-1.3	-2.2	-6.5	278	4.7	1.7	-2.6
heart	g	1.42	-6.4	-10.6	-15.6	1.04	-2.0	-9.2	-8.2
	% of bw	0.425	-5.1	-8.7	-9.8	0.374	-6.4	-10.7	-5.8
thyroid	g	1.42	-28.2	-31.3	-25.3	1.04	-1.3	6.3	-11.8
	% of bw	0.425	-27.2	-29.8	-20.2	0.374	-5.7	4.5	-9.5

Statistically signficant differences from control are underlined ($p < 0.05$).

There were no NNC 90-1170 treatment related microscopic pathology findings in heart, pancreas, and thyroid. Panniculitis occurred at the injection site, but this was attributed to the vehicle because of the similar incidence and severity in control and high dose groups.

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS Males Grp 1 0 mg/kg /day	Grp 2 0.1 mg/kg /day	Grp 3 0.250 mg/kg /day	Grp 4 1.0 mg/kg /day	Females Grp 1 0 mg/kg /day	Grp 2 0.1 mg/kg /day	Grp 3 0.250 mg/kg /day	Grp 4 1.0 mg/kg /day
INJECTION/TREATMENT SITE		(10)			(10)	(10)			(10)
Haemorrhage, subcutaneous.									
minimal		0			0	0			1
mild		0			1	0			0
Total Incidence		0			1	0			1
Panniculitis.									
minimal		2			4	1			1
mild		0			0	1			2
Total Incidence		2			4	2			3

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

[P62]

In study 201075, hepatic microsomes were prepared from control and 0.1 or 1 mg/kg NNC 90-1170 treated rats (5/sex/dose) for determining activity of CYPs 1A2, 2A1, 2B1/2, 2C11, 2E1, 3A1/2 and 4A isozymes using isozyme selective probe substrates. Total CYP liver content in male and females was unaffected by liraglutide treatment. CYP2A1-mediated testosterone 7α-hydroxsylase activity was significantly decreased ~2 fold in males treated with 1 mg/kg liraglutide for 4 weeks.

Table 12 7α-OH Testosterone Formation in Male Rat Microsomes (Groups 1, 2 and 4)

Group	Animal ID	pmol 7α/ min/mg protein	Group Mean ± S.E.	pmols/min/ nmol P450	Group Mean ± S.E.	pmols/min/ g liver	Group Mean ± S.E.	nmols/min/ liver	Group Mean ± S.E.
1	1	193.75	200.00 ± 22.64	280.797	282.816 ± 35.468	4012.56	3473.10 ± 325.77	68.494	56.481 ± 5.491
1	2	182.50		260.714		4232.18		60.986	
1	3	128.75		178.819		2652.25		37.768	
1	4	235.00		293.750		3717.70		64.130	
1	6	260.00		400.000		2750.80		51.027	
2	11	255.00	199.50 ± 14.89	326.923	255.057 ± 29.318	4467.60	3430.17 ± 396.73	76.039	58.329 ± 8.668
2	12	201.25		314.453		3990.79		73.670	
2	13	170.00		236.111		2944.40		54.884	
2	14	192.50		229.167		3545.85		59.393	
2	15	178.75		168.632		2202.20		27.660	
4	31	96.36	112.32 ± 14.94*	110.753	171.440 ± 35.683	1739.21	2071.84 ± 250.96*	24.645	29.560 ± 2.642**
4	32	131.52		292.256		2840.72		37.554	
4	33	153.75		199.675		2160.19		33.505	
4	34	66.25		92.014		1355.48		23.607	
4	35	113.75		162.500		2263.63		28.431	

*= p<0.05, **= p<0.01 = Significant Difference, S.E. = Standard Error
(Calculated from Students Paired T-Test)

[Study 201075 P39]

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing.

Plasma Toxicokinetics

Parameter		AUC_{0-24} (nM.h)			Cmax (nM)		
NNC 90-1170 Dose (mg/kg/day)		0.1	0.25	1	0.1	0.25	1
Sex	Day						
Male	1	479	1,298	8,384	37	93	1,195
	28	549	1,915	11,993	44	136	413
Female	1	422	1,994	5,737	39	155	763
	28	460	2,485	6,155	32	173	371

Study title: NNC 90-1170: 13 Week subcutaneous toxicity study in rats with recovery period

Key study findings:

- 4 unscheduled deaths were not considered treatment-related.
- The NOAEL was 0.25 mg/kg/day liraglutide, based on clinical signs of thin appearance (males only) and hunched posture at 1 mg/kg high dose during the first week of treatment. Rolling / high stepping gait and piloerection noted during the high dose groups during treatment persisted in high dose recovery group males and females.
- Dose-dependent decreased absolute heart weight in males at ≥ 0.1 mg/kg was not considered adverse because there was no significant effect on relative heart weight at any dose and no correlative histopathology findings. However, CPK increased > 20% in week 13 at all liraglutide doses in males and females, but the isozyme affected was not determined.
- In males, decreased body weight, decreased body weight gain and transiently decreased food consumption were considered pharmacologic effects (decreased gastric emptying and appetite suppression).
- Body weight gain was significantly higher in high dose group recovery males compared to controls, and by the end of the 4 week recovery period, there was no significant difference in body weight between control and high dose groups.
- Injection site reactions were attributed to the vehicle because the incidence and severity of panniculitis and/or subcutaneous fibrosis was similar between control and high dose groups.

Study no.: 980189
Module and page #: Module 4.2.3.2.1, pages 1- 547
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 11 March 1999
GLP compliance: Yes (OECD Principles of GLP, [redacted])
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch 433-980813 (2 mg/mL) or 317901-01 (5 mg/mL), purity 87.6 – 87.8 % (certificate of analysis page 112 - 114)

Methods

Doses: 0 (vehicle), 0.1, 0.25, 1 mg/kg/day NNC 90-1170

Rationale for dose selection: Doses were selected based on results from a 28 day repeat dose toxicity study in rats taking into account the MTD and expected human exposure.

Species/strain: Crl:CD® (SD) IGS BR rats (Sprague Dawley)
Number/sex/group: The study design is summarized in the table below.
10/sex/dose main study
5/sex/ control and high dose recovery groups (4 weeks)
5/sex/dose satellite toxicokinetic groups

Group	Treatment (mg/kg/day)		Animal Numbers					
			Main Study		Recovery Study		Satellite Study	
			Males	Females	Males	Females	Males	Females
1	Control	0	1-10	71-80	41-45	111-115	51-55	121-125
2	Low	0.1	11-20	81-90	-	-	56-60	126-130
3	Intermediate	0.25	21-30	91-100	-	-	61-65	131-135
4	High	1.00	31-40	101-110	46-50	116-120	66-70	136-140

[P22]

Route, formulation, volume: subcutaneous injection (rotating between 4 sites on the dorsal surface) once a day (at 2 different sites if the dose volume exceeded 0.5 mL/injection), 2 - 5 mg/mL NNC 90-1170 solution in the vehicle (0.71 mg/ml disodium monohydrogenphosphate dihydrate, 0.62 mg/ml monosodium hydrogenphosphate dihydrate, 38 mg/ml mannitol and 5 mg/ml phenol), 1 mL/kg.
Age: ~7 weeks
Weight: ~85 g males, ~70 g females
Sampling times: Tail vein blood for toxicokinetic analysis was collected from 1 male and 1 female toxicokinetic group rat from each dose group (except controls) study day 1 and in week 13 prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours after dosing. Two rat/dose were bled 48 and 60 hours after dosing after the last dose in week 13.
Unique study design and Protocol deviations
Anti-NNC 90-1170 antibodies were detected using a screening assay precipitating Ig bound radiolabeled acylated GLP-1 (SOP 878-LP-07031).

Eosin-stained sperm from the cauda epididymis from control and high dose rats was examined microscopically (one thousand sperm/rat).

Results:

Mortality:
The deaths of 4 female rats during the treatment or recovery periods were not considered related to NNC 90-1170. Two rats died during the treatment period (one in the control group on day 87 and one at 0.1 mg/kg on day 87) and 2 died during recovery (one control on day 100 and 1 at 1 mg/kg on day 114).

Group	Animal No/Sex	KP/ DB*	Day of Death	Notable Findings Clinical Signs (immediately prior to death)	Major Necropsy Findings
1	4♂	KP	87	Right eye bulging, damaged and dark, right eyelids swollen and abnormally coloured, hence killed prematurely	Lesion on right eye, right eye dark and damaged, right eyelid swollen, dark skin on right eyelid, mandibular lymph nodes dark, right eye opaque.
2	17♂	DB	87	Coat stained brown on head, cage stained pink	Skin staining on head, mandibular lymph nodes enlarged, trachea contains blood, all lobes of lung spongy
1	41♂	KP	100	Firm swelling on right side of perigenital area, scabs on right side of perigenital area, lesion on right side of perigenital area, killed due to open lesion	Irregular skin lesion on right perigenital area (20x13 mm)
4	46♂	KP	114	High stepping gait, rolling gait, piloerection. Accidental injury (animal fell and was injured during routine data collection on day of week 17 bleed), hence was killed prematurely.	Urinary bladder distended by contents (20x15 mm), thymus speckled (1x1 mm)

*DB – Died at bleed, KP – killed prematurely

[P32]

Clinical signs: *Observed daily with detailed physical examinations once a week.*

Nearly half the high dose group males (4/10) were noted as thin with evident weight loss during the first few days of treatment with hunched posture occurring in high dose groups of both sexes during the first week. Rolling gait, piloerection, and high stepping gait were noted in the high dose group during treatment with clinical signs of toxicity persisting in the recovery period. Tables below show the number of rats affected (at least one occasion) in each dose group (10 /sex/dose main study, 5/sex/dose recovery).

Main Study

Observation/Finding	Group/Dose Level (mg/kg/day)							
	1♂ (0)	2♂ (0.10)	3♂ (0.25)	4♂ (1.0)	1♀ (0)	2♀ (0.10)	3♀ (0.25)	4♀ (1.0)
Rolling gait	0	0	0	10	0	0	0	10
Piloerection	0	0	0	10	0	0	0	10
High stepping gait	0	0	0	9	0	0	0	5
Hunched posture	0	0	0	5	0	0	0	5
Thin/weight loss	0	0	1	4	0	0	0	0

[P44]

Recovery Study

Observation/Finding	Group/Dose Level (mg/kg/day)			
	1♂ (0)	4♂ (0.10)	1♀ (0.25)	4♀ (1.0)
Rolling gait	0	5	0	5
Piloerection	0	5	1	5
High stepping gait	0	5	0	5

[P44]

Body weights: *Weighed daily with daily weights reported for the first week of treatment and reported weekly thereafter.*

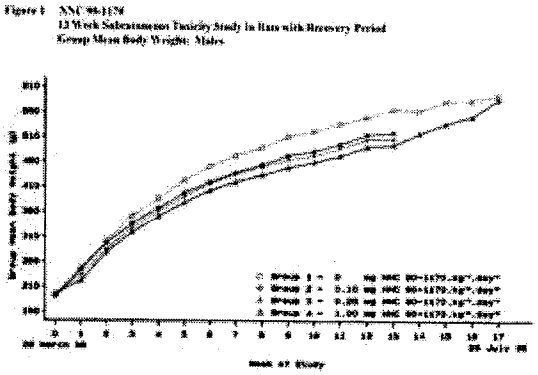
On day 91, group mean body weight compared to controls was up to 12.3% lower in males (dose-dependent) at $\geq$ 0.25 mg/kg with correlative, dose-dependent decreased body weight gain from 14.4 to 19.8% lower than controls at $\geq$ 0.25 mg/kg. Lower body weight and body weight gain in NNC 90-1170 female groups were not significantly different from controls. There were no statistically significant effects of NNC 90-1170 treatment on body weight or body weight gain in females.

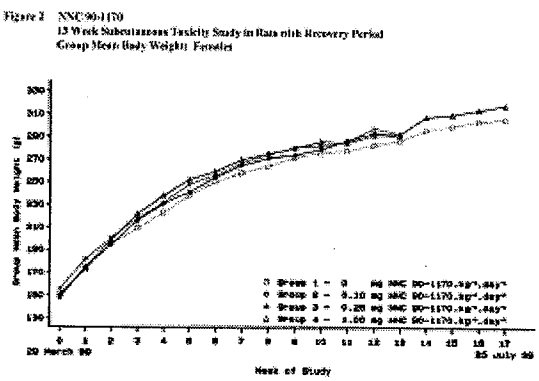
During the 4 week recovery period, body weight gain was 132% higher in high dose recovery males compared to concurrent controls.

Treatment Period

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
Parameter									
Body weight	N (day 91)	14	9	10	15	14	9	10	15
	g, day 0	193	190	192	197	152	148	156	152
	g, day 91	561	519	507	492	285	290	292	292
	% difference from control, day 91	0.0	-7.5	-9.6	-12.3	0.0	1.8	2.5	2.5
Body weight gain (day 0 to day 91)	g	368	329	315	295	133	142	136	140
	% of pretest body weight	191	173	164	150	88	96	87	92
	% difference from control	0.0	-10.6	-14.4	-19.8	0.0	6.8	2.3	5.3

Statistically significant differences from control are underlined ($p < 0.05$).





[P42-3]

Recovery Period

Sex		Male		Female	
NNC 90-1170 (mg/kg/day)		0	5	0	5
Parameter					
Body weight	N	5	5	4	4
	g, day 91	551	486	282	291
	g, day 119	594	586	306	318
	% difference from control, day 119	0.0	-1.3	0.0	3.9
Body weight gain (day 91 to day 119)	g	43	100	24	27
	% of pretest body weight	7.8	20.6	8.5	9.3
	% difference from control	0.0	132.6	0.0	12.5

Food and water consumption: *Measured and recorded twice weekly during the first 4 weeks of dosing, then weekly thereafter. Water consumption was monitored by weekly visual inspection.*

In males, food consumption was transiently decreased compared to concurrent controls up to day 3 at 0.1 mg/kg, up to day 7 at 0.25 mg/kg, and up to day 10 at 1 mg/kg. In females, food consumption was transiently, and sporadically, decreased up to day 14 at 0.25 mg/kg and up to day 10 at 1 mg/kg. Food consumption returned to control group levels by the third week. There were no significant differences in food consumption between control and high dose recovery groups during the recovery period.

Table 6 NNC 90-1170
13 Week Subcutaneous Toxicity Study in Rats with Recovery Period
Food Consumption (g.animal^{-1}.day^{-1}): Main Study ANCOVA: Males

Group/Dose Level (mg.kg^{-1}.day^{-1})		Pretrial (Days)		Treatment Period (Days)					
		-4	0	3	7	10	14	17	21
1 (0)	Number	7	7	7	7	7	7	7	7
	Mean	24.9	26.4	29.7	31.8	30.9	33.0	31.9	30.3
	SE	0.3	0.6	0.7	0.4	0.9	0.6	0.7	0.7
2 (0.1)	Number	5	5	5	5	5	5	5	5
	Mean	24.9	26.4	24.2	30.5	31.0	32.0	32.5	30.9
	SE	0.3	0.8	0.8	0.5	1.0	0.7	0.8	0.8
	Prob.	na	na	Cs					
3 (0.25)	Number	5	5	5	5	5	5	5	5
	Mean	25.0	26.4	20.2	29.2	30.2	30.7	31.5	31.0
	SE	0.3	0.8	0.8	0.5	1.0	0.6	0.8	0.8
	Prob.	na	na	Cs	bs		ac		
4 (1.0)	Number	7	7	6	7	7	7	7	7
	Mean	25.2	26.4	14.9	26.3	27.9	30.8	30.7	30.2
	SE	0.3	0.6	0.8	0.4	0.9	0.6	0.7	0.7
	Prob.	na	na	cs	cs	bc			

Sexes tested combined (c) and significantly different from the Control: ac P<0.05, bc P<0.01, cc P<0.001
Sexes tested separately (s) and significantly different from the Control: as P<0.05, bs P<0.01, cs P<0.001

na Not appropriate

Table 8 NNC 90-1170
13 Week Subcutaneous Toxicity Study in Rats with Recovery Period
Food Consumption (g.animal^{-1}.day^{-1}): Main Study ANCOVA: Females

Group/Dose Level (mg.kg^{-1}.day^{-1})		Pretrial (Days)		Treatment Period (Days)					
		-4	0	3	7	10	14	17	21
1 (0)	Number	7	7	7	7	7	6	7	7
	Mean	20.4	20.7	22.1	20.6	20.5	21.7	22.2	20.6
	SE	0.2	0.5	0.5	0.4	0.4	0.3	0.3	0.6
2 (0.1)	Number	5	5	5	5	5	5	5	5
	Mean	19.4	20.7	20.1	21.9	21.4	21.5	22.9	21.4
	SE	0.3	0.6	0.6	0.4	0.5	0.4	0.4	0.7
	Prob.	na	na						
3 (0.25)	Number	5	5	5	5	5	5	5	5
	Mean	20.5	20.7	16.4	22.3	20.3	21.1	21.7	20.8
	SE	0.3	0.6	0.6	0.5	0.5	0.4	0.4	0.8
	Prob.	na	na	Ca	as		ac		
4 (1.0)	Number	7	7	6	7	7	7	7	7
	Mean	19.9	20.7	13.2	20.3	20.1	21.6	22.6	21.8
	SE	0.2	0.5	0.5	0.4	0.4	0.3	0.3	0.6
	Prob.	na	na	cs		bc			

Sexes tested combined (c) and significantly different from the Control: ac P<0.05, bc P<0.01, cc P<0.001
Sexes tested separately (s) and significantly different from the Control: as P<0.05, bs P<0.01, cs P<0.001

na Not appropriate

[P51, 54]

There were no treatment-related differences in water consumption monitored by visual inspection.

Ophthalmoscopy: *Anterior, lenticular, and fundic areas of both eyes were examined by indirect ophthalmoscopy for all rats prior to initiating treatment and for control and high dose groups only in study weeks 12 and 16 (week 3 of recovery period). Examinations were performed after instilling a mydriatic (1% tropicamide).*

There were no treatment-related findings.

Hematology: *Orbital sinus blood (3 mL) from isoflurane anesthetized rats was collected during treatment week 13 and during week 17. Parameters were RBC, Hct, Hb, MCV, MCH, MCHC, plat, retic, total and differential WBC. Coagulation parameters were PT and APTT.*

In week 13, absolute lymphocyte count in high dose group females were elevated 60% compared to controls. At the end of recovery, lymphocyte count in high dose recovery females were similar to concurrent controls, primarily due to an increase in the control group.

Serum chemistry: *Orbital sinus blood (3 mL) from isoflurane anesthetized rats was collected during treatment week 13 and during week 17. Parameters were AST, ALT, ALP, CPK, LDH, total bilirubin, gluc, urea, creatinine, Ca, Na, K, Cl, P, total protein, albumin, globulin, A/G ratio,chol.*

In week 13, albumin was 5.6 – 8.3% lower than concurrent controls at $\geq$ 0.1 mg/kg in females. Lower albumin resulted in significantly decreased A/G ratio at 1 mg/kg. There were no statistically significant changes in protein parameters in males during treatment, but in recovery,

total protein and globulin were significantly lower in high dose recovery males compared to concurrent controls.

In week 13, CPK was elevated 29% in high dose males and 54% in mid-dose females, but the increase was not considered toxicologically relevant because of the small magnitude and absence of correlative histopathology.

Serum Chemistry (main study n = 9/10, recovery n = 4/5)

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
Parameter	Week	Absolute Value	% Difference from Control			Absolute Value	% Difference from Control		
CPK (IU/L)	13	105	161.9	21.0	28.6	144	23.6	54.2	26.4
	Recovery	109	-	-	8.3	93	-	-	18.3
Total protein (g/L)	13	75	-4.0	-2.7	-1.3	77	-2.6	-2.6	-3.9
	Recovery	72	-	-	-6.9	76	-	-	2.6
albumin (g/L)	13	32	0.0	-3.1	0.0	36	-5.6	-5.6	-8.3
	Recovery	31	-	-	-3.2	36	-	-	2.8
globulin (g/L)	13	43	-7.0	-4.7	-2.3	41	0.0	0.0	0.0
	Recovery	41	-	-	-9.8	40	-	-	2.5
A/G ratio	13	0.7	14.3	14.3	14.3	0.9	-11.1	-11.1	-11.1
	Recovery	0.8	-	-	0.0	0.9	-	-	0.0

Statistically signficant differences from control are underlined ($p < 0.05$).

Increased CPK in main study 0.1 mg/kg males was due to > 10 fold increase in rat 12. The increase occurred in the absence of correlative histopathology findings.

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)	Animal	CPK
2 (0.1)	11	101
	12	1614
	13	114
	14	139
	15	101
	16	123
	17	-
	18	92
	19	94
	20	101
	Number	9
	Mean	275
	SD	502
	P-Value[(2)]	ns

[P331]

Urinalysis: *Urine was collected from fasted, water deprived rats (selected from each dose group) in metabolism cages for 4 hours. Parameters were volume, specific gravity, protein, Labstix (pH, glucose, ketones, urobilinogen, bilirubin, blood pigments) and sediment microscopy.*

There were no biologically relevant treatment-related urinalysis parameter changes. A small magnitude, but statistically significant, decrease in specific gravity in high dose group females occurred in the absence of correlative changes in other urinalysis parameters or kidney histopathology findings. There were no treatment-related changes at the end of recovery.

Organ weights: *Paired organs weighed separately, but total organ weight was reported.*

There were no treatment-related organ weight changes. Absolute heart weight decreased 11.4 – 17.0% at ≥ 0.1 mg/kg in males. Decreased absolute heart weight wasn't considered biologically relevant because mean group heart weight wasn't significantly different from control for any NNC 90-1170 group, absolute and relative heart weight in females was unaffected by treatment, the magnitude of decreased relative heart was lower due to dose-related decreased body weight at ≥ 0.1 mg/kg, and there were no correlative histopathology findings.

Organ Weights, Main Study

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
N		9	9	10	10	10	10	10	10
		Value	% Difference from Control[b]			Value	% Difference from Control[b]		
body (g)		555	-8.6	-10.5	-13.3	278	1.8	2.5	1.8
brain	g	2.14	-1.9	-1.9	-3.7	1.92	2.1	1.0	3.1
	% of bw	0.386	7.4	9.6	11.1	0.691	0.3	-1.4	1.3
heart	g	1.76	-11.4	-17.0	-14.8	1.04	0.0	-3.8	-2.9
	% of bw	0.317	-3.0	-7.4	-1.7	0.374	-1.8	-6.2	-4.6

Gross Pathology and Histopathology: *Tissues collected for microscopic examination are shown in the histopathology inventory table. Tissues were fixed in 10% neutral buffered formalin. Optic nerve and eyes were fixed in Davidson's fluid. Testes were fixed in Bouin's and a transverse section as stained with PAS-H. Sperm from the cauda epididymis were fixed in methanol. Tissue sections were stained with eosin and hematoxylin, and examined microscopically.*

Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Sperm Analysis

There were no differences in the incidence of sperm defects between control and high dose groups.

Table 26 NNC 90-1170
13 Week Subcutaneous Toxicity Study in Rats with Recovery Period
Sperm Abnormality Assessment: Summary of Results

Animal Dose Group	No. of Rats Examined	% Abnormal Sperm			
		Including Tail Defects		Excluding Tail Defects	
		Mean	SD	Mean	SD
Vehicle Control	9	0.1	0.2	0.0	0.1
High Dose	10	0.1	0.1	0.0	0.0

[P77]

There were no NNC 90-1170 related pathology findings in heart, pancreas, or thyroid. A low incidence of panniculitis or fibrosis occurred at the injection site of control and high dose groups, but it was attributed to the vehicle because the incidence and severity were similar in control and high dose groups.

Toxicokinetics: *Plasma NNC 90-1170 was quantified using a radioimmunoassay according to SOP 878-LP-08004.*

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing.

Table 2 Pharmacokinetic parameters after s.c. administration of three different dose levels of NNC 90-1170/ kg to Sprague Dawley Rats.

Week	Gender	Dose mg/kg/day	C_{max} nmol/l	t_{max} H	AUC h·nmol/l	AUC(0-t) h·nmol/l	V_z/f l/kg	Cl/f ml/min/kg	$t_{½}$ h
1	Female	0.1	76	6	844	781	0.27	0.53	6
		0.25	307	6	2582	2356	0.26	0.43	7
		1	1159	4	13536	12591	0.16	0.33	6
	Male	0.1	62	4	776	724	0.27	0.57	5
		0.25	227	8	2721	2490	0.20	0.41	6
		1	920	4	11948	10404	0.23	0.37	7
13	Female	0.1	53	2	938	865	0.29	0.47	7
		0.25	368	4	3922	3731	0.17	0.28	7
		1	768	6	12849	12208	0.30	0.35	10
	Male	0.1	39	4	825	643	1.12	0.54	24
		0.25	144	8	2715	2447	0.55	0.41	16
		1	358	12	9840	9188	0.47	0.45	12

[P469]

Summary and Conclusions

In a 13 week repeat subcutaneous dose toxicity study of 0, 0.1, 0.25, or 1 mg/kg/day NNC 90-1170 in Sprague Dawley rats (10/sex/dose) with a 4 week recovery period (5/sex/control and high dose) and a toxicokinetic satellite group (5/sex/dose), there were 4 unscheduled deaths that were considered unrelated to treatment.

The NOAEL was 0.25 mg/kg/day NNC 90-1170 based on clinical signs of thin appearance (males only) and hunched posture at 1 mg/kg during the first week of treatment and rolling / high stepping gait and piloerection at 1 mg/kg/day that persisted in high dose recovery group males and females.

Compared to concurrent controls, body weight was dose-dependently decreased up to 13.6% and body weight gain decreased up to 19.1% at ≥ 0.1 mg/kg in males. By the end of the 4 week recovery period, body weight gain was significantly greater in the high dose recovery group males (~130%) and body weight was similar in control and high dose group males. There was no significant effect on body weight or body weight gain in females during treatment or recovery periods. Food consumption was transiently decreased during the first week of dosing at ≥ 0.1 mg/kg in males and at ≥ 0.25 mg/kg in females, but the effect was transient and it returned to control group levels by the third week. Decreased body weight gain and transiently decreased food consumption were considered pharmacologic effects (decreased gastric emptying and appetite suppression).

NNC 90-1170 had no toxicologically relevant effects on ophthalmology, hematology, coagulation, serum chemistry, or urinalysis parameters and it didn't affect organ weight, the incidence of sperm abnormalities, or macroscopic or microscopic pathology.

Decreased absolute heart weight compared to controls at ≥ 0.1 mg/kg in males was in large part attributable to lower body weight in NNC 90-1770 treated groups. Relative heart weight decreased < 10% in all treated groups. Injection site reactions were attributed to the vehicle because the incidence and severity of panniculitis and/or subcutaneous fibrosis was similar between control and high dose groups.

Study title: NNC 90-1170: 26 Week subcutaneous toxicity study in rats

Key study findings:

- 3 unscheduled deaths were not treatment-related.
- The NOAEL and MTD were ≥ 1 mg/kg/day, the highest dose tested.
- Injection site reaction consisted of macroscopic reddened injection site and microscopic focal dermatitis, focal panniculitis and subcutaneous fibrosis and hemorrhage occurred in both NNC 90-1170 and control groups with a similar incidence and severity, so they were attributed to injection of the vehicle.
- Dose-dependent decreased absolute heart weight was not considered adverse because relative heart weight decreased < 10% in all treated groups.
- In the exocrine pancreas, the incidence of up to mild acinar cell atrophy was increased at 1 mg/kg in both males and females and the incidence of minimal focal inflammation was increased in high dose females.
- There was no evidence of pancreas cell proliferation by immunohistochemical staining for PCNA and no proliferative thyroid C-cell lesions were noted.
- Decreased body weight gain and transiently decreased food consumption were considered pharmacologic effects (decreased gastric emptying and appetite suppression) and did not adversely affect the health of the rats.
- Anti-NNC 90-1170 antibodies were not assayed and pancreas weight was not reported.

Study no.: 200239
Module and page #: Module 4.2.3.2.1, pages 1- 455
Conducting laboratory and location: ——————————— b(4)
Date of study initiation: 11 December 2000
GLP compliance: Yes (OECD Principles of GLP, ———————)
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170 (5 mg/mL), batch 317011 (pH 7.4), purity 98.6 % (certificate of analysis page 94)

Methods

Doses: 0 (vehicle), 0.1, 0.25, 1 mg/kg/day NNC 90-1170

Rationale for dose selection: Doses were selected based on the MTD and anticipated humans exposure

Species/strain: Crl:CD® (SD) IGS BR rats (Sprague Dawley)
Number/sex/group: 15/sex/dose, the study design is summarized in the table below.

Group	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	
			Males	Females
1	Control	0	1-15	61-75
2	Low	0.10	16-30	76-90
3	Intermediate	0.25	31-45	91-105
4	High	1.0	46-60	106-120

[P18]

Route, formulation, volume: subcutaneous injection (rotating between 4 sites on the dorsal surface) once a day (at 2 different sites if the dose volume exceeded 0.5 mL/injection), 5 mg/mL NNC 90-1170 solution in the vehicle (0.71 mg/ml disodium monohydrogenphosphate dihydrate, 0.62 mg/ml monosodium hydrogenphosphate dihydrate, 36.9 mg/ml mannitol and 5 mg/ml phenol), 1 mL/kg.
Age: ~5 weeks

Weight: 148 206 g males, 108 – 147 g females
Sampling times: Tail vein blood for toxicokinetic analysis was collected from all main study rats on study day 1 and in week 26 prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours after dosing.
Unique study design and Protocol deviations
Dosing was suspended on day 72 due to adverse weather conditions, but this didn't affect the integrity of the study.

Cell proliferation in the pancreas was assessed by staining tissue sections from control and high dose groups with anti-PCNA antibody.

During week 26, analysis of the dosing formulation showed that samples from 0.1 mg/kg and 1 mg/kg were mislabeled. However, week 26 TK analysis shows plasma exposures in week 26 were similar to those in week 1 and exposure increased with dose. Any error in that may have occurred in the formulation analysis did not affect the integrity of the study.

Results:

Mortality:

There were 3 unscheduled deaths: one control group male (group 1), one male at 0.1 mg/kg (group 2) and one female at 1 mg/kg. None of the unscheduled deaths were considered treatment-related.

Group	Animal No/Sex	KP/DB	Week of Death	Notable Findings Clinical Signs (immediately prior to death)	Major Necropsy Findings
1	2M	KP	13	Right eye bulging, dry and damaged. Killed for humane reasons.	Right eye bulging
2	25M	KP	23	Limping, swollen right hind limb, one area of sparse hair. Killed for humane reasons.	Right hind foot enlarged
4	110F	DB	13	No abnormalities detected	All lung lobes dark, thymus pale, dark focus on thymus

DB – Died at bleed, KP – killed prematurely

Clinical signs: *Observed daily with detailed physical examinations once a week and palpation once a week from week 13 onward.*

Transient minor skin scabbing occurred in liraglutide-treated groups, but not in controls.

Body weights: *Weighed daily, but reported weekly throughout the study.*

On day 181, group mean body weight compared to controls was up to 13.6% lower in males (dose-dependent) at $\geq$ 0.1 mg/kg with correlative, dose-dependent decreased body weight gain up to 19.1% compared to controls at $\geq$ 0.25 mg/kg. Lower body weight and body weight gain in NNC 90-1170 female groups were not significantly different from controls. It's notable that day 0 group mean weights were identical for all male dose groups and all female dose groups.

Treatment Period

Parameter	Sex / NNC 90-1170 (mg/kg/day)	Male 0	Male 0.1	Male 0.25	Male 1	Female 0	Female 0.1	Female 0.25	Female 1
Body weight	N	15	15	15	15	15	15	15	15
	g, day 0	183.2	175.5	177.3	178.7	127.9	122.6	122.9	124.5
	g, day 181	639.5	562.4	554.3	539.2	344.7	323.7	318.4	319.6
	% difference from control, day 181	0.0	-12.1	-13.3	-15.7	0.0	-6.1	-7.6	-7.3
Body weight gain (day 0 to day 181)	g	456.3	386.9	377.0	360.5	216.8	201.1	195.5	195.1
	% of pretest body weight	249.1	220.5	212.6	201.7	169.5	164.0	159.1	156.7
	% difference from control	0.0	-15.2	-17.4	-21.0	0.0	-7.2	-9.8	-10.0

Statistically significant differences from control are underlined ($p < 0.05$).

Figure 1 NNC 90-1170
26 Week Subcutaneous Toxicity Study in Rats
Mean Body Weight (g): Males

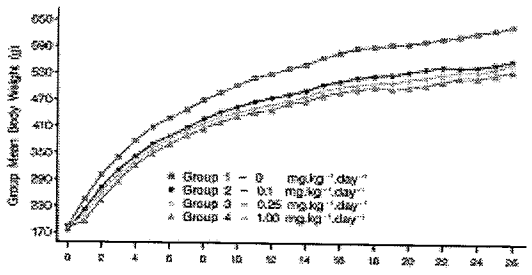
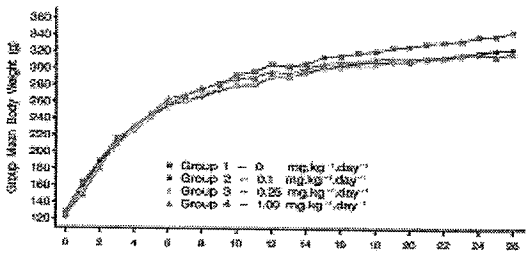


Figure 2 NNC 90-1170
26 Week Subcutaneous Toxicity Study in Rats
Mean Body Weight (g): Females



[P92-3]

Food consumption: *Measured and recorded weekly.*

There was a transient statistically significant decrease in food consumption during the first week of dosing (day 6) in males at ≥ 0.1 mg/kg and in females at ≥ 0.25 mg/kg and in the second week (day 13) in both sexes at 1 mg/kg. Food consumption returned to control group levels by the third week.

Table 4 NNC 90-1170
26 Week Subcutaneous Toxicity Study in Rats
Food consumption (Covariance Analysis): Males

Group/Dose Level (mg.kg.day⁻¹)		Pretrial (Day) -1	6	13	21	28	35
1 (0)	Number	3	3	3	3	3	3
	Mean	24.1	28.6	30.6	31.2	31.3	30.6
	SE	0.7	0.6	0.6	0.5	0.6	0.6
2 (0.10)	Number	3	3	3	3	3	3
	Mean	24.1	24.3	30.0	31.1	29.6	29.4
	SE	0.7	0.7	0.6	0.6	0.7	0.7
	Prob.		bs				
3 (0.25)	Number	3	3	3	3	3	3
	Mean	24.1	21.2	27.5	30.1	29.0	29.1
	SE	0.7	0.6	0.5	0.5	0.6	0.6
	Prob.		cs				
4 (1.00)	Number	3	3	3	3	3	3
	Mean	24.1	17.5	24.9	28.7	28.8	28.3
	SE	0.7	0.6	0.5	0.5	0.6	0.6
	Prob.		cs	as			

Sexes tested combined (c) and significantly different from the control: as P≤0.05, bs P≤0.01, cs P≤0.001
Sexes tested separately (s) and significantly different from the control: as P≤0.05, bs P≤0.01, cs P≤ 0.001

Table 5 NNC 90-1170
26 Week Subcutaneous Toxicity Study in Rats
Food consumption (Covariance Analysis): Females

Group/Dose Level (mg.kg⁻¹.day⁻¹)		Pretrial (Day) -1	6	13	21	28	35
1 (0)	Number	3	3	3	3	3	3
	Mean	17.3	20.8	22.4	20.9	22.1	22.5
	SE	0.4	0.5	2.4	1.0	1.0	0.8
2 (0.10)	Number	3	3	3	3	3	3
	Mean	17.3	19.7	22.0	21.7	21.5	21.9
	SE	0.4	0.5	2.2	0.9	0.9	0.7
	Prob.						
3 (0.25)	Number	3	3	3	3	3	3
	Mean	17.3	17.5	19.8	21.0	21.3	21.1
	SE	0.4	0.4	1.9	0.7	0.8	0.6
	Prob.		bs				
4 (1.00)	Number	3	3	3	3	3	3
	Mean	17.3	13.7	20.9	22.4	23.2	22.5
	SE	0.4	0.4	1.8	0.7	0.7	0.6
	Prob.		cs	ac			

Sexes tested combined (c) and significantly different from the control: ac P≤0.05, bc P≤0.0
Sexes tested separately (s) and significantly different from the control: as P≤0.05, bs P≤0.01

[P42-3]

There were no treatment-related differences in water consumption monitored by visual inspection.

Ophthalmoscopy: *Anterior, lenticular, and fundic areas of both eyes were examined by indirect ophthalmoscopy for all rats prior to initiating treatment and for control and high dose groups*

only in study weeks 12 and 25. Examinations were performed after instilling a mydriatic (1% tropicamide).

There were no treatment-related findings.

Hematology: *Orbital sinus blood (3 mL) from isoflurane anesthetized rats was collected during treatment weeks 13 and 25. Parameters were RBC, Hct, Hb, MCV, MCH, MCHC, plat, retic, total and differential WBC. Coagulation parameters were PT.*

There were no treatment-related findings.

Serum chemistry: *Orbital sinus blood (3 mL) from isoflurane anesthetized rats was collected during treatment weeks 13 and 25. Parameters were AST, ALT, ALP, CPK, LDH, total bilirubin, gluc, urea, creatinine, Ca, Na, K, Cl, P, total protein, albumin, globulin, A/G ratio,chol, TG.*

There were no findings considered biologically relevant because the magnitude of changes were small, changes in week 13 didn't recur in week 25, and/or they lacked a dose-response. Statistically significant changes in week 13 occurred in both sexes (increased urea at 0.1 and 0.25 mg/kg, potassium and total protein at 0.25 mg/kg) and in females (decreased chloride and increased phosphate at 1 mg/kg, increased LDH at ≥ 0.25 mg/kg). In week 25, statistically significant changes occurred in males (increased chloride at 1 mg/kg) and in females (decreased sodium and increased phosphate at 0.1 and 0.25 mg/kg). High dose group male rat 51 had an elevated CPK in week 25 (851 vs control group mean of 130), no LDH value reported because of an inadequate sample size, a necropsy finding of minimum focal myocarditis, and no skeletal muscle findings.

Urinalysis: *Urine was collected overnight from fasted, water deprived rats (selected from each dose group) in metabolism cages for 24 hours after the previous dose during the 4 hour collection period. Parameters were volume, specific gravity, protein, Labstix (pH, glucose, ketones, urobilinogen, blood pigments) and sediment microscopy.*

There were no biologically relevant treatment-related urinalysis parameter changes.

Organ weights: *Paired organs weighed separately, but total organ weight was reported.*

At necropsy, body weight decreased up to 15.9% compared to controls and up to 8.9% in females at ≥ 0.1 mg/kg. In males, decreased body weight was dose-related. Absolute heart weight was decreased up to 19.9% in males and up to 13.8% in females, but due to decreased body weight, relative weight of heart decreased < 10% in all NNC 90-1170 groups. Pancreas weight was not reported.

Organ Weights, Main Study

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
N		14	14	15	15	15	15	15	14
		Value	% Difference from Control[b]			Value	% Difference from Control[b]		
body (g)		565	-12.5	-14.4	-15.9	318	-5.6	-8.9	-7.7
brain	g	2.20	-0.4	-1.3	-3.1	2.02	-0.5	-1.5	-0.5
	% of bw	0.389	13.7	15.2	15.2	0.635	5.5	8.2	7.8
heart	g	1.74	-15.2	-19.9	-16.8	1.26	-9.2	-13.8	-14.6
	% of bw	0.308	-3.1	-6.5	-1.0	0.396	-3.8	-5.4	-7.5

[b]Statistically significant differences from control are underlined ($p < 0.05$).

Gross Pathology and Histopathology: *Tissues collected for microscopic examination are shown in the histopathology inventory table. Tissues were fixed in 10% neutral buffered formalin. Optic nerve and eyes were fixed in Davidson's fluid. Testes were fixed in Bouin's. Tissue sections were stained with eosin and hematoxylin, and examined microscopically. Pancreas sections form*

control and high dose groups were stained with a mouse anti-proliferating cell nuclear antigen (PCNA) antibody to detect proliferating cells in the pancreas

Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

NECROPSY FINDINGS	GROUP TOTALS							
	Males				Females			
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 2 0.10 mg/kg /day	Grp 3 0.25 mg/kg /day	Grp 4 1.00 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 0.10 mg/kg /day	Grp 3 0.25 mg/kg /day	Grp 4 1.00 mg/kg /day
INJECTION/TREATMENT SITE								
Reddened	6	5	6	10	7	11	10	6
SKIN AND SUBCUTIS								
Scab	1		3			1		
Hair loss	1		7	3	3	1		6
Staining		1		1				1

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

[Compiled from Table 20]

There were no gross pathology or histopathology findings considered related to NNC 90-1170. Injection site reactions of macroscopic reddened injection site and microscopic focal dermatitis, focal panniculitis and subcutaneous fibrosis and hemorrhage occurred in both NNC 90-1170 and control groups with a similar incidence and severity, so they were attributed to injection of the vehicle. There were no substantive differences between control and high dose group pancreas tissue sections stained for PCNA. In the exocrine pancreas, the incidence of up to mild acinar cell atrophy was increased at 1 mg/kg in both males and females and the incidence of minimal focal inflammation was increased in high dose females.

HISTOLOGICAL FINDINGS	GROUP TOTALS			
	Males		Females	
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 4 1.00 mg/kg /day	Grp 1 0 mg/kg /day	Grp 4 1.00 mg/kg /day
CAECUM	(15)	(15)	(15)	(15)
No abnormality detected	11	10	14	8*
Nematodes	4	5	1	7*
COLON	(15)	(15)	(15)	(15)
No abnormality detected	15	14	15	12
Nematodes	0	1	0	3
INJECTION/TREATMENT SITE	(15)	(15)	(15)	(15)
Dermatitis, focal				
minimal	1	2	1	3
mild	0	2	1	0
Total Incidence	1	4	2	3
Fibrosis, subcutaneous				
minimal	4	4	4	3
mild	7	7	0	0
Total Incidence	11	11	4	3
Haemorrhage, subcutaneous				
minimal	2	0	2	1
mild	2	7	5	3
moderate	0	1	0	0
Total Incidence	4	8	7	4
Panniculitis, focal				
minimal	4	5	8	6
mild	6	4	2	1
Total Incidence	10	9	10	7
LUNG	(15)	(15)	(15)	(15)
Alveolar macrophage accumulation, focal				
minimal	2	6	3	3
mild	2	1	2	2
moderate	0	1	0	0
Total Incidence	4	8	5	5
Inflammatory cell infiltration, perivascular				
minimal	0	3	0	0
Total Incidence	0	3	0	0
PANCREAS (EXOCRINE)	(15)	(15)	(15)	(15)
No abnormality detected	7	8	14	10
Acinar cell atrophy				
minimal	2	2	0	1
PANCREAS (EXOCRINE)	(15)	(15)	(15)	(15)
Acinar cell atrophy				
mild	0	3	0	1
Total Incidence	2	5	0	2
Inflammation, focal				
minimal	5	3	1	4
mild	1	0	0	0
Total Incidence	6	3	1	4
RECTUM	(15)	(15)	(15)	(15)
No abnormality detected	11	11	13	10
Nematodes	4	4	2	5

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

[Compiled from Table 21]

Toxicokinetics: Venous blood samples for TK analysis were taken prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours on day 1 and in week 26. Plasma NNC 90-1170 was quantified using an immunoassay according to SOP 878-LP-08005.04 (LLOQ 450 pM)

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing.

TK-parameter values on Day 1:

Period	Dose (mg/kg)	Gender	C_{max} (nmol/l)	t_{max} (h)	AUC (h·nmol/l)
Day 1	0.10	Male	69.7	6.0	868
		Female	84.3	6.0	875
		Mean	77.0	6.0	871
	0.25	Male	180	4.0	2130
		Female	196	6.0	2130
		Mean	188	5.0	2130
	1.00	Male	675	6.0	9460
		Female	560	8.0	7290
		Mean	618	7.0	8380

TK-parameter values after 26 weeks:

Period	Dose	Gender	C_{max} (nmol/l)	t_{max} (h)	AUC_t (h·nmol/l)
Week 26	0.10	Male	24.8	8.0	379
		Female	44.5	6.0	583
		Mean	34.7	7.0	481
	0.25	Male	85.2	12.0	1270
		Female	128	6.0	1900
		Mean	106	9.0	1580
	1.00	Male	468	8.0	5640
		Female	668	6.0	6840
		Mean	568	7.0	6240

[P182]

Summary and Conclusions

In a 26 week repeat subcutaneous dose toxicity study of 0, 0.1, 0.25, or 1 mg/kg/day NNC 90-1170 in Sprague Dawley rats (15/sex/dose), there were 3 unscheduled deaths that were considered unrelated to treatment. The MTD was 1 mg/kg/day, the highest dose tested. The NOAEL was 0.25 mg/kg/day based on clinical signs of toxicity at 1 mg/kg/day that were not reversed during a 4 week recovery period.

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing.

Compared to concurrent controls, body weight was dose-dependently decreased up to 13.6% and body weight gain was decreased up to 19.1% at $\geq$ 0.1 mg/kg in males, but there was no significant effect on body weight or body weight gain in females. Food consumption was transiently decreased during the first week of dosing at $\geq$ 0.1 mg/kg in males and at $\geq$ 0.25 mg/kg in females, but the effect was transient and it returned to control group levels by the third week. Decreased body weight gain and transiently decreased food consumption are considered pharmacologic effects of NNC 90-1170.

NNC 90-1170 had no toxicologically relevant effects on clinical signs, ophthalmology, hematology, coagulation, serum chemistry, or urinalysis parameters and it didn't affect organ weight, or macroscopic or microscopic pathology. Dose-related decreased absolute heart weight compared to controls at $\geq$ 0.1 mg/kg in males and females was in large part attributable to lower body weight in NNC 90-1770 treated groups. Relative heart weight decreased < 10% in all treated groups. Injection site reaction consisted of macroscopic reddened injection site and microscopic focal dermatitis, focal panniculitis and subcutaneous fibrosis and hemorrhage occurred in both NNC 90-1170 and control groups with a similar incidence and severity, so they were attributed to injection of the vehicle. There was no evidence of pancreas cell proliferation by immunohistochemical staining for PCNA and no proliferative thyroid C-cell lesions were noted. In the exocrine pancreas, the incidence of up to mild acinar cell atrophy was increased at 1 mg/kg in both males and females and the incidence of minimal focal inflammation was increased in high dose females.

Monkeys

Study titles: NNC 90-1170: Subcutaneous maximum tolerated dose study in cynomolgus monkeys (2 studies, 970455 & 980181)

Key study findings:

- No unscheduled deaths occurred.
- In cynomolgus monkeys, the MTD in a 3-day ascending dose study with a 4 day washout between doses was 5 mg/kg/day NNC 90-1170, the highest dose tested.
- The MTD in a 14-day repeat dose study was 4 mg/kg/day NNC 90-1170, the only dose tested.
- Decreased body weight and transiently decreased food consumption were considered pharmacologic effects of NNC 90-1170.

Results and Conclusions

The maximum tolerated subcutaneous dose of NNC 90-1170 in cynomolgus monkeys was determined in 2 studies: a dose escalation study using doses of 0.1, 0.5, 2.5, or 5 mg/kg administered for 3 days with a 4 day washout period between doses and a 14-day repeat dose study of 4 mg/kg/day NNC 90-1170. Both studies used 2 male and 2 female monkeys and no control group. Study parameters were clinical signs, body weight, food consumption, toxicokinetics, hematology, coagulation, serum chemistry, urinalysis, fecal occult blood, organ weight (adrenal, heart, kidneys, liver, lung, ovaries, spleen and testes only), and macroscopic pathology.

In an ascending dose study of 0.1, 0.5, 2.5, or 5 mg/kg/day, treatment lasted for a total of 4 weeks with 3 consecutive days of dosing following by a 4 day washout period every week. The maximum tolerated dose was $\geq$ 5 mg/kg/day NNC 90-1170 in both males and females. The only pathology finding was injection site reaction characterized as an enlarged lesion and thickened vein noted at necropsy in male 2. There were no unscheduled deaths or treatment-related changes in food consumption, hematology & coagulation parameters, serum chemistry parameters, urinalysis parameters, or organ weights. Fecal occult blood tests were negative and there was no treatment-related effect on plasma glucose. Over the 4 week treatment period, both females lost 0.1 kg body weight (5 – 5.6% of their body weight). Decreased body weight is considered a pharmacologic effect of GLP-1R agonists.

Treatment Period

	Sex	Male		Female	
	Monkey #	1	2	3	4
Body weight	kg, day 1	1.9	1.9	1.8	2.0
	kg, week 4	1.9	1.9	1.7	1.9
	change (week 4 - day 1), kg	0.0	0.0	-0.1	-0.1
	% difference from day 1	0.0	0.0	-5.6	-5.0

A glucose tolerance test was performed 3 to 4 weeks after the treatment period (weeks 7 and 8) to evaluate responsiveness to 0.1 mg/kg NNC 90-1170 (0.5g/kg glucose i.v 2 hours after dosing). Two hours after administering a single subcutaneous dose, 0.1 mg/kg NNC 90-1170 reduced plasma glucose levels from ~50 – 80 minutes after glucose loading.

Table 7 Glucose Tolerance Test

Dose Level (mg.kg^{-1}.day^{-1})	Animal Number/Sex	Time after NNC 90-1170/Vehicle Administration			Time After Glucose Administration								
		pd	ipd	+ 1 h	+ 2 min	+ 10 min	+ 20 min	+ 30 min	+ 40 min	+ 50 min	+ 60 min	+ 70 min	+ 80 min
0.1 mg.kg^{-1} NNC 90-1170	1♂	3.91	5.78	4.47	19.23	12.41	9.01	6.24	6.16	2.60	2.88	2.70	3.18
	3♀	3.45	3.16	2.87	20.44	11.10	5.31	3.06	3.55	2.34	2.45	3.00	3.05
Vehicle	2♂	4.09	4.91	4.40	20.69	12.94	10.02	7.54	6.88	5.66	4.49	4.51	4.41
	4♀	4.84	3.86	5.21	20.74	13.52	11.63	8.88	2.32	5.24	5.11	4.62	4.25

Dose Level (mg.kg^{-1}.day^{-1})	Animal Number/Sex	Time after NNC 90-1170/Vehicle Administration			Time After Glucose Administration								
		pd	ipd	+ 1 h	+ 2 min	+ 10 min	+ 20 min	+ 30 min	+ 40 min	+ 50 min	+ 60 min	+ 70 min	+ 80 min
Vehicle	1♂	3.79	4.42	3.54	17.21	11.37	10.07	8.49	7.93	6.69	5.16	4.19	4.21
	3♀	4.05	2.95	3.30	17.26	10.62	7.83	5.95	4.22	4.35	3.98	4.02	3.69
0.1 mg.kg^{-1} NNC 90-1170	2♂	4.79	2.89	4.05	18.70	12.36	9.11	8.53	6.94	4.51	2.11	2.80	2.55
	4♀	4.99	4.14	3.65	20.63	14.18	10.60	7.91	4.99	2.59	1.95	1.92	2.14

pd = Predose
ipd = Immediately predose

[P39]

In a 14 day repeat dose study of 4 mg/kg/day NNC 90-1170 (2/sex/dose, study 980181), the MTD was ≥ 4 mg/kg in the absence of a dose limiting toxicity. There were no unscheduled deaths or treatment related effects on urinalysis parameters or organ weights. Screens for plasma anti-NNC 90-1170 antibodies and fecal occult blood were both negative. Plasma toxicokinetic analysis on days 1 and 14 showed systemic exposure in all treated monkeys with no substantial sex differences in exposure and minimal plasma accumulation, based on $AUC_{0\text{-}24h}$.

Table 1: Individual and mean pharmacokinetic parameters on day 1 and on day 14.

Day	Animal No.	AUC(0-24) (h-nmol/l)	AUC(0-24) Ratio (day 14/day1)	t_{max} (h)	C_{max} (nmol/l)	C_{max} Ratio (day 14/day1)	MRT(0-24) (h)
1	1 (♂)	59585	-	8	3963	-	10.7
	2 (♂)	68944	-	8	3683	-	11.6
	3 (♀)	53275	-	6	2902	-	11.1
	4 (♀)	43894	-	8	2776	-	11.0
	Mean	56425	-	7.5	3331	-	11.1
	SD	10546	-	1	582	-	0.4
	CV%	19	-	13	17	-	3
14	1 (♂)	65176	1.09	6	5382	1.36	9.7
	2 (♂)	79730	1.16	8	5902	1.60	10.3
	3 (♀)	54371	1.02	6	5986	2.06	9.9
	4 (♀)	45690	1.04	6	2420	0.87	11.1
	Mean	61242	1.08	6.5	4923	1.47	10.2
	SD	14678	0.06	1	1690	0.50	0.6
	CV%	24	6	15	34	34	6

[P120]

Injection site reactions were characterized by clinical signs of thickening of the skin around the injection site from study day 4 onward and at necropsy, subcutaneous reddening of the injection site in all monkeys. Decreased RBC parameters (RBC count, Hb, Hct), and in all but one male, increased reticulocytes were consistent with a mild regenerative anemia.

Parameter	Sex / Monkey # / Day	Male 1	Male 2	Female 3	Female 4
RBC (x 10^12/L)	Pretreatment				
	12				
	% change	-13.6	-10.4	-12.8	-13.8
Hb (g/dL)	Pretreatment				
	12				
	% change	-13.7	-11.3	-13.1	-15.9
Hct (L/L)	Pretreatment				
	12				
	% change	-11.8	-10.8	-12.0	-13.7
Ret (%)	Pretreatment				
	12				
	% change	-20.0	100.0	250.0	125.0

b(4)

Serum chemistry changes considered possibly related to treatment were increased CPK in 2 monkeys (male 1 and female 4) and decreased glucose.

Parameter	Sex / Monkey # / Day	Male 1	Male 2	Female 3	Female 4
CPK (IU/L)	Pretreatment				
	12				
	Fold change	4.4	0.4	1.1	4.0
Glucose (mM)	Pretreatment				
	12				
	% change	-11.9	-11.2	-21.9	-24.6

b(4)

By the end of the treatment period, monkeys lost 0.1 – 0.2 kg (4.5 – 10.5%) of their pre-treatment body weight (day 1).

Treatment Period

	Sex / Monkey #	Male 1	Male 2	Female 3	Female 4
Body weight	kg, day 1				
	kg, day 15				
	change (day 15 - day 1), kg	-0.1	-0.1	-0.2	-0.1
	% difference from day 1	-4.5	-4.5	-10.5	-6.3

b(4)

Food consumption was transiently decreased during the first week of treatment in both males (%) and females (&).

Mean Food Consumption for Male and Female Animals (g/sex/week)

Animals	Week -2	Week -1	Week 1	Week 2
%	134	146	123	136
&	119	126	111	128

[P26]

Decreased body weight, transiently reduced food consumption, and decreased plasma glucose were considered pharmacologic effects of NNC 90-1170.

Study title: NNC 90-1170: 28 Day subcutaneous toxicity study in cynomolgus monkeys

Key study findings:

- No unscheduled deaths occurred.

- The NOAEL was 5 mg/kg/day NNC 90-1170 in males and females, the highest dose tested.
- Injection site reactions consisting of reddening and subcutaneous thickening with microscopic fasciitis, hemorrhage, and pigmented macrophages were attributed to injection of the vehicle.
- Decreased body weight and transiently decreased food consumption were considered pharmacologic effects.

Study no.: 980184
Module and page #: Module 4.2.3.2.1, pages 1- 296 b(4)
Conducting laboratory and location: [redacted]
Date of study initiation: 6 November 1998
GLP compliance: Yes (OECD Principles of GLP)
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170 (~2 mg/mL), batches433-980902-01 & 433-980908-01, purity 97.4% by HPLC (certificate of analysis page 94 for 433-980902-01)

Methods

Doses: 0 (vehicle), 0.05, 0.5, 5 mg/kg/day NNC 90-1170

Rationale for dose selection: Doses were selected based on results from dose-range finding studies ([redacted] studies 568707 / 571217). b(4)

Species/strain: cynomolgus monkeys (purpose bred)
Number/sex/group: The study design is summarized in the table below.
Main study: 3/sex/dose

Group No.	Treatment (mg/kg/day)	Dose Concentration (mg/ml)	Animal No.	
			Males	Females
1	0	0	1-3	13-15
2	0.05	0.02	4-6	16-18
3	0.5	0.20	7-9	19-21
4	5.0	2.00	10-12	22-24

[P17]

Route, formulation, volume: subcutaneous injection once a day at 2 different sites each day, NNC 90-1170 solution in the vehicle (0.71 mg/ml disodium monohydrogenphosphate dihydrate, 0.62 mg/ml monosodium hydrogenphosphate dihydrate, 38 mg/ml mannitol and 5 mg/ml phenol), 2.5 mL/kg.
Age: not stated, but based on weight, males and females were probably not sexually mature
Weight: 1.6 – 2.0 kg males and females
Sampling times: Femoral vein blood for toxicokinetic analysis was collected from all monkeys on study days 1 and 28 prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours after dosing. Protease inhibitors EDTA, bacitracin, and aprotonin were added to blood samples.
Unique study design and Protocol deviations
Samples were screened for anti-NNC 90-1170 antibodies using blood samples taken on day 1, and after the last dose, prior to dosing and 24 hours afterward. Antibodies were detected by precipitating Ig bound radioactivity from plasma incubated with [^{125}I]acyl GLP-1 for up to 24 hours (SOP only, assay not validated).

Feces was tested for occult blood.

Results and Conclusions

In a 28 day repeat oral dose toxicity study of 0 (vehicle), 0.05, 0.5, or 5 mg/kg/day NNC 90-1170 (study 980184) injected subcutaneously once a day (2.5 mL/kg, 2 sites/day) in cynomolgus monkeys (3/sex/dose), study observations were clinical signs, body weight, food consumption, water consumption, ophthalmoscopy, toxicokinetics, determination of anti-NNC 90-1170 antibodies, hematology, coagulation, serum chemistry, urinalysis, fecal occult blood, organ weights, and gross and microscopic pathology.

Toxicokinetic analysis of plasma NNC 90-1170 quantified using a RIA assay showed peak (Cmax) and total 24 hour (AUC_{0-24}) exposure increased with dose across the dose range on both days 1 and 28 in both sexes (Table 2). Exposure in males was > 2 fold higher than females in the high dose group on day 28, but otherwise, there were no substantive sex differences. Plasma NNC 90-1170 did not accumulate after 28 days of dosing.

Table 2: Mean (±SD) pharmacokinetic parameters after s.c. administration of three different dose levels of NNC 90-1170/ kg to Cynomolgus Monkeys. A: 0.05 mg/kg; B: 0.5 mg/kg; C: 5.0 mg/kg (n=3).

A

Para-meter	Unit of measure	Day 1				Day 28			
		Male		Female		Male		Female	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC(0-24)	h·nmol/l	257	64	210	62	233	99	170	28
C_{max}	nmol/l	16	4	13	4	15	6	11	2
t_{max}	h	12	0	11	2	11	2	6	2
MRT(0-24)	h	11	0.8	11	0.3	10	0.4	10	0.2

B

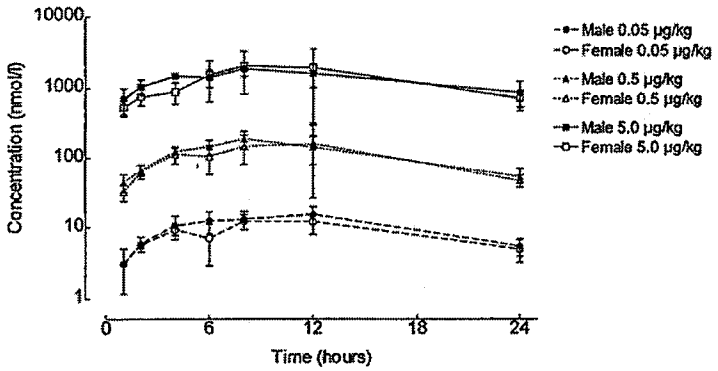
Para-meter	Unit of measure	Day 1				Day 28			
		Male		Female		Male		Female	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC(0-24)	h·nmol/l	2755	838	2613	1498	2285	847	1430	109
C_{max}	nmol/l	191	57	183	119	157	63	127	9
t_{max}	h	8	0	8	4	7	2	6	2
MRT(0-24)	h	11	0.4	11	0.3	10	0.4	10	0.2

C

Para-meter	Unit of measure	Day 1				Day 28			
		Male		Female		Male		Female	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC(0-24)	h·nmol/l	32031	9499	32829	20127	35381	12700	14938	2453
C_{max}	nmol/l	1903	433	2293	1509	2679	1239	945	243
t_{max}	h	8	1	11	2	7	1.0	7	2
MRT(0-24)	h	11	0.6	11	0.9	10	0.35	10	0.56

[P268]

Figure 1: Mean (±SD) plasma concentration profiles on day 1 after s.c. administration of 3 different dose levels of NNC 90-1170 to male and female Cynomolgus Monkeys (n=3).



[P269]

Clinical signs of subcutaneous thickening at the injection site was attributed to the vehicle because it occurred in all treated monkeys, including controls, from week 3 to the end of the study.

Table 1 Clinical Signs: Group Mean Incidence

Clinical Sign	Week Number	Sex/Group/Dose level/mg/kg/day							
		Males				Females			
		1 (0)	2 (0.05)	3 (0.5)	4 (5)	1 (0)	2 (0.05)	3 (0.5)	4 (5)
Subcutaneous thickening at injection site	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	18	13	20	12	13	14	14	21
	4	21	21	21	21	15	21	21	21

Maximum incidence per week = no. of animals per group (3) x days (7) = 21

[P38]

There were no treatment-related changes in water consumption, ophthalmology parameters, hematology parameters, serum chemistry parameters, or urinalysis parameters. Compared to concurrent controls at the end of treatment, body weight was 10% lower in males at ≥ 0.5 mg/kg and in females at 5 mg/kg with decreased body weight gain at ≥ 0.5 mg/kg in both sexes.

Treatment Period

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
Parameter									
Body weight	N	3	3	3	3	3	3	3	3
	kg, day 0	1.9	1.9	2.1	2.0	1.8	1.8	1.9	1.9
	kg, day 28	2.0	2.0	1.8	1.8	1.9	1.9	1.9	1.7
	% difference from control, day 28	0.0	0.0	-10.0	-10.0	0.0	0.0	0.0	-10.5
Body weight gain (day 0 to day 28)	kg	0.1	0.1	-0.3	-0.2	0.1	0.1	0.0	-0.2
	% of pretest body weight	5.3	5.3	-14.3	-10.0	5.6	5.6	0.0	-10.5
	% difference from control	0.0	0.0	-400.0	-300.0	0.0	0.0	-100.0	-300.0

Food consumption was dose-dependently decreased during the first week of treatment, but the effect was transient with return to control group levels in the second week (Tables 6 &7, food consumption of days 1 – 14).

Table 6
(continued) Group Mean Food Consumption : Males

Group/Treatment (mg/kg/day)		Treatment Period (Days)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 (0)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	147	130	127	130	127	123	143	130	147	130	120	127	133	130
	SD	15	10	12	10	12	6	6	17	6	17	17	6	6	0
2 (0.05)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	120	113	110	123	113	123	130	120	137	137	137	140	137	140
	SD	10	6	0	12	6	12	0	10	6	6	6	0	6	17
3 (0.5)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	113	107	113	117	120	123	127	133	123	123	120	130	127	130
	SD	6	12	6	6	10	15	21	6	12	6	10	10	12	10
4 (5)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	103	107	110	107	110	123	113	117	130	127	117	133	137	117
	SD	12	6	0	12	0	6	6	6	10	6	12	6	15	6

[P44]

Table 7 Group Mean Food Consumption: Females

Group/Treatment (mg/kg/day)		Pretrial (Days)													
		-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
1 (0)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	127	137	130	137	127	123	143	143	117	143	137	140	133	127
	SD	12	6	17	6	12	35	15	25	6	21	6	10	15	6
2 (0.05)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	113	120	113	130	127	137	130	133	123	120	117	127	127	123
	SD	12	10	12	10	12	6	17	15	15	10	6	6	12	6
3 (0.5)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	123	130	133	137	133	137	140	143	130	140	133	133	143	130
	SD	21	10	6	6	6	32	0	6	10	26	12	15	6	10
4 (5)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	130	130	123	127	117	140	133	137	117	127	127	120	117	143
	SD	26	20	21	23	23	10	15	6	6	15	12	10	12	6

[P46]

Water consumption also tended to be lower than concurrent controls when it was measured on days 20 – 23.

Table 8 Group Mean Water Consumption (Days 20 to 23): Males

Group/Dose Level (mg/kg/day)		Day of Study: Volume of Water Consumed (ml)			
		20	21	22	23
1 (0)	Number	3	3	3	3
	Mean	146	217	293	698
	SD	14	29	142	150
2 (0.05)	Number	3	3	3	3
	Mean	92	183	500	425
	SD	14	76	50	43
3 (0.5)	Number	3	3	3	3
	Mean	83	242	275	375
	SD	58	88	139	25
4 (5)	Number	3	3	3	3
	Mean	17	200	317	367
	SD	29	132	138	76

Table 9 Group Mean Water Consumption Days (20 to 23): Females

Group/Dose Level (mg/kg/day)		Day of Study: Volume of Water Consumed (ml)			
		20	21	22	23
1 (0)	Number	3	3	3	3
	Mean	125	317	363	550
	SD	43	126	202	87
2 (0.05)	Number	3	3	3	3
	Mean	50	217	350	483
	SD	0	58	25	165
3 (0.5)	Number	3	3	3	3
	Mean	75	192	333	417
	SD	43	50	104	118
4 (5)	Number	3	3	3	3
	Mean	42	150	250	483
	SD	52	100	87	176

[P49-50]

Absolute and relative weight of pancreas was ~30% higher than concurrent controls in NNC 90-1170 treated male groups, but not in females. In the absence of correlative histopathology, this finding was not considered relevant to NNC 90-1170 toxicity.

Organ Weights, Main Study (n = 3/sex/dose)

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
		Value	% Difference from Control			Value	% Difference from Control		
body (kg)		1.90	5.3	-10.5	-5.3	1.80	5.6	5.6	-5.6
brain	g	68.120	-2.5	-1.5	-4.4	58.750	-0.7	13.7	4.6
	% of bw	3.585	-7.4	10.1	0.9	3.264	-6.0	7.7	10.8
heart	g	7.610	2.8	-13.1	-11.2	7.110	-1.3	0.3	-7.9
	% of bw	0.401	-2.4	-2.9	-6.2	0.395	-6.5	-5.0	-2.5
pancreas	g	2.780	30.2	34.2	32.4	3.530	-10.2	-20.4	-5.1
	% of bw	0.146	23.7	50.0	39.7	0.196	-14.9	-24.6	0.5
thymus	g	2.680	27.6	-34.7	-15.7	3.010	-17.3	0.3	-43.5
	% of bw	0.141	21.2	-27.0	-11.0	0.167	-21.6	-4.9	-40.2

Injection site reactions had histopathology findings of minimal to moderate subacute or chronic fasciitis, hemorrhage, and pigmented macrophages, but the findings were attributed to the vehicle because the incidence and severity were similar in control and NNC 90-1770 treated groups.

HISTOLOGICAL FINDINGS	GROUP TOTALS							
	Males				Females			
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 2 0.05 mg/kg /day	Grp 3 0.5 mg/kg /day	Grp 4 5.0 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 0.05 mg/kg /day	Grp 3 0.5 mg/kg /day	Grp 4 5.0 mg/kg /day
INJECTION/TREATMENT SITE	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Fasciitis								
minimal	0	1	0	1	1	1	1	0
mild	1	1	3	1	1	1	1	1
moderate	2	1	0	1	0	0	1	2
Total Incidence	3	3	3	3	2	2	3	3
Pigmented macrophages								
minimal	1	2	1	1	0	0	1	3
mild	2	0	1	0	0	1	1	0
moderate	0	0	0	0	0	1	0	0
Total Incidence	3	2	2	1	0	2	2	3
Necrosis, needle track, unilateral.								
minimal	0	0	0	0	0	1	0	0
Total Incidence	0	0	0	0	0	1	0	0
Haemorrhage								
minimal	0	0	0	1	1	1	0	1
mild	1	2	2	2	2	2	2	0
Haemorrhage								
moderate	2	1	1	0	0	0	1	2
Total Incidence	3	3	3	3	3	3	3	3
Granuloma, unilateral.								
mild	0	0	0	0	0	1	0	1
Total Incidence	0	0	0	0	0	1	0	1

Figures in brackets represent the number of animals from which this tissue was examined microscopically

[P80-81]

The NOAEL was 5 mg/kg/day NNC 90-1170 based on the absence of toxicity at any dose in males or females. Injection site reactions consisting of reddening and subcutaneous thickening with microscopic fasciitis, hemorrhage, and pigmented macrophages were attributed to injection of the vehicle. However, it's notable that although pigmented macrophages occurred in control and treated males, they only occurred in liraglutide-treated females. Decreased body weight and transiently decreased food consumption were considered pharmacologic effects of NNC 90-1170.

Study title: NNC 90-1170: 13 Week subcutaneous toxicity study in the cynomolgus monkey with a recovery period

Key study findings:

- No unscheduled deaths occurred.
- The NOAEL was 0. 5 mg/kg/day NNC 90-1170 in males based on increased eosinophils and injection site reactions at 5 mg/kg/day. In females, the NOAEL was < 0.05 mg/kg/day based on increased eosinophils and injection site reaction s at ≥ 0.05 mg/kg.
- Subcutaneous thickening (clinical sign) with correlative microscopic chronic fasciitis, characterized by fibrosis and variable mixed, diffusely distributed, mononuclear cell infiltrates, localized edema, and multifocal perivascular infiltration of lymphocytes and eosinophils and increased blood eosinophils(week 13), occurred at 5 mg/kg in males and at ≥ 0.05 mg/kg in females. Chronic fasciitis was not reversed after a 2 week recovery period in high dose females.
- A mild anemia characterized by reduced RBC parameters (RBC count, Hb, Hct) occurred at ≥ 0.5 mg/kg/day NNC 90-1170 in males and females in week 13.
- Decreased body weight and transiently decreased food consumption were considered pharmacologic effects.

Study no.: 990191
Module and page #: Module 4.2.3.2.1, pages 1- 388 b(4)
Conducting laboratory and location: [redacted]
Date of study initiation: 11 August 1999
GLP compliance: Yes (OECD Principles of GLP)
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170 (5 mg/mL), batches 317902, purity 96.5 % by HPLC (certificate of analysis page 138 - 140)

Methods

Doses: 0 (vehicle), 0.05, 0.5, 5 mg/kg/day NNC 90-1170

Rationale for dose selection: The high dose was selected based on prior use of the same high dose of 5 mg/kg/day NNC 90-1170 in a 4 week study in which a dose limiting toxicity was not observed at 5 mg/kg.

Species/strain: cynomolgus monkeys (purpose bred)
Number/sex/group: The study design is summarized in the table below.
Main study: 4/sex/dose
2 week recovery: 2/sex/control and high dose groups

Group Number	Treatment (mg/kg/day)	Dose Concentration (mg/ml)	Animal Number			
			Main Study		Recovery Study	
			Male	Female	Male	Female
1	0	0	1-4	21-24	17,18	37,38
2	0.05	0.02	5-8	25-28	-	-
3	0.5	0.2	9-12	29-32	-	-
4	5.0	2.0	13-16	33-36	19,20	39,40

[P21]

3 monkeys received veterinary care for cuts or wounds during the study (female 21 at 0 mg/kg on day 45 (tail amputated after it was caught in the cage), male 18 at 0 mg/kg on

days 1 – 3 (cheeck cut), and female 30 at 0.5 mg/kg on day 89 (cheek pouch cut, surgically debrided)).

Route, formulation, volume: subcutaneous injection once a day at 2 different sites each day, NNC 90-1170 solution in the vehicle (0.71 mg/ml disodium monohydrogenphosphate dihydrate, 0.62 mg/ml monosodium hydrogenphosphate dihydrate, 38 mg/ml mannitol and 5 mg/ml phenol), 2.5 mL/kg.
Age: 11 – 19 months (males and females probably not sexually mature)
Weight: 1.8 – 2.5 kg males and females
Sampling times: Femoral vein blood for toxicokinetic analysis was collected from all monkeys on study day 1 and in weeks 6 and 13 prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours after dosing. Protease inhibitors EDTA, bacitracin, and aprotonin were added to blood samples.
Unique study design and Protocol deviations
Anti-NNC 90-1170 antibodies were detected and characterized.

Feces was tested for occult blood.

Results:

Mortality:

There were no unscheduled deaths.

Clinical signs: *Observed daily.*

Subcutaneous thickening at the injection site was attributed to the vehicle because it occurred in all dose groups, including controls, by week 6 in most monkeys and it wasn't reversed after a 2 week recovery period. Vomiting occurred 30 minutes after dosing on a single occasion in 2 high dose males (in study weeks 1 and 3) and in one the males, it occurred once prior to dosing (study week 4). Due to its infrequency, its relation to treatment was equivocal.

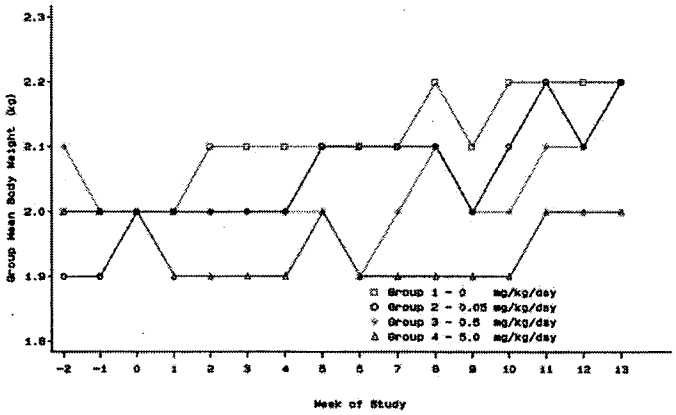
Body weights: *Weighed once a week starting 2 weeks prior to dosing, at the end of treatment or recovery, and prior to necropsy.*

After 13 weeks, body weight was 9.1% lower in males at 5 mg/kg. NNC 90-1170 and 4.3 – 8.7% lower in females at ≥ 0.5 mg/kg. Lower body weight was likely due to transiently decreased food consumption during the first week of treatment, a pharmacologic effect. There were no substantive differences in body weight or body weight gain between control and high dose treated monkeys at the end of a 2 week recovery period.

Treatment Period

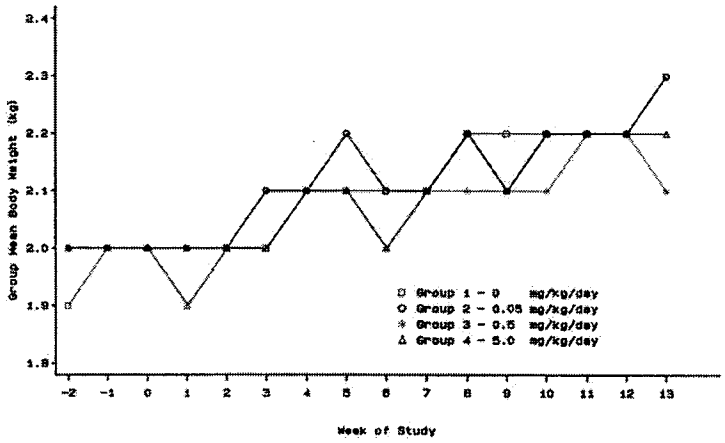
Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
Parameter									
Body weight	N	6	4	4	6	6	4	4	6
	kg, week 0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	kg, week 13	2.2	2.2	2.2	2.0	2.3	2.3	2.1	2.2
	% difference from control, week 13	0.0	0.0	0.0	-9.1	0.0	0.0	-8.7	-4.3
Body weight gain (week 0 to week 13)	kg	0.2	0.2	0.2	0.0	0.3	0.3	0.1	0.2
	% of pretest body weight	10.0	10.0	10.0	0.0	15.0	15.0	5.0	10.0
	% difference from control	0.0	0.0	0.0	-100.0	0.0	0.0	-50.0	-33.3

Figure 1 Group Mean Body Weight (kg): Males



[P136]

Figure 2 Group Mean Body Weight (kg): Females



[P137]

Recovery Period

Sex		Male		Female	
NNC 90-1170 (mg/kg/day)		0	5	0	5
Parameter	N	2	2	2	2
Body weight	kg, week 14	2.2	2.1	2.2	2.2
	kg, week 15	2.3	2.2	2.2	2.2
	% difference from control, week 15	0.0	-4.3	0.0	0.0
Body weight gain (week 14 to week 15)	kg	0.1	0.1	0.0	0.0
	% of pretest body weight	4.5	4.8	0.0	0.0
	% difference from control	0.0	0.0	N/C	N/C

N/C = not calculable (denominator = 0)

Food consumption: *Recorded daily with weekly values reported.*

Food consumption transiently decreased during the first week of treatment in all NNC 90-1170 groups and monkeys were allowed overnight access to food during the first 2 days of the study to stimulate food consumption. By week 2, food consumption was unaffected by treatment.
Ophthalmoscopy: *Anterior, lenticular, and fundic areas of both eyes of sedated monkeys examined by indirect ophthalmoscopy prior to initiating treatment, during treatment weeks 6 and 13, and in the last week of recovery. Examinations were performed after instilling a mydriatic (1% tropicamide).*

There were no treatment-related findings.
ECG: *Not recorded.*
Anti-liraglutide antibodies: *Anti-liraglutide antibodies in femoral vein blood samples (protease inhibitors added) taken prior to dosing and in weeks 6 and 13 and in recovery week 2 were detected by precipitating IgG bound radioactivity after incubating plasma with [^{125}I]acylated GLP-1 overnight (protocol 878-LP-08006.03).*

No samples were positive for anti-liraglutide antibodies.
Hematology: *Femoral vein blood samples from overnight fasted monkeys were taken prior to treatment, during treatment weeks 6 and 13, and in recovery week 2. Hematology parameters were RBC, Hct, Hb, MCV, MCH, MCHC, plat, retic, total and differential WBC. Romanowsky stained bone marrow smears were taken at necropsy, but in the absence of hematology findings, they were not evaluated. Coagulation parameters were APTT, PT.*

RBC parameters were reduced in week 13 in males and females. Hemoglobin was 3 – 5% lower than concurrent controls at ≥ 0.5 mg/kg, and RBC was 1 – 5% lower at 5 mg/kg. Mild anemia persisted in high dose recovery males with lower RBC, Hb, and Hct compared to controls with increased reticulocytes consistent with a regenerative response.

In week 6, eosinophils were 267 – 1100% higher than concurrent controls at 5 mg/kg. In week 13, eosinophils were increased at 5 mg/kg in males and at ≥ 0.05 mg/kg in females. Increased eosinophils were considered fully (males) or partially (females) reversed after a 2 week recovery period.

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
Parameter	N / Week	6 Absolute Value	4	4 % Difference from Control	6	6 Absolute Value	4	4 % Difference from Control	6
RBC (x 10^12/L)	6	6.79	1.5	-2.5	-4.4	6.59	-1.5	7.6	3.2
	13	7.00	3.4	-2.6	-5.4	6.81	0.9	3.4	-0.9
	Recov (n = 2)	7.14	-	-	-10.4	6.70	-	-	4.3
Hb (g/dL)	6	13.6	-5.1	-6.6	-5.1	13.0	1.5	2.3	3.1
	13	13.7	-0.7	-5.1	-5.1	13.4	1.5	-3.0	-3.0
	Recov (n = 2)	13.5	-	-	-9.6	12.6	-	-	6.3
Hct (L/L)	6	0.447	-1.1	-2.2	-3.6	0.437	-0.5	2.1	1.8
	13	0.463	-0.2	-3.2	-4.3	0.452	-0.2	-2.9	-1.5
	Recov (n = 2)	0.464	-	-	-6.7	0.452	-	-	6.2
Ret (%)	6	0.8	0.0	-12.5	-12.5	0.6	-16.7	-33.3	-33.3
	13	0.4	25.0	25.0	25.0	0.4	0.0	25.0	0.0
	Recov (n = 2)	0.6	-	-	117	0.2	-	-	250
Eosin (x 10^9/L)	6	0.02	450.0	-100.0	1100	0.06	33.3	133	267
	13	0.13	-15.4	92.3	300	0.02	600	1200	1600
	Recov (n = 2)	0.14	-	-	-42.9	0.00	-	-	0.16[a]

Statistically signficant differences from control are underlined ($p < 0.05$).

[a]% difference not calculable (denominator = 0), absolute value shown.

Serum chemistry: *Femoral vein blood samples from overnight fasted monkeys were taken prior to treatment, during treatment weeks 6 and 13, and in recovery week 2. Parameters were AST, ALT, ALP, CPK, LDH, total bilirubin, gluc, urea, creatinine, Ca, Na, K, Cl, P, total protein, albumin, globulin, A/G ratio,chol, TG.*

Compared to concurrent controls, alkaline phosphatase activity was significantly decreased up to 39.2% in males and up to 32.7% in females at ≥ 0.5 mg/kg in week 6. Decreased ALP at ≥ 0.5 mg/kg persisted in week 13, but the decrease was no longer statistically significant. At the end of a 2 week recovery period, ALP in high dose recovery groups persisted in males (-43.8%) and in females (-23.7%).

Total bilirubin was increased compared to concurrent controls at 5mg/kg in males in week 6 and in week 13, at 0.05 and 0.5 mg/kg in females in week 13, and in high dose recovery group females in week 2 of the recovery period.

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
Parameter	N / Week	6 Absolute Value	4	4 % Difference from Control	6	6 Absolute Value	4	4 % Difference from Control	6
ALP (IU/L)	6	1661	-21.1	-34.7	-39.2	1460	-2.2	-7.8	-32.7
	13	1451	-7.2	-24.8	-34.3	1315	9.3	-11.0	-20.2
	Recov (n = 2)	1545	-	-	-43.8	1493	-	-	-23.7
Total bilirubin (uM)	6	3.6	-16.7	-13.9	41.7	4.0	15.0	2.5	-15.0
	13	2.8	17.9	0.0	39.3	3.3	33.3	36.4	15.2
	Recov (n = 2)	3.2	-	-	-9.4	2.5	-	-	40.0

Statistically signficant differences from control are underlined ($p < 0.05$).

Urinalysis: *Urine was collected overnight from fasted, water restricted monkeys using metabolism trays. Urinalysis parameters were volume, specific gravity, Labstix (pH, protein, glucose, ketones, urobilinogen, bilirubin, blood pigment), Na, K, Cl, and sediment microscopy.*

There were no treatment-related urinalysis parameter changes.

Organ weights: *Paired organs weighed separately, but total organ weight was reported.*

No treatment-related organ weight changes o occurred.

Gross Pathology and Histopathology: *Tissues collected for microscopic examination are shown in the histopathology inventory table. Tissues were fixed in 10% neutral buffered formalin. The gall bladder was drained and weighed with the liver. One lung lobe was perfusion fixed. Optic nerve and eyes were fixed in Davidson's fluid. Formalin fixed frozen sections of liver and kidneys were stained with oil red O to ascertain the presence of fat. Tissue sections were stained with eosin and hematoxylin, and examined microscopically.*

Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Treatment-related pathology findings occurred at the injection site. Clinical signs of thickening at the injection site had correlative histopathology findings of chronic fasciitis in control NNC 90-1170 treated monkeys. Chronic fasciitis was characterized by fibrosis and variable mixed, diffusely distributed, mononuclear cell infiltrates and it was attributed to injection of the vehicle. Chronic active fasciitis was NNC 90-1170 treatment related at 5 mg/kg males and at $\geq$ 0.05 mg/kg females. Chronic fasciitis was considered active when localized edema and multifocal perivascular infiltration of lymphocytes and eosinophils also occurred. Increased eosinophils in week 13 at 5 mg/kg in males and at $\geq$ 0.05 mg/kg in females is consistent with increased eosinophils in chronic active fasciitis. After a 2 week recovery period, chronic active fasciitis and hemorrhage persisted in high dose females. In high dose recovery males, chronic fasciitis persisted, but it was no longer active.

Histopathology findings considered equivocally related to treatment occurred in trachea (inflammatory cell infiltration in males at $\geq$ 0.05 mg/kg in male and at 0.05 and 5 mg/kg in females), lungs (alveolar macrophage accumulation at $\geq$ 0.5 mg/kg in males and at 5 mg/kg in females, congestion / hemorrhage, considered agonal by the sponsor at 0.05 and 5 mg/kg in males and at $\geq$ 0.05 mg/kg in females), and thyroid (fat infiltration in males at 5 mg/kg).

Histopathology, Main Study (n = 4/sex/dose)

Organ	Finding	Severity	Sex	Male				Female			
			NNC 90-1170 (mg/kg/day)	0	0.05	0.5	5	0	0.05	0.5	5
Injection / Treatment site	reddened (macroscopic)			4	4	4	4	3	4	3	4
	chronic fasciitis	mild		0	1	1	1	1	0	0	0
		moderate		4	2	3	0	3	1	0	0
		marked		0	0	0	0	0	0	1	0
		severe		0	1	0	0	0	0	0	0
		total affected		4	4	4	1	4	1	1	0
	chronic active fasciitis	moderate		0	0	0	0	0	2	3	2
		marked		0	0	0	3	0	1	0	2
		total affected		0	0	0	3	0	3	3	4
Trachea	inflammatory cell infiltration	minimal		0	2	3	2	1	2	0	2
Lung	alveolar macrophage accumulation			0	0	1	1	0	0	0	1
	congestion / hemorrhage			0	1	0	1	0	1	1	1
Thyroid	fat infiltration	minimal / mild		0	0	1	2	1	0	0	1
Parathyroid	cyst			0	1	0	1	0	1	1	0

Histopathology, Recovery (n = 2/sex/dose)

Organ	Finding	Severity	Sex	Male		Female	
			NNC 90-1170 (mg/kg/day)	0	5	0	5
Injection / Treatment site	reddened (macroscopic)			0	0	1	2
	chronic fasciitis	minimal		2	1	0	0
		mild		0	1	2	0
		total affected		2	2	2	0
	chronic active fasciitis	mild		0	0	0	1
		moderate		0	0	0	1
		total affected		0	0	0	2
	hemorrhage			0	0	0	2

Toxicokinetics: Venous blood samples for TK analysis were taken prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours on day 1 and in weeks 6 and 13. Plasma NNC 90-1170 was quantified using an immunoassay according to SOP 878-LP-08005. NNC 90-1170 was quantified in predose samples taken on day 1 and 8 hour samples taken on days 1, 42, and 91.

NNC 90-1170 was detected in samples from 3/6 control group males(monkeys #1 (8 hours post dose on days 42, 91), #4 (predose on day 1 and 8 hours postdose on days 1, 42, 91), #17 X (predose on day 1 and 8 hours postdose on days 1, 42, 91), 1/6 control group females (#24, predose on day 1 and 8 hours post dose on days 1, 42), and 1/6 males in the 0.05 mg/kg group prior to dosing on day 1. Although NNC 90-1170 was detected in control group monkeys and in a LD male prior to dosing on day 1, the levels much lower near the limit of detection (> 400 to < 900 pg/mL) and < 10 fold lower than levels taken 8 hours after dosing the low dose group > 10,000 pg/mL)

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing.

NNC 90-1170 Toxicokinetics

NNC 90-1170 dose (mg/kg/day)	Plasma [NNC 90-1170], nM[A]					
	Day 1		Day 42		Day 91	
	M	F	M	F	M	F
0.05	15	14	16	14	13	17
0.5	149	210	174	176	140	164
5	2,215	2,257	1,953	2,564	1,764	1,641

[A]8 Hours Postdose, 6/sex/0 or 5 mg/kg, 4/sex/dose 0.05 or 0.5 mg/kg

Summary and Conclusions

In a 13 week repeat subcutaneous dose toxicity study of 0, 0.05, 0.5, or 5 mg/kg/day NNC 90-1170 in cynomolgus monkeys (4/sex/dose) with a 2 week recovery period (2/sex/ control and high dose), there were no unscheduled deaths. The NOAEL was 0. 5 mg/kg/day NNC 90-1170 based on increased eosinophils and injection site reactions in males. In females, the NOAEL was < 0.05 mg/kg/day based on increased eosinophils and injection site reaction s at ≥ 0.05 mg/kg. Liraglutide was detected in blood samples in some monkeys prior to initiating treatment and in some control group monkeys. Because plasma levels in treatment naive and control group monkeys were much lower than levels in treated low dose monkeys, the finding does not affect the integrity of the study.

Clinical signs of subcutaneous thickening at the injection site occurred in all treated monkeys, so it was attributed to vehicle, and it wasn't fully reversed after a 2 week recovery period. Macroscopic injection site pathology consisted of reddening in nearly all monkeys. Chronic fasciitis, characterized by fibrosis and variable mixed, diffusely distributed, mononuclear cell infiltrates occurred at the injection site of all treated monkeys, including controls. Chronic fasciitis at the injection site was characterized as active only in NNC 90-1170 treated monkeys, with localized edema, multifocal perivascular infiltration of lymphocytes and eosinophils, and correlative increased blood eosinophils(week 13) in addition to chronic fasciitis at 5 mg/kg in males and at ≥ 0.05 mg/kg in females. After a 2 week recovery period, vehicle -related chronic fasciitis at the injection site persisted in control and high dose males and control females, but NNC 90 1170-related chronic active fasciitis and elevated blood eosinophils persisted and microscopic hemorrhage emerged at the injection site of high dose recovery females.

There were no treatment-related changes in ophthalmoscopy, ECG, or urinalysis parameters and no treatment-related organ weight changes. Body weight decreased 9.1% in males at 5 mg/kg and up to 8.7% in females at ≥ 0.5 mg/kg, but NNC 90-1170 only transiently decreased food consumption in the first week of treatment. Decreased body weight, considered a pharmacologic effect of liraglutide, was reversed at the end of a 2 week recovery period.

RBC parameters (RBA, Hb, Hct) were slightly decreased in males and females in week 13 at 5 mg/kg, and reticulocytes were mildly elevated at the end of recovery indicating a slight regenerative anemia. Elevated eosinophils in week 13 and at 5 mg/kg in males and at ≥ 0.05 mg/kg in females correlated with chronic active fasciitis in the same dose groups.

Compared to concurrent controls, ALP was up to 39.2% lower in males and up to 32.7% in lower females at ≥ 0.5 mg/kg in weeks 6 and 13. Decreased ALP persisted after a 2 week recovery.

There were no treatment-related organ weight changes. Histopathology findings in trachea, lung, and thyroid were considered equivocally related to treatment because the low incidence and/or the absence of a dose-response.

Analysis of plasma NNC 90 1170 prior to dosing and 8 hours after on day 1 and 8 hours after dosing on days 42 and 91 showed systemic exposure increased with subcutaneous dosing

across the dose range with no substantial difference between sexes. No blood samples were positive for anti-liraglutide antibodies using a precipitation format screening assay.

Study title: NNC 90-1170; 52 Week subcutaneous toxicity study in cynomolgus monkeys with 4 week recovery period

Key study findings:

- No unscheduled deaths occurred.
- The NOAEL for both local and systemic toxicity was < 0.05 mg/kg/day based on injection site reactions in males and females and inflammatory cell infiltrates in kidney (females) or stomach pylorus (females) at ≥ 0.05 mg/kg.
- The incidence of thickened injection site increased with dose, along with correlative subcutaneous inflammatory cell infiltration, at ≥ 0.05 mg/kg. In the 5 mg/kg high dose group, microscopic injection site findings were subcutaneous sclerosis, foreign material, and foreign body giant cells. Treatment-related injection site reactions persisted after a 4 week recovery, but their severity was somewhat diminished.
- Increased relative weight of pancreas at ≥ 0.05 mg/kg/day in males and at ≥ 0.5 mg/kg/day in females had correlative increased mass of pancreatic exocrine cells and ducts at 5 mg/kg/day (the only dose in which pancreatic cell type mass was quantified).
- A mild anemia occurred at ≥ 0.5 mg/kg/day liraglutide and it was accompanied by a regenerative response in high dose groups in week 52. Anemia was reversed during recovery. Increased total bilirubin at ≥ 0.5 mg/kg/day in males and at 5 mg/kg/day in females suggests anemia may be hemolytic.
- Body weight decreased up to 18% at ≥ 0.5 mg/kg in males only, but food consumption only transiently decreased on study day 1 at ≥ 0.5 mg/kg in males and at 5 mg/kg in females. Reduced body weight is considered a pharmacologic effect of GLP-1R agonists.
- Three high dose monkeys had confirmed anti-NNC 90-1170 antibody responses and the antibodies cross reacted with GLP-1. The sensitivity of the immunoprecipitation assay may not be adequate or plasma NNC 90-1170 may interfere with the screening assay.

Study no.: 200241
Module and page #: Module 4.2.3.2.1, pages 1- 531 b(4)
Conducting laboratory and location: [redacted]
Date of study initiation: 21 January 2001
GLP compliance: Yes (OECD Principles of GLP, [redacted]
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170 (5 mg/mL), batches 317010, 317011, 317012, purity 98.2 % for batch 317011(certificate of analysis page 107)

Methods

Doses: 0 (vehicle), 0.05, 0.5, 5 mg/kg/day NNC 90-1170

Rationale for dose selection: The high dose was selected based on prior use of the same high dose of 5 mg/kg/day NNC 90-1170 in a 13 week study in which a dose limiting toxicity was not observed. Sponsor states dose limited by injection volume.

Species/strain: cynomolgus monkeys (purpose bred)
Number/sex/group: The study design is summarized in the table below.

Group Number	Treatment (mg/kg/day)	Dose Concentration (mg/ml)	Animal Number			
			Main Study		Recovery Study	
			Male	Female	Male	Female
1	0	0	1-4	21-24	17,18	37,38
2	0.05	0.02	5-8	25-28	-	-
3	0.5	0.2	9-12	29-32	-	-
4	5	2.0	13-16	33-36	19,20	39,40

[N000 4.2.3.2.1 P20]

During the course of the study, monkeys 7, 25, 6, and 31 received veterinary treatment for liquid feces (monkeys 6 and 7), an infection (monkey 25), and separate incidences of liquid feces with clinical signs of illness and a tail wound (female 31). Female 31 wasn't treated for 8 days (starting day 7 in week 33) because of dehydration caused by persistent liquid feces.

Route, formulation, volume: subcutaneous injection once a day at 2 different sites each day, NNC 90-1170 solution in the vehicle (0.71 mg/ml disodium monohydrogenphosphate dihydrate, 0.62 mg/ml monosodium hydrogenphosphate dihydrate, 36.9 mg/ml mannitol and 5 mg/ml phenol), 2.5 mL/kg.
Age: 12 – 17 months (males and most females probably not sexually mature)
Weight: 1.6 – 2.3 kg males and females
Sampling times: Femoral vein blood for toxicokinetic analysis was collected from all monkeys on study day 1 and in week 52 prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours after dosing. Samples were taken from recovery group monkeys 36, 48, and 60 hours after the last dose.
Unique study design and Protocol deviations
Anti-NNC 90-1170 antibodies were detected and characterized.

Mass of beta and non-beta pancreatic cells were assessed in 0 and 5 mg/kg/day groups (report 040301) by double immunohistochemical staining (guinea-pig anti-insulin, monoclonal mouse anti-glucagon, and rabbit anti-somatostatin antibodies) using stereological methods (unspecified). Parallel sections of pancreas were immunohistochemically stained for exocrine duct cells (using anti-cytokeratin CK-7) and for exocrine cells using amylase (report 040301). Parameters reported were total cell mass (mg) and relative cell mass (mg cell mass / kg body weight).

Results:
Mortality:
There were no unscheduled deaths.
Clinical signs: *Observed daily.*
Subcutaneous thickening at the injection site was attributed to the vehicle because it occurred in all dose groups, including controls, by week 13 in most monkeys. However, severity was higher in NNC 90-1170 treated monkeys (moderate to severe) compared to controls (slight to moderate). Subcutaneous thickening at injection site was not reversed after a 4 week recovery period in high dose monkeys, but it reversed in controls. Liquid feces was also noted in all dose groups, but 3 monkeys in NNC 90-1170 groups required veterinary care to treat it (Males 6 & 7 in the 0.05 mg/kg/day group, and female 31 in the 0.5 mg/kg/day group). In female 31, liraglutide treatment was suspended for 8 days starting in week 33 to treat dehydration from persistent liquid feces.

Table 1 % Incidences of Most Commonly Observed Clinical Observations: Males

Observation	Group/Dose Level (mg/kg/day)	Weeks of Study																
		1-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32	33-36	37-40	41-44	45-48	49-52	R1	R2	R3	R4
Subcutaneous thickening of injection sites	1 (0)	0	19	19	65	79	81	83	83	83	83	100	100	100	100	100	100	2
	2 (0.05)	0	31	56	75	78	100	100	100	100	100	100	100	100	-	-	-	-
	3 (0.5)	0	31	51	96	100	100	98	100	100	100	100	100	100	-	-	-	-
	4 (5.0)	0	64	100	100	100	100	99	100	100	100	100	100	100	100	100	100	100

Table 1 (continued) % Incidences of Most Commonly Observed Clinical Observations: Females

Observation	Group/Dose Level (mg/kg/day)	Weeks of Study																
		1-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32	33-36	37-40	41-44	45-48	49-52	R1	R2	R3	R4
Subcutaneous thickening of injection sites	1 (0)	0	37	60	86	100	100	100	100	100	100	100	100	100	100	100	100	0
	2 (0.05)	0	13	51	75	75	98	100	100	100	100	100	100	100	-	-	-	-
	3 (0.5)	0	37	94	71	75	75	97	100	100	100	100	100	100	-	-	-	-
	4 (5.0)	0	81	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

[N000 4.2.3.2.1 P41-2]

Body weights: *Weighed once a week starting 2 weeks prior to dosing, at the end of treatment or recovery, and prior to necropsy.*

At the end of the treatment period, group mean body weight was 14 – 18% lower than controls in males at 0.5 and 5 mg/kg. NNC 90-1170 didn't decrease body weight of females.

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
Parameter									
Body weight	N	6	4	4	6	6	4	4	6
	kg, week 0	2.0	2.0	2.0	2.0	2.1	2.1	2.1	2.1
	kg, week 52	2.8	2.9	2.3	2.4	2.6	2.8	2.7	2.6
	% difference from control, week 52	0.0	3.6	-17.9	-14.3	0.0	7.7	3.8	0.0
Body weight gain (week 0 to week 52)	kg	0.8	0.9	0.3	0.4	0.5	0.7	0.6	0.5
	% of pretest body weight	40.0	45.0	15.0	20.0	23.8	33.3	28.6	23.8
	% difference from control	0.0	12.5	-62.5	-50.0	0.0	40.0	20.0	0.0

Figure 1 Group Mean Body Weight (kg): Males

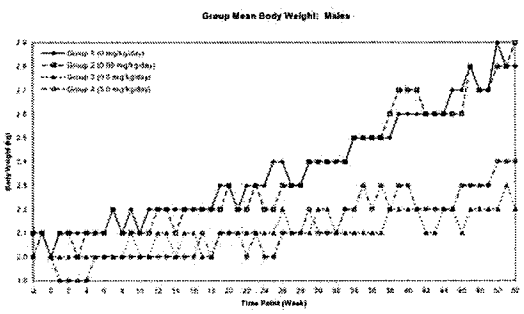
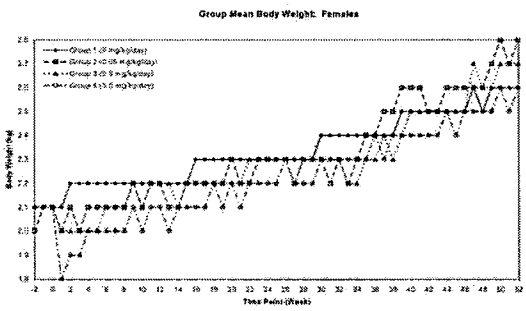


Figure 2 Group Mean Body Weight (kg): Females



[N000 4.2.3.2.1 P105-106]

Food consumption: *Recorded daily with weekly values reported.*

Food consumption was transiently decreased on day 1 in males at 0.5 and 5 mg/kg and in females at 5 mg/kg. There were no other treatment-related effects on food consumption.

Ophthalmoscopy: *Anterior, lenticular, and fundic areas of both eyes of sedated monkeys examined by indirect ophthalmoscopy prior to initiating treatment, during treatment weeks 13,*

26, 39, and 52, and in the last week of recovery. Examinations were performed after instilling a mydriatic (1% tropicamide).

There were no treatment-related findings.

ECG: *Lead II electrocardiographs recorded prior to treatment, during treatment on day 2 and weeks 26 and 52, and during recovery week 4. ECG parameters were heart rate, wave forms, and ECG intervals including PR, QRS, and QT.*

There were no treatment-related findings. The table below summarizes effects on heart rate showing the mean heart rate at baseline in each dose group (pretreatment) and the % change from pretreatment prior to dosing and 9 hours after dosing on study day 2 and in weeks 26 and 52.

Heart Rate Effects

Sex		Males				Females			
LGT Dose (mg/kg)		0	0.05	0.5	5	0	0.05	0.5	5
Study Day	Time								
Pre-treatment (bpm)		238	217	255	256	238	249	250	243
		% Change from Pre-treatment							
Day 2	Predose	0.4	12.4	3.9	4.7	6.7	6.4	5.2	5.8
	+ 9h	-1.3	20.3	5.1	5.5	5.5	4.8	2.4	7.4
Week 26	Predose	-6.7	16.1	0.0	0.4	5.9	-2.8	4.4	5.3
	+ 9h	-0.4	18.9	2.4	-1.2	5.5	0.8	3.6	3.3
Week 52	Predose	-14.3	4.6	1.2	0.8	-6.3	-9.2	-2.4	6.6
	+ 9h	-9.2	6.9	-0.4	1.2	-2.1	2.4	2.0	6.6
Recovery		-9.2	-	-	0.0	-0.8	-	-	4.5

N = 6 in 0 & 5 mg/kg groups, N = 4 in 0.05 & 0.5 mg/kg groups

Anti-liraglutide antibodies: *Anti-liraglutide antibodies in femoral vein blood samples (protease inhibitors added) taken in weeks 13, 26, 39, and 52 and in recovery week 4 were detected by precipitating IgG bound radioactivity after incubating [^{125}I]NNC 90-1170 with serum overnight. Positive samples were confirmed by adding unlabeled NNC 90-1170 during incubation to decrease IgG bound radioactivity. GLP-1 cross-reactivity of anti-NNC 90-1170 antibodies was determined adding unlabeled GLP-1 during the overnight incubation with [^{125}I]NNC 90-1170 in of confirmed positive samples.*

Three high dose monkeys were positive for anti-NNC 90-1170 antibodies: male 15 in week 52 and at the end of recovery, male 20 and female 40. All confirmed anti-NNC 90-1170 sera cross reacted with GLP-1.

Hematology: *Femoral vein blood samples from overnight fasted monkeys were taken prior to treatment, during treatment weeks 13, 26, 39, and 52, and in recovery week 4. Hematology parameters were RBC, Hct, Hb, MCV, MCH, MCHC, plat, retic, total and differential WBC. Coagulation parameters were APTT, PT.*

A mild regenerative anemia was characterized by reduced RBC parameters (RBC, Hb, Hct) at $\geq$ 0.5 mg/kg from week 26 to week 52 and increased absolute reticulocytes at 5 mg/kg in week 52. RBC parameters were similar to controls at the end of recovery. There were no treatment-related changes in coagulation parameters.

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
Parameter[a]	N / Week	6 Absolute Value	4	4	6	6 Absolute Value	4	4	6
			% Difference from Control				% Difference from Control		
RBC (x 10^12/L)	13	6.77	-3.1	-5.5	-3.0	7.04	-0.7	-5.4	-4.0
	26	6.81	-7.2	-9.7	-9.7	6.80	2.2	-4.9	-7.1
	39	6.67	-0.3	-7.0	-5.4	6.59	3.6	-1.4	-4.4
	52	7.09	-4.8	-11.6	-10.3	6.73	-0.9	-1.2	-8.3
Hb (g/dL)	13	13.5	-5.9	-5.2	-3.7	14.0	-1.4	-6.4	-4.3
	26	13.2	-6.8	-6.8	-9.8	13.2	0.8	-6.1	-7.6
	39	13.0	-3.1	-4.6	-5.4	12.8	0.8	-3.9	-4.7
	52	13.8	-7.2	-10.9	-10.1	13.2	-2.3	-3.0	-5.3
Hct (L/L)	13	0.441	-5.4	-6.1	-4.5	0.445	0.4	-2.0	-0.9
	26	0.428	-10.3	-9.3	-10.7	0.414	2.4	-2.7	-5.6
	39	0.435	-4.6	-10.1	-7.8	0.416	3.6	1.7	-1.4
	52	0.470	-9.1	-11.3	-10.6	0.434	0.0	1.2	-5.1
Ret (%)	13	0.3	66.7	33.3	33.3	0.4	0.0	0.0	0.0
	26	0.7	-28.6	-28.6	14.3	0.7	0.0	-28.6	14.3
	39	0.4	0.0	-25.0	50.0	0.6	0.0	33.3	16.7
	52	0.5	0.0	0.0	40.0	0.7	14.3	-14.3	57.1

Statistically signficant differences from control are underlined ($p < 0.05$).

Serum chemistry: *Femoral vein blood samples from overnight fasted monkeys were taken prior to treatment, during treatment weeks 13, 26, 39, and 52, and in recovery week 4. Parameters were AST, ALT, ALP, CPK, LDH, total bilirubin, gluc, urea, creatinine, Ca, Na, K, CL, P, total protein, albumin, globulin, A/G ratio,chol, TG.*

Alkaline phosphatase activity decreased in males at 5 mg/kg with the decrease attaining statistical significance compared to concurrent controls at 5 mg/kg in weeks 26 and 52. In females, total bilirubin increased at 5 mg/kg in both sexes and the increase was significantly higher than controls in weeks 13 and 52 in males and in weeks 13, 39, and 52 in females. Treatment-related changes were reversed at the end of recovery. The ALP isozyme with decreased activity in high dose groups was not determined.

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
Parameter[a]	N / Week	6 Absolute Value	4	4	6	6 Absolute Value	4	4	6
			% Difference from Control				% Difference from Control		
ALP (IU/L)	13	1583	-14.5	-10.9	-25.4	1888	-1.7	-0.8	-20.1
	26	1643	-24.5	-23.7	-37.5	1424	7.0	19.1	-18.5
	39	1874	-7.5	-32.3	-28.6	1469	23.0	33.4	-0.7
	52	2236	-21.7	-31.2	-35.8	1671	12.6	32.5	-12.4
Total Bilirubin (uM)	13	2.5	20.0	16.0	40.0	3.5	-11.4	8.6	22.9
	26	3.1	3.2	19.4	71.0	3.8	-2.6	18.4	34.2
	39	2.3	-8.7	43.5	65.2	2.4	-4.2	-8.3	45.8
	52	2.8	-10.7	46.4	78.6	4.0	-17.5	-5.0	27.5

Statistically signficant differences from control are underlined ($p < 0.05$).

Urinalysis: *Urine was collected overnight from fasted, water restricted monkeys using metabolism trays. Urinalysis parameters were volume, specific gravity, Labstix (pH, protein, glucose, ketones, urobilinogen, bilirubin, Na, K, Cl, and sediment microscopy.*

There were no treatment-related urinalysis parameter changes.

Organ weights: *Paired organs weighed separately, but total organ weight was reported.*

At necropsy, body weight was up to 24.2% lower than concurrent controls at ≥ 0.5 mg/kg NNC 90-1170 in males, but the decrease didn't reach statistical significance.

Relative pancreas weight (relative to body weight) dose-dependently increased 52.7 – 111.4% at ≥ 0.05 mg/kg in males and 46.0 – 78.5% at ≥ 0.5 mg/kg in females with statistically significant increases in absolute pancreas weight at ≥ 0.5 mg/kg in both sexes.

Relative heart weight (relative to body weight) increased 22.9 – 48.1% compared to controls and absolute heart weight was 11.4 to 15.3% at ≥ 0.05 mg/kg in males only, but increased heart weight didn't reach statistical significance.

Absolute thymus weight decreased 31.3 to 41.7% in males at ≥ 0.05 mg/kg, but decreased relative weight was diminished because of decreased body weight a ≥ 0.5 mg/kg. Absolute weight of thymus increased 76.3% and relative weight increased 71.5% at 0.5 mg/kg in females, but it didn't occur at any other dose.

Although group mean relative weight of thyroid increased 39% at 0.05 mg/kg and 78% at 5 mg/kg in females, in the absence of relation to dose or correlative histopathology, thyroid weight changes in females were considered incidental.

Organ weight changes in heart, pancreas, and thyroid lacked correlative histopathology and reversed after a 4 week recovery period.

Organ Weights, Main Study (n = 4/sex/dose)

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.05	0.5	5	0	0.05	0.5	5
		Value	% Difference from Control[a]			Value	% Difference from Control[a]		
body (kg)		2.93	-6.1	-24.2	-23.2	2.48	6.9	2.8	2.0
brain	g	73.580	6.1	-5.4	-5.3	68.406	-10.5	3.9	-0.4
	% of bw	2.511	13.0	24.8	23.3	2.758	-16.3	1.0	-2.4
heart	g	8.733	15.3	12.6	11.4	9.120	-5.1	1.8	-2.2
	% of bw	0.298	22.9	48.6	45.1	0.368	-11.2	-1.0	-4.2
pancreas	g	3.712	43.3	55.0	62.3	3.569	15.1	50.1	82.1
	% of bw	0.127	52.7	104.6	111.4	0.144	7.7	46.0	78.5
thymus	g	5.521	-31.3	-40.5	-41.7	2.867	14.9	76.3	21.8
	% of bw	0.188	-26.8	-21.5	-24.1	0.116	7.5	71.5	19.4
thyroid	g	0.337	-9.0	-14.8	-11.5	0.215	48.8	2.2	82.0
	% of bw	0.011	-3.0	12.5	15.2	0.009	39.2	-0.6	78.4

[a]Statistically significant differences from control are underlined ($p < 0.05$).

Organ Weights, Recovery (n = 2/sex/dose)

Sex		Male		Female	
NNC 90-1170 (mg/kg/day)		0	5	0	5
		Value	% Difference from Control	Value	% Difference from Control
body (kg)		2.60	-1.9	2.70	-7.4
brain	g	77.830	-4.3	66.530	-0.4
	% of bw	2.993	-2.5	2.464	7.5
heart	g	10.480	2.7	9.650	0.8
	% of bw	0.403	4.7	0.357	8.9
pancreas	g	4.440	24.1	5.170	-1.5
	% of bw	0.171	26.5	0.191	6.3
thymus	g	3.390	20.6	3.520	0.9
	% of bw	0.130	23.0	0.130	8.9
thyroid	g	0.292	11.3	0.275	-1.1
	% of bw	0.011	13.5	0.010	6.8

Statistically significant absolute organ weight changes occurring in pituitary gland (low and intermediate dose males and females) and brain (low dose females) were not considered toxicologically relevant because of the small magnitude and absence of a dose-response.

Gross Pathology and Histopathology: *Tissues collected for microscopic examination are shown in the histopathology inventory table. Tissues were fixed in 10% neutral buffered formalin. The gall bladder was drained and weighed with the liver. One lung lobe was perfusion and fixed. Optic nerve and eyes were fixed in Davidson's fluid. Formalin fixed frozen sections of liver and kidneys were stained with oil red O to ascertain the presence of fat. Tissue sections were stained with eosin and hematoxylin, and examined microscopically.*

Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Macroscopic thickening at the injection site at ≥ 0.05 mg/kg had correlative microscopic subcutaneous inflammatory cell infiltration at ≥ 0.05 mg/kg and in high dose groups, subcutaneous foreign material, foreign body giant cells, and sclerosis. Fibrosis or sclerosis at the injection site occurred in all treated monkeys, but mild to marked sclerosis only occurred in high dose groups. Sclerosis was characterized as dense, amorphous acellular collagen (stained green with Masson's trichrome stain). At 5 mg/kg, amorphous, eosinophilic foreign material and foreign body giant cells (fused macrophages with disorganized nuclei) occurred subcutaneously at injection sites. Reddening at the injections site occurred in all monkeys, and it correlated with microscopic hemorrhage (not included in table). After a 4 week recovery, injection site treatment-related toxicity was not reversed with macroscopic findings of thickened and/or dark area and microscopic findings of subcutaneous sclerosis, inflammatory cell infiltration, and foreign body giant cells.

Mild thymus atrophy occurred at 5 mg/kg in males, and it correlated with reduced size in one high dose male. Thymus atrophy occurring in one male at 0.5 mg/kg and in a control group female were considered incidental. Changes in relative amounts of specific cell-types were not reported.

Microscopic findings of inflammatory cell infiltration in kidneys occurred at 5 mg/kg/day in males and at ≥ 0.05 mg/kg/day in females. Inflammatory cell infiltration in lamina propria of stomach pylorus occurred at ≥ 0.05 mg/kg/day in males and at 0.05 and 5 mg/kg/day in females.

Histopathology, Main Study (n = 4/sex/dose)

Organ	Finding	Severity	Sex	Male				Female			
			NNC 90-1170 (mg/kg/day)	0	0.05	0.5	5	0	0.05	0.5	5
Injection / Treatment site	thickened (macroscopic)			1	2	3	4	1	3	3	4
	reddened (macroscopic)			4	4	4	4	4	4	4	4
	subcutaneous fibrosis	minimal		0	0	0	0	2	0	0	0
		mild		4	1	4	0	2	4	4	2
		moderate		0	3	0	1	0	0	0	1
		total affected		4	4	4	1	4	4	4	3
	subcutaneous sclerosis	mild		0	0	0	1	0	0	0	1
		moderate		0	0	0	1	0	0	0	0
		marked		0	0	0	1	0	0	0	0
		total affected		0	0	0	3	0	0	0	1
	subcutaneous foreign body giant cells	minimal		0	0	0	1	0	0	0	0
		mild		0	0	0	1	0	0	0	3
		moderate		0	0	0	1	0	0	0	0
		total affected		0	0	0	3	0	0	0	3
	subcutaneous inflammatory cell infiltration	minimal		1	0	1	1	0	0	0	2
		mild		0	1	2	1	1	3	0	1
		moderate		0	1	0	1	0	0	2	0
		total affected		1	2	3	3	1	3	2	3
	subcutaneous foreign material			0	0	0	1	0	0	0	2
Kidney	focal interstitial inflammatory cell infiltration			3	0	2	4	0	2	2	3
	positive oil red O staining for fat	minimal		0	1	2	1	1	0	2	1
Pancreas	no abnormality detected			4	3	4	3	4	3	3	4
Stomach	pylorus, inflammatory cell infiltrate in lamina propria			1	2	2	2	0	4	0	2
Thymus	small (macroscopic)			0	0	0	1	0	0	0	0
	atrophy	mild		0	0	1	3	1	0	0	0

Histopathology, Recovery (n = 2/sex/dose)

Organ	Finding	Severity	Sex	Male		Female	
			NNC 90-1170 (mg/kg/day)	0	5	0	5
Injection / Treatment site	thickened (macroscopic)			0	1	0	1
	dark (macroscopic)			0	1	0	1
	subcutaneous fibrosis	minimal		2	0	1	0
	subcutaneous sclerosis	moderate		0	2	0	2
	subcutaneous foreign body giant cells	minimal		0	1	0	1
		moderate		0	1	0	1
		total affected		0	2	0	2
	subcutaneous inflammatory cell infiltration	minimal		0	1	0	0
		mild		0	0	0	1
		total affected		0	1	0	1

In high dose monkeys, NNC 90-1170 had no effect on the overall structure of pancreatic islets or insulin staining intensity. Liraglutide significantly lowered the relative volume fraction of both beta cells (-34.5%, $p<0.05$) and non-beta islet cells (-44.6 %, $p<0.01$, exocrine duct cells

and amylase immunoreactive cells), but without having any significant effect on the absolute mass of beta cells and non-beta islet cells or relative beta- or non-beta islet cell mass (mg cells / kg b.w.).

Table 1 Pancreatic beta cell and non-beta cell volume fractions in %, absolute mass in mg, and absolute mass relative to body weight in mg/kg

Group	Sex	BodyWeight	PancWeight	Vvol beta %	Vvol non-beta %	Absolute mg beta	Absolute mg non-beta	Rel beta mg/kg	Rel non-beta mg/kg
Vehicle (mean ± SEM)	M+F	2.70	3.96	1.69	2.33	56.3	77.5	20.7	28.7
	(4+4)	± 0.12	± 0.35	± 0.17	± 0.28	± 7.0	± 10.5	± 2.4	± 3.8
Liraglutide 5mg/kg/day	M+F	2.39	6.02	1.10	1.29	60.8	70.4	24.8	29.5
	(4+4)	± 0.11	± 0.44	± 0.12	± 0.16	± 9.0	± 10.4	± 2.7	± 4.0
Treatment effect 2-way ANOVA	P=	0.035	0.002	0.016	0.006	N.S.	N.S.	N.S.	N.S.

[P18]

Five mg/kg/day NNC 90-1170 had no effect on the relative volume fraction of duct cells (anti-CK-7 positive) and exocrine cells (anti-amylase positive) or structure of the exocrine pancreas, but absolute mass of duct cells increased 67% and exocrine cell mass increased 64% compared to controls.

Table 2 Pancreatic duct cell and exocrine cell volume fractions in %, absolute mass in mg, and absolute mass relative to bodyweight in mg/kg

Group	Sex	BodyWeight	PancWeight	Vvol ducts %	Vvol exocrine %	Absolute mg ducts	Absolute mg exocrine	Rel ducts mg/kg	Rel exocrine mg/kg
Vehicle (mean ± SEM)	M+F	2.70	3.96	6.63	89.35	225	3007	84	1116
	(4+4)	± 0.12	± 0.35	± 0.66	± 0.55	± 31	± 319	± 12	± 113
Liraglutide 5mg/kg/day	M+F	2.39	6.02	6.77	90.84	376	4944	154	2064
	(4+4)	± 0.11	± 0.44	± 0.47	± 0.48	± 46	± 343	± 13	± 91
Treatment effect 2-way ANOVA	P=	0.035	0.002	N.S.	N.S.	0.015	0.006	0.002	0.001

[P19]

Toxicokinetics: Venous blood samples for TK analysis were taken prior to dosing and 1, 2, 4, 6, 8, 12, and 24 hours on day 1 and in week 52. Samples were taken 36, 48, and 60 hours after the last dose. Plasma NNC 90-1170 was quantified using an immunoassay according to SOP 878-LP-08005.

Plasma NNC 90-1170 concentrations increased with subcutaneous dose across the dose range with no substantive sex difference in exposure or accumulation with repeat dosing.

Single Dose

Dose (mg/kg)	Period	Gender		C_{max} (nmol/l)	t_{max} (h)	AUC (h·nmol/l)
0.05	Day 1	Female	Mean	35.1	7.5	560
			SD	7.2	1.0	111
		Male	Mean	24.5	7.0	407
			SD	4.2	1.2	21
0.5		Female	Mean	450	7.0	7870
			SD	91.3	1.2	1369
		Male	Mean	384	7.5	6550
			SD	54	1.0	927
5		Female	Mean	5160	9.0	118000
			SD	809	2.4	22601
		Male	Mean	4900	8.0	87800
			SD	828	2.2	8325

[N000 4.2.3.2.1 P233]

Steady State

Period	Dose (mg/kg)	Gender		C_{max} (nmol/l)	t_{max} (h)	AUC_τ (h·nmol/l)
Week 52	0.05	Female	Mean	62.4	7.5	1110
			SD	53.0	1.0	1219
		Male	Mean	29.9	8.5	523
			SD	3.0	2.5	34
	0.5	Female	Mean	406	7.5	6430
			SD	117	1.0	1423
		Male	Mean	439	7.0	7610
			SD	123	1.2	2313
	5	Female	Mean	3370	5.7	56300
			SD	1130	2.0	22289
		Male	Mean	3680	4.7	62100
			SD	1927	1.6	30902

[N000 4.2.3.2.1 P234]

Summary and Conclusions

In a 52 week repeat subcutaneous dose toxicity study of 0, 0.05, 0.5, or 5 mg/kg/day NNC 90-1170 in cynomolgus monkeys (4/sex/dose) with a 4 week recovery period (2/sex/ control and high dose), there were no unscheduled deaths. The NOAEL was < 0.05 mg/kg/day based on injection site reactions in males and females and inflammatory cell infiltrates in kidney (females) or stomach pylorus (females) at ≥ 0.05 mg/kg. Clinical signs of reddening at the injection site occurred in all treated monkeys, including controls, and it had macroscopic pathology findings of reddening with correlative microscopic hemorrhage. Thickened injection site mainly occurred in NNC 90-1170 treated monkeys. The incidence of thickened injection site increased with dose, along with correlative subcutaneous inflammatory cell infiltration, at ≥ 0.05 mg/kg. In the 5 mg/kg high dose group, microscopic injection site findings were subcutaneous sclerosis, foreign material, and foreign body giant cells. Treatment-related injection site reactions persisted after a 4 week recovery, but their severity was somewhat diminished.

Toxicokinetic analysis on day 1 and in week 52 showed systemic exposure increased with NNC 90-1170 subcutaneous dosing across the dose range with no substantial difference between sexes or accumulation with repeat dosing. Three high dose monkeys had confirmed anti-NNC 90-1170 antibody responses and the anti-liraglutide antibodies cross reacted with GLP-1. Based on the assay format (precipitation of IgG bound radioactivity after addition of exogenous [^{125}I]NNC 90-1170) and since 2 of the monkeys in the high dose recovery group didn't test positive in week 52, the plasma liraglutide might have interfered with the assay.

There were no treatment-related changes in ophthalmology, ECG, or urinalysis parameters. Body weight decreased up to 18% compared to controls at ≥ 0.5 mg/kg in males only, but food consumption was only transiently decreased on study day 1 at ≥ 0.5 mg/kg in males and at 5 mg/kg in females. Reduced body weight is considered a pharmacologic effect of GLP-1R agonists. A mild anemia characterized by decreased RBC, Hb, and Hct occurred at ≥ 0.5 mg/kg from week 26 to week 52, and at 5 mg/kg, reticulocyte count increased in week 52 suggesting a regenerative response. In the absence of evidence of cholestatis and consistent with a mild anemia, elevated total bilirubin at ≥ 0.5 mg/kg in males and at 5 mg/kg in females may be due to hemolysis. Alkaline phosphatase dose-dependently decreased at ≥ 0.05 mg/kg in males, but the specific isozyme affected wasn't identified.

Relative weight of pancreas dose-dependently increased at ≥ 0.05 mg/kg in males and at ≥ 0.5 mg/kg in females. Quantification of pancreatic cells showed increased relative weight of pancreas was due to increased mass of exocrine cells and pancreatic ducts and at 5 mg/kg/day NNC 90-1170. Relative weight (normalized to body weight) of heart was increased in males at ≥ 0.05 mg/kg, but it lacked correlative histopathology findings. Inflammatory cell infiltrates occurred in kidneys (focal interstitial at 5 mg/kg in males and at ≥ 0.05 mg/kg in females) and in the stomach pylorus (in lamina propria at ≥ 0.05 mg/kg in males and at 0.05 and 5 mg/kg in females). Thymus atrophy occurred at 5 mg/kg in males with correlative decreased thymus weight at ≥ 0.05 mg/kg males, and in one high dose male, atrophy correlated to small thymus.

Histopathology inventory (General toxicity studies ≥ 13 weeks)

Study	204082 (13 week)	980189 (13 week)	200239 (26 week)	990191 (13 week)	200241 (52 week)
Species	Mouse	Rat	Rat	Monkey	Monkey
Abnormalities	X	X	X	X	
Identification		X (microchip)		X (tattoo)	X (tattoo)
Adrenals	X	X*	X*	X*	X*
Aorta	X	X	X	X	X
Bone	X (femur, rib))				X
Bone Marrow	X	X	X	X	X
Brain	X*	X*	X*	X*	X*
Bronchi					
Cecum	X	X	X	X	X
Cervix					
Colon	X	X	X	X	X
Diaphragm					
Duodenum	X	X	X	X	X
Epididymis	X*	X*	X*		
Esophagus	X	X	X	X	X
Eyes	X	X	X	X	X
Fallopian tube					
Gall bladder	X (w/ liver)			X	X
Gross lesions					
Harderian gland	X	X	X		
Heart	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X
Injection site	X	X	X	X	X
Jejunum	X	X	X	X	X
Joint (with femur and tibia)					
Kidneys	X* (+ ureters)	X*	X*	X*	X*
Lachrymal gland					
Larynx					
Liver	X*	X*	X*	X*	X*
Lungs	X*	X*	X*	X*	X*
Lymph nodes, cervical					
Lymph nodes, submandibular	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X
Mammary gland	X	X	X	X	X
Nasal cavity (skull)	X				
Optic nerves	X	-	X	X	X
Oviducts					
Ovaries	X*	X*	X*	X*	X*
Pancreas	X	X	X	X*	X*
Parathyroid					
Peripheral nerve					
Pharynx					
Pituitary	X	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*
Rectum	X	X	X	X	X
Salivary gland (submaxillary)	X	X*	X*	X	X
Sciatic nerve	X	X	X	X	X
Seminal vesicles	X	X	X	X	X
Skeletal muscle	X (thigh)	X	X (thigh)	X	X
Skin	X	X	X	X	X
Spinal cord	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*
Sternum	X	X (+ rib)	X (+ rib)	X	X
Stomach	X	X	X	X	X
Teeth					
Testes	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*
Thyroid (w/ parathyroid)	X	X*	X*	X *	X*
Tongue	X	X	X	X	X
Trachea	X	X	X	X	X
Ureters					
Urinary bladder	X	X	X	X	X
Uterus (w/ cervix)	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X
Zymbal gland					

*Organs weighed, paired organs weighed separately with total weight reported

2.6.6.4 Genetic toxicology

Study title: Reverse mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan-requiring strains of Escherichia coli

Key findings:

- In experiment 1, NNC 90-1170 significantly increased the number of revertant colonies in the presence and absence of rat liver S9 metabolic activation in strain TA98 at (-S9 5000 mcg/mL, +S9 6 mcg/mL) and in the presence of S9 in strain 1537 (+S9 30 and 150 mcg/mL).
- NNC 90-1170 did not increase the number of revertant colonies in experiment 2, a confirmatory assay,.
- Exposure to the test article was limited to 1 hour at 37C in solution, the highest dose of 3750 mcg/mL in the presence of S9 was not based on cytotoxicity or solubility. The limit dose is based on test article mass / plate, not test article concentration.
- The sponsor did not demonstrate that NNC 90-1170 interferes with standard format Ames assays (preincubation or plate incorporation) prior to conducting a nonstandard format Ames assay.
- Historical control data from bacterial reverse mutation assays using standard formats may not apply to the assay format used for liraglutide.
- Statistically significantly increases in the number of revertants were not indicated in summary tables from either experiment.

Study no.: 665/230 (———); 980191 (Novo Nordisk) b(4)
Volume #, and page #: 4.2.3.3.1 page 1 – 89
Conducting laboratory and location: ———

GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, Lot # NN22119804, purity 90%
Formulation/vehicle: white powder in vehicle of phosphate buffer pH 7.4 (4 mM monosodium dihydrogenphosphate dihydrate, 4 mM disodium dihydrogenphosphate dihydrate, adjust pH to 7.4) Drug solutions were filter sterilized before use.

Methods

Strains/species/cell line:

S. typhimurium strains TA 98, TA100, TA1535, TA1537
E. coli strains WP2pKM101, WP2uvrA

3 plates/strain/dose with or without S9

Liver S9 mix for metabolic activation from Aroclor 1254 treated male SD rats was purchased from ———. Activity of S9 was verified by ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens and CYP450 alkoxyresorufin-O-dealkylase activities. b(4)

Doses used in definitive study:
Experiment 1
Dose range-finding study doses:

- S9: 8, 40, 200, 1000, 5000 mcg/mL
+ S9: 6, 30, 150, 750, 3750 mcg/mL

Experiment 2

All strains except TA98:
- S9: 50, 158, 500, 1581, 5000 mcg/mL
+ S9: 37.5, 118.6, 375, 1186, 3750 mcg/mL

TA98 only:
-S9: 50, 158, 500, 1581, 5000 mcg/mL
+ S9: 6, 37.5, 118.6, 375, 1186, 3750 mcg/mL

Basis of dose selection:
The maximum dose in the absence of S9 was the limit dose, 5000 mcg/plate. The maximum dose in the presence of S9 was 3750. The maximum dose is the presence of S9 was reduced due to the addition of S9 mix. Lower doses were ~ ½ log unit serial dilutions starting at the highest dose.

Cytotoxicity was not observed for any bacterial strain except thinning of the bacterial lawn for the strain TA100 occurred at the highest concentration in the presence of S9 in the dose-range findings study.

Negative controls:
Phosphate buffer

Positive controls: (prepared in DMSO, except MNNG prepared in water)
(-) S9
Sodium azide (0.75 mcg/plate): TA100 or TA1535
ICR-191 (0.5 mcg/mL): TA1537
2-Nitrofluorene (25 mcg/mL): TA98
4-Nitroquinoline-1-oxide (1 mcg/mL): TA100
N-methyl-N'-nitro-N-nitrosoguanidine : 2.5 mcg/mL TA1535
7.5 mcg/mL E coli

(+) S9
2-aminoanthracene (AAN): 20 mcg/mL E coli
5 mcg/mL TA1535
2.5 mcg/mL TA98, TA100, TA1537

Incubation and sampling times:

This assay was performed using a modified preincubation method in the presence and absence of rat liver S9. Both an exploratory and confirmatory assays used the same assay format. Treatments were performed in triplicate in each experiment.

Bacterial pellets containing ~ 2 x 10^9 cells were resuspended in 1 mL of the treatment solution (with or without S9) and incubated at 37C for 1 hour. After incubation, bacteria were pelleted by centrifugation, resuspended in phosphate buffer, and an aliquot was added to molten soft agar then poured over minimal agar plates. Plates were incubated for 1 to 3 days at 37C, then the number of revertant colonies or continuity of the bacterial lawn was determined.

Assay acceptance criteria were:
1. the number of revertants in concurrent solvent controls are within the historical solvent control range.
2. positive controls induced a clear increase in the number of revertant colonies

3. no more than 5% of plates were lost to contamination or other unforeseen events.

Evaluation criteria were:

1. the assay is valid.
2. Dunnett's test was significant ($p < 0.01$) with a dose-related increase in mutation frequency.
3. Any positive trend was reproducible.

Results

Study outcome:

In experiment 1, there was no evidence of toxicity at any dose, except some bacterial lawn thinning occurred in TA100 plates treated with 3750 mcg/mL NNC 90-1170 in the presence of S9 metabolic activation. No toxicity occurred at any dose with or without S9 in experiment 2.

In experiment 1, NNC 90-1170 statistically significantly increased the number of revertant colonies in the presence and absence of S9 in strain TA98 at (-S9 5000 mcg/mL, +S9 6 mcg/mL) and in the presence of S9 in strain 1537 (+S9 30 and 150 mcg/mL). Because the increases lacked a dose response and because they were not reproducible, they were considered incidental.

Experiment 1

Summary of mean revertant colonies (-S-9)

Substance	Dose Level µg/ml	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP2 uvrA pKM101
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
4mM phosphate buffer	1000 µl	17 ± 5 (M)	111 ± 13	15 ± 4	5 ± 3	35 ± 11	110 ± 8
NNC 90-1170, Glipacyf	8	26 ± 3 (M)	105 ± 7	16 ± 3	8 ± 6 (M)	33 ± 3	111 ± 22
	40	17 ± 2 (M)	106 ± 10	13 ± 1	7 ± 1 (M)	29 ± 3	126 ± 11
	200	24 ± 2 (M)	107 ± 15	13 ± 1	4 ± 2 (M)	32 ± 3	119 ± 12
	1000	21 ± 4 (M)	104 ± 12	12 ± 3	4 ± 3 (M)	28 ± 7	118 ± 7
	5000	38 ± 5 (M)	95 ± 14 (S)	18 ± 6	6 ± 1 (M)	36 ± 12	111 ± 9
Positive controls	Compound	2NF	NQO	MNNG	ICR-191	MNNG	MNNG
	Dose Level	25 µg/ml	1 µg/ml	2.5 µg/ml	0.5 µg/ml	7.5 µg/ml	7.5 µg/ml
	Mean ± SD	264 ± 23 (M)	272 ± 18	1077 ± 65	217 ± 25	216 ± 26	306 ± 17

SD Standard deviation

2NF 2-Nitrofluorene
NQO 4-Nitroquinoline 1-oxide
MNNG N-Methyl-N'-nitro-N-nitrosoguanidine
ICR-191 ICR-191 mutagen

[P29]

Summary of mean revertant colonies (+S-9)

Substance	Dose Level µg/ml	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP2 uvrA pKM101
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
4mM phosphate buffer	750 µl	21 ± 5 (M)	98 ± 16	14 ± 5	4 ± 1 (M)	30 ± 4	113 ± 6
NNC 90-1170, Glipacyl	6	46 ± 3 (M)	122 ± 15	17 ± 5	5 ± 1 (M)	33 ± 6	112 ± 9
	30	26 ± 9 (M)	102 ± 9	15 ± 3	9 ± 3 (M)	27 ± 5	111 ± 3
	150	24 ± 6 (M)	109 ± 12	14 ± 3	9 ± 2 (M)	30 ± 5	119 ± 22
	750	21 ± 3 (M)	121 ± 10	13 ± 7	5 ± 2 (M)	33 ± 2	117 ± 14
	3750	24 ± 3 (M)	113 ± 13 (S)	14 ± 4	4 ± 2 (M)	29 ± 4	105 ± 12
Positive controls	Compound	AAN	AAN	AAN	AAN	-	AAN
	Dose Level	2.5 µg/ml	2.5 µg/ml	5 µg/ml	2.5 µg/ml	-	20 µg/ml
	Mean ± SD	83 ± 60 (M)	326 ± 75	71 ± 7	8 ± 4 (M)	-	447 ± 33

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn
M : Plate counted manually

[P30]

Experiment 2

Summary of mean revertant colonies (-S-9)

Substance	Dose Level µg/ml	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP uvrA pKM101
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
4mM phosphate buffer	1000 µl	20 ± 5	88 ± 7	10 ± 4	3 ± 1 (M)	34 ± 8	93 ± 12
NNC 90-1170, Glipacyl	50	20 ± 11	87 ± 7	14 ± 6	3 ± 2 (M)	31 ± 6	117 ± 10
	158.1	13 ± 5	99 ± 11	11 ± 2	5 ± 2 (M)	28 ± 5	102 ± 9
	500	11 ± 3	91 ± 13	15 ± 3	4 ± 2 (M)	25 ± 4	102 ± 16
	1581	14 ± 4	99 ± 11	12 ± 2	5 ± 2 (M)	23 ± 3	116 ± 5
	5000	16 ± 1	81 ± 7	15 ± 2	6 ± 2 (M)	27 ± 6	107 ± 11
Positive controls	Compound	2NF	NQO	MNNG	ICR-191	MNNG	MNNG
	Dose Level	25 µg/ml	1 µg/ml	2.5 µg/ml	0.5 µg/ml	7.5 µg/ml	7.5 µg/ml
	Mean ± SD	262 ± 110	280 ± 14	1152 ± 46	128 ± 46 (M)	288 ± 32	204 ± 56

SD Standard deviation

2NF 2-Nitrofluorene
NQO 4-Nitroquinoline 1-oxide
MNNG N-methyl-N'-nitro-N-nitrosoguanidine
ICR-191 ICR-191 mutagen

[P43]

Summary of mean revertant colonies (+S-9)

Substance	Dose Level µg/ml	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP2 uvrA pKM101
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
4mM phosphate buffer	750 µl	13 ± 4	95 ± 10	12 ± 3	7 ± 2	18 ± 1	84 ± 15
NNC 90-1170 Glipcyl	6	19 ± 4	-	-	-	-	-
	37.5	16 ± 4	75 ± 10	11 ± 5	8 ± 3	18 ± 5	81 ± 15
	118.6	17 ± 3	84 ± 8	10 ± 3	5 ± 1	21 ± 7	76 ± 4
	375	19 ± 4	91 ± 16	11 ± 4	5 ± 3	20 ± 6	76 ± 13
	1186	18 ± 2	90 ± 4	11 ± 3	10 ± 3	21 ± 4	87 ± 2
	3750	14 ± 5	86 ± 20	10 ± 4	7 ± 1	24 ± 5	85 ± 13
Positive controls	Compound	AAN	AAN	AAN	AAN	-	AAN
	Dose Level	2.5 µg/ml	2.5 µg/ml	5 µg/ml	2.5 µg/ml	-	20 µg/ml
	Mean ± SD	156 ± 34	198 ± 9	27 ± 5	14 ± 5	-	615 ± 37

SD Standard deviation

AAN 2-Aminoanthracene

[P44]

Appendix E: Historical negative (solvent) control values for *S. typhimurium* strains

Strain	- or + S-9	Mean of spontaneous revertants	SD	Range* lower	Range* upper
TA98	-	29	7.3	10	48
	+	34	7.3	15	53
TA100	-	112	18.3	65	160
	+	130	16.0	89	171
TA1535	-	17	5.1	4	30
	+	21	5.3	7	34
TA1537	-	13	5.8	1	28
	+	14	6.7	1	32

* 99% confidence limits about the mean

The above are pooled data from at least 20 consecutive experiments over the period 23.6.97 to 29.7.97.

Appendix F: Historical negative (solvent) control values for *E. coli* strains

Strain	- or + S-9	Mean of spontaneous revertants	SD	Range* lower	Range* upper
WP2 pKM101	-	26	7.8	6	46
	+	42	10.9	14	70
WP2 uvrA pKM101	-	121	23.2	61	181
	+	146	33.3	60	232

* 99% confidence limits about the mean

The above are pooled data from 23 consecutive experiments over the period 7.1.97 to 13.10.97

[P61, 62]

Study title: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes

Key findings:

- NNC 90-1170 was negative for structural chromosomal and numerical aberrations in cultured human peripheral blood lymphocytes in the presence or absence of rat liver S9 metabolic activation.
- Stability of the test article under the assay conditions are unknown.

Study no.: 665/513 [redacted]); 203114 (Novo Nordisk) b(4)

Module and page #: 4.2.3.3.1, pages 1 - 335 b(4)
Conducting laboratory and location: [redacted]

GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch # AB-4A-K4-001, and purity was not specified.
Formulation/vehicle: solution in aqueous vehicle.
Date of study initiation: June 20, 2003

Methods
Strains/species/cell line: Culture human peripheral blood lymphocytes (PBLs) from 3 healthy adult female donors

Liver S9 mix for metabolic activation from Aroclor 1254 treated male SD rats was purchased b(4) from [redacted] .

Doses used in definitive study:
All doses tested
112.6, 140.7, 175.9, 219.9, 343.6, 429.5, 536.9, 671.1, 838.9, 1049, 1311, 1638, 2048, 2560, 3200, 4000, 5000 mcg/mL NNC 90-1170

Doses Scored to Determine Mitotic Index
3 hr incubation + 17 hr recovery –/+ S9: 1049, 1311, 1638, 2048, 2560, 3200, 4000, 5000 mcg/mL

20 hr incubation + 0 hr recovery -S9: 671.1, 838.9, 1049, 1311, 1638, 2048, 2560, 3200, 4000, 5000 mcg/mL

Doses Scored for Chromosomal Aberrations
3 hr incubation + 17 hr recovery –/+ S9: 5000, 3200, 2048 mcg/mL

20 hr incubation + 0 hr recovery -S9: 5000, 4000, 3200 mcg/mL

Negative controls:
Water for injection

Positive controls:
- S9, 4-nitroquinoline-1-oxide (NQO), 2.7 or 5.5 mcg/mL
+S9, cyclophosphamide (CPA), 3.4, 6.9, or 13.7 mcg/mL

Basis of dose selection:
In the absence of any effect on mitotic index under any treatment condition, the highest NNC 90-1170 dose selected was 5,000 mcg/mL, the limit dose.

Incubation and sampling times:
PBLs were exposed to the test article in the absence or presence of rat liver S9 for 3 hours followed by a 17 hour recovery period (+S9 and –S9) or for 20 hours with no recovery period (-S9 only). Duplicate cultures were performed for each treatment condition. To arrest cells in metaphase, colchicine (1 mcg/mL) was added 2 hours prior to harvesting. At harvesting, cells were pelleted, resuspended on hypotonic KCl, then cells were fixed by adding an equal volume of

ice cold methanol/glacial acetic acid. Cells resuspended in fixative were dropped onto microscope slides, stained with Giemsa, and dried and mounted. The effect of treatment on mitotic index was determined from at least 1000 cells/treatment. One hundred cells containing 44 – 46 chromosomes from each culture were scored for chromosome aberrations. Polyploid, endoreduplicated, or hyperploid cells were recorded separately.

Results

Study validity:

Criteria for a valid assay were:

1. binomial dispersion test demonstrates acceptable heterogeneity between replicate cultures.
2. proportion of cells with structural aberrations (excluding gaps) in negative controls is within the historical control range.
3. at least 160 analyzable cells at each dose.
4. positive control statistically significantly increases the number of cells with structural aberrations.

Criteria for a positive response were:

1. statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) in a test article treated group.
2. proportion of cells with structural aberrations at one or more concentration exceeds the historical negative control range in replicate cultures.

Historical control group data from solvent controls are shown in the summary table from report Appendix E below.

Appendix E
NNC 90-1170: historical ranges for solvent controls

Sex and S-9 treatment	Category	Total number of cells scored	Aberrant cells scored per 100 cells		
			Mean	Calculated normal range #	Observed Range
Female -S-9	Structural aberrations including gaps	6600	2.33	3-8	0-9
	Structural aberrations excluding gaps	6600	1.50	3-6	0-7
	Polyploid Cells	5216	0.21	1-2	0-2
	Numerical aberrations	5216	0.31	1-2	0-2
Female +S-9 (Aroclor 1254)	Structural aberrations including gaps	5600	1.66	2-6	0-6
	Structural aberrations excluding gaps	5600	0.95	2-4	0-4
	Polyploid Cells	5018	0.36	1-2	0-3
	Numerical aberrations	5018	0.30	1-2	0-3

Calculated in October 2002
Calculated range = 99% confidence interval

[P41]

The assay was considered valid.

Study outcome:

Inhibition of the mitotic index by NNC 90-1170 was < 50% at all doses under all treatment conditions (data not shown), therefore the highest dose selected for scoring was 5000 mcg/mL, the limit dose. At least 200 cells from 3 dose groups from each assay condition was scored. The following NNC 90-1170 concentrations were scored:

3 hr incubation + 17 hr recovery –/+ S9: 5000, 3200, 2048 mcg/mL

20 hr incubation + 0 hr recovery -S9: 5000, 4000, 3200 mcg/mL

The frequency of cells with structural or numerical aberrations in all NNC 90-1170 treated groups was within the historical control group range.

Table 1
3 hour treatment -S-9, 17 hour recovery (3+17),
Donor sex: female

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Mitotic Index (mean)
Solvent	A	100	1	1	7.3
	B	100	1	1	11.1
	C	ND	ND	ND	8.8
	D	ND	ND	ND	8.1
	Totals	200	2	2	(8.8)
2048	A	100	0	0	9.2
	B	100	1	1	8.3
	Totals	200	1	1	(8.8)
3200	A	100	0	0	8.1
	B	100	1	1	8.6
	Totals	200	1	1	(8.4)
5000	A	100	0	0	10.1
	B	100	0	0	8.0
	Totals	200	0	0	(9.1)
NQO, 5.495	A	100	17	16	
	B	100	15	14	
	Totals	200	32	30[a]	

Binomial Dispersion Test $\chi^2_{2,0.01} = 2.01$, not significant
Note: solvent replicates C and D scored for mitotic index only
[a] Statistical significance $p \leq 0.001$
ND = not determined
Numbers highlighted exceed historical negative control range (Appendix E)

Table 7
3 hour treatment -S-9, 17 hour recovery (3+17),
Donor sex: female

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
2048	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
3200	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
5000	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
NQO, 5.495	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0

** Total cells examined for numerical aberrations
For abbreviations and classification see Appendix B

[P32, 38]

Table 2
3 hour treatment +S-9, 17 hour recovery (3+17),
Donor sex: female

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Mitotic Index (mean)
Solvent	A	100	0	0	10.1
	B	100	0	0	9.8
	C	ND	ND	ND	9.4
	D	ND	ND	ND	10.4
	Totals	200	0	0	(9.9)
2048	A	100	1	0	10.5
	B	100	0	0	12.2
	Totals	200	1	0	(11.4)
3200	A	100	2	1	9.0
	B	100	1	0	9.5
	Totals	200	3	1	(9.3)
5000	A	100	1	0	8.7
	B	100	0	0	8.7
	Totals	200	1	0	(8.7)
CPA, 6.868	A	100	31	30	
	B	100	22	21	
	Totals	200	53	51[a]	

Binomial Dispersion Test χ^2= 1.01, not significant
Note: solvent replicates C and D scored for mitotic index only
[a] Statistical significance $p \leq 0.001$
ND = not determined
Numbers highlighted exceed historical negative control range (Appendix E)

Table 8
3 hour treatment +S-9, 17 hour recovery (3+17),
Donor sex: female

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
2048	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
3200	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
5000	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
CPA, 6.868	A	100	0	0	0	0	0
	B	101	1	0	0	1	1.0
	Total	201	1	0	0	1	0.5

** Total cells examined for numerical aberrations
For abbreviations and classification see Appendix B

[P33,39]

Table 3
20 hour treatment -S-9, 0 hour recovery (20+0),
Donor sex: female

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Mitotic Index (mean)
Solvent	A	100	2	1	5.4
	B	100	1	0	6.2
	C	ND	ND	ND	6.5
	D	ND	ND	ND	6.7
	Totals	200	3	1	(6.2)
3200	A	100	2	0	5.9
	B	100	1	0	6.5
	Totals	200	3	0	(6.2)
4000	A	100	0	0	6.0
	B	100	1	1	5.4
	Totals	200	1	1	(5.7)
5000	A	100	0	0	6.1
	B	100	2	1	6.7
	Totals	200	2	1	(6.4)

Binomial Dispersion Test χ^2= 3.02, not significant
Note: solvent replicates C and D scored for mitotic index only
ND = not determined

Table 9
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 1
Donor sex: female

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
3200	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
4000	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
5000	A	101	0	0	1	1	1.0
	B	101	0	0	1	1	1.0
	Total	202	0	0	2	2	1.0

** Total cells examined for numerical aberrations
For abbreviations and classification see Appendix B

[P34, 40]

Key findings:

- A 4-day repeat dose bone marrow erythrocyte micronucleus assay in male rats treated with 0, 7.5, 15, or 30 mg/kg/day NNC 90-1170 was negative.

Study no.: 665/231 (———); 980192 (Novo Nordisk) b(4)
Module and page #: 4.2.3.3.2.1, pages 1 - 45
Conducting laboratory and location: ———

Date of study initiation: July 27, 1998
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, Batch # NN22119804, 97.7% by RP-HPLC (certificate of analysis on page 40-41)

Methods

Strains/species/cell line: Crl:BR(CD) rats
Range-finding study: male and female rats (5/sex/dose)
Definitive study: male rats only (7/dose)

Doses used in definitive study:
0 (untreated), 0 (vehicle), 7.5, 15, or 30 mg/kg NNC 90-1170 formulated as a solution in 4 mM phosphate buffer (pH 7.4) administered subcutaneously once a day for 4 days to CD rats (7/sex/dose)

Basis of dose selection:
A dose-range finding study using single sc of 0 (vehicle, 4 mM phosphate buffer) or 40 mg/kg NNC 90-1170 administered to rats (4 - 5/sex/dose) showed NNC 90-1170 decreased body weight gain and body weight (summary table below), decreased food consumption (day 1 food consumption was 32 g/day for males and 22 g/day for females in controls, and 8 g/day for males and 7 g/day for females in the 40 mg/kg NNC 90-1170 group), and decreased bone marrow polychromatic erythrocyte / normochromatic erythrocyte (PCE/NCE) ratio (table below). Decreased PCE/NCE ratio demonstrates exposure and toxicity in bone marrow. Based on body weight loss, decreased food consumption, and bone marrow toxicity, 40 mg/kg NNC 90-1170 exceeded the MTD for a multiple dose study.

Body Weight

Treatment (mg/kg/day)	Group	Animal number/sex	Day 1	Day 2	Day 3	Day 4	Day 5
Vehicle control	1	674 M	288	294	302	307	316
		675 M	316	322	331	339	342
		676 M	318	326	336	342	348
		677 M	311	317	329	337	343
		678 M	301	301	313	318	321
	3	684 F	209	212	215	215	218
		685 F	201	205	210	211	208
		686 F	212	215	220	226	230
		687 F	220	219	217	224	231
		688 F	217	209	216	220	223
NNC90-1170, Glipacyl, 40	2	679 M	298	271	258	251	246
		680 M	309	284	274	271	273
		681 M	308	276	267	267	267
		682 M	314	283	273	281	287
	4	689 F	214	195	188	194	193
		690 F	211	191	186	189	198
		691 F	204	191	183	188	191
		692 F	204	183	176	181	181

Treatment group (mg/kg/day)	Kill time (hours)	Sex	Group mean ratio PCE/NCE (± standard deviation)	
			Per sex	Per treatment group
Vehicle control	24	Male	0.70	0.63 ± 0.15
		Female	0.56	
40	24	Male	0.24	0.21 ± 0.15
		Female	0.18	

[P23, 24]

Negative controls:
Vehicle (4 mM phosphate buffer, pH 7.4)
Untreated

Positive controls:
Cyclophosphamide (CPA) solution in physiologic saline, 4 mg/mL

Incubation and sampling times:

Male rats were subcutaneously injected with 0, 7.5, 15 or 30 mg/kg/day NNC 90-1170 for 4 days and sacrificed 24 hours after the last dose. CPA was administered as a single intraperitoneal injection with rats terminated 24 hours after dosing. Because NNC 90-1170 affected hematology parameters in some repeat dose toxicity studies, hematology parameters (RBC, Hb, Hct, Retic, WBC with differential) were assessed in both dose-ranging findings and definitive *in vivo* micronucleus studies. Blood samples from both range-finding and definitive studies clotted before analysis, so the studies were repeated taking blood by cardiac puncture from halothane anesthetized animals.

Bone marrow was obtained from one femur per rat. Bone marrow cell smears were prepared and stained with acridine orange. The PCE/NCE ratio was determined after counting at least 1000 total erythrocytes per rat. At least 2000 PCEs per rat were evaluated for micronuclei.

Results
Study validity

Assay acceptance criteria were:

1. The concurrent vehicle control mean value is within the historical control group range.
2. At least 7 rats/dose available for analysis.
3. The positive control group clearly demonstrates a statistically significant increased frequency of MN-PCE compared to the concurrent vehicle control group.

The sponsor's criteria for a positive test were:

1. statistically significant increase in the frequency of MN-PCE compared to the vehicle control mean at 1 dose.
2. the frequency of MN-PCE exceeds the historical vehicle control range.

The group mean incidence of MN-PCE in untreated and vehicle treated control groups (Appendix A table) were within the historical range for males (0 – 1.2 MN-PCE / 1000 PCE). Historical control group data are shown in table from Appendix G. The incidence of MN-PCE in positive control group was higher than the vehicle control group.

Appendix A: Summary of group mean data

Treatment group (mg/kg/day)	Mean ratio PCE/NCE ± sd	Group mean frequency of micronucleated PCE (per 1000 ± sd)
Vehicle control	0.98 ± 0.44	0.14 ± 0.24
Untreated control	1.16 ± 0.39	0.14 ± 0.24
CPA, 40+	0.58 ± 0.74	4.50 ± 2.90

+ Administered as a single dose
sd Standard deviation

[P30, edited to include only control group data]

Appendix G: Historical vehicle control data

Sex		Group Mean Ratio PCE/NCE*	Group mean frequency of micronucleated PCE (per 1000)*	Animals (%) with 0.1 (or more) micronuclei (per 2000 PCE scored)**					
				0	1	2	3	4	5(+)
Males	Mean	1.26	0.36	47	37	12	2	0	1
	Range	b(4)	b(4)						
Females	Mean	1.06	0.37	52	31	12	3	2	0
	Range	b(4)	b(4)						

b(4)

* Average of group means from 28 consecutive studies at September 1997
Data from 24 and 48 hour sampling times are combined
** Individual animal profile based on the above 28 experiments; data from 277 males and 255 females

[P39]

Study outcome:

Daily subcutaneous injections of 0, 7.5, 15, or 30 mg/kg NNC 90-1170 for 4 days showed NNC 90-1170 reduced body weight gain, body weight, and food consumption at all doses. Food consumption decreased ~50% in NNC 90-1170 treated groups over the 4 day period. Bone marrow toxicity of NNC 90-1170 was evident from the reduced PCE/NCE ratio (Appendix A Summary Table below), decreased reticulocytes, and suppression of WBCs (see summary table below for WBC and reticulocyte data).

Hematology Parameters

NNC 90-1170 Dose (mg/kg/day)	0.0	7.5	15.0	30.0
Parameter	Absolute Value	% Difference from Control		
WBC (x 10^9/L)	14.9	-44.3	-43.6	-38.9
HB (g/dL)	12.5	5.6	8.0	13.6
PCV (%)	39.6	1.3	4.0	7.6
N (x 10^9/L)	3.64	-57.4	-64.3	-61.8
L (x 10^9/L)	10.59	-42.7	-36.9	-32.6
M (x 10^9/L)	0.31	-64.5	-58.1	-64.5
E (x 10^9/L)	0.10	200.0	0.0	80.0
B (x 10^9/L)	0.05	-60.0	-40.0	-40.0
LUC (x 10^9/L)	0.26	-23.1	-38.5	-15.4
RETI (%)	4.3	-62.8	-41.9	-65.1

Micronucleus assay results are summarized in the table from Appendix A. The group mean frequency of MN-PCE in NNC 90-1170 treated male rats was not significantly increased over concurrent vehicle controls. Group mean frequency of MN-PCE were 2-fold higher than concurrent controls in 7.5 and 30 mg/kg NNC 90-1170 groups, but the frequencies in both NNC 90-1170 groups were within the historical control group range and the highest frequency of MN-PCE in individual rats in both groups was ≤ 3, therefore the increased group mean frequencies were not considered biologically significant.

Appendix A: Summary of group mean data

Treatment group (mg/kg/day)	Mean ratio PCE/NCE ± sd	Group mean frequency of micronucleated PCE (per 1000 ± sd)
Vehicle control	0.98 ± 0.44	0.14 ± 0.24
Untreated control	1.16 ± 0.39	0.14 ± 0.24
7.5	0.44 ± 0.27	0.50 ± 0.58
15	0.33 ± 0.22	0.21 ± 0.39
30	0.42 ± 0.16	0.29 ± 0.57
CPA, 40+	0.58 ± 0.74	4.50 ± 2.90

+ Administered as a single dose
sd Standard deviation

Appendix E: Individual animal data: Main study

Treatment (mg/kg/day)	Animal number	PCE/NCE Ratio	PCE*	MN†	MN/1000
Vehicle	566				
	570				
	560				
	557				
	555				
	577				
	562				
Untreated	540				
	538				
	539				
	542				
	572				
	541				
	574				
7.5	551				
	537				
	545				
	550				
	569				
	571				
	553				
15	578				
	573				
	549				
	563				
	544				
	568				
	554				
30	559				
	552				
	564				
	565				
	575				
	547				
	561				
CPA, 40+	576				
	546				
	548				
	556				
	558				
	567				
	543				

† MN (micronucleated PCE) observed
+ Administered as a single dose
* PCE counted

[P30, 36-7]

b(4)

Study title: NNC 90-1170: Assessment of micronucleus frequencies on microscope slide preparations from rats

Key findings:

- NNC 90-1170 did not increase the incidence of micronucleated polychromatic erythrocytes in bone marrow from rats treated with up to 1 mg/kg NNC 90-1170 for 28 days.
- Results of micronucleus frequencies are not valid because:
  - Results from males and females were combined.
  - Peripheral blood from rats is not appropriate for assessment of MN-PCEs when only 2000 PCEs/rat are examined.
  - An inadequate number of peripheral blood samples/sex were assessed at 0.1 mg/kg (males on days 4, 7, 21, 28 and females on days 7, 21, 28), 0.25 mg/kg (males on days 4, 28 and females on days 4, 7, 21) and 1 mg/kg (males on days 0, 4, 28 and females on days 4, 7, 21).

Study no.: 665/255-D5140 ( ); 990072 (Novo Nordisk)
Module and page #: 4.2.3.3.2.1, pages 1 - 37
Conducting laboratory and location: b(4)

Date of study initiation: December 4, 1998
GLP compliance: Yes

QA report: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch433-980813-01, 97.9% (4 week rat toxicity study, study 980183)

Methods

MN-PCEs were assessed in samples of venous blood and bone marrow taken from male and female Sprague Dawley rats in the 4 week toxicity study (study 980183) treated with 0 (vehicle), 0.1, 0.25, or 1 mg/kg/day NNC 90-1170. Blood samples were taken prior to dosing and on study days 4, 7, 14, 21, and 28. Femur bone marrow samples were taken at terminal sacrifice (day 28). Bone marrow samples and acridine orange stained blood samples produced at [redacted] Research were shipped to [redacted] for evaluation. [redacted] provided positive control blood samples by treating rats (5/sex) with cyclophosphamide (20 mg/kg/day CPA in saline injected 24 and 72 hours prior to sampling) and bone marrow sampling at terminal sacrifice (24 hours after the last of 2 doses). Blood samples from CPA treated rats and all bone marrow samples were stained with acridine orange for evaluation of polychromatic erythrocytes (type I and type II reticulocytes in peripheral blood samples). b(4

Basis of dose selection:
The high dose of 1 mg/kg/day NNC 90-1170 in the 4 week repeat-dose toxicity study in rats was the MTD established in a 7-day repeat dose study (study 980180).

Negative controls:
Vehicle (0.71 mg/mL disodium monohydrogenphosphate dihydrate, 0.62 mg/mL monosodium hydrogenphosphate dihydrate, 38 mg/mL mannitol, and 5 mg/mL phenol, pH 7.4)

Positive controls:
20 mg/kg cyclophosphamide (CPA) solution in physiologic saline, 4 mg/mL stock, 10 mL/kg injection volume

Results

Study validity
Assay acceptance criteria were:

1. The positive control (CPA treated rats) statistically significantly increases the frequency of MN-PCE in bone marrow and peripheral blood.

The sponsor's criteria for a positive test were:

1. a statistically significant increase in the frequency of MN-PCE compared to the vehicle control mean at 1 dose in at least 1 sample time.

The incidence of MN-PCE in positive control group (male and female data combined) was higher than the vehicle control group.

Peripheral Blood

Appendix A: Summary of group mean data, Peripheral blood

Group mean ratio †

Treatment group	Days after start of treatment					
(mg/kg/day)	0	4	7	14	21	28
Vehicle	7.84	-	-	-	-	4.74
CPA, 20+	-	0.87	-	-	-	-

† Relative proportions of type I and type II reticulocytes in the reticulocyte population

Group mean frequency of micronucleated reticulocytes, per 1000 (SD)

Treatment group (mg/kg/day)	Days after start of treatment					
	0	4	7	14	21	28
Vehicle	0.20 (0.26)	-	-	-	-	0.25 (0.35)
CPA, 20+	-	16.69 (4.00)	-	-	-	-

+ Administered as two doses only
NS Not significant
SD Standard deviation
‡ Measured against vehicle control at 0 hours

[P24, edited to remove results from NNC 90-1170 groups]

Bone Marrow

Appendix B: Summary of group mean data, Bone marrow

Treatment group (mg/kg/day)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE per 1000 (SD)
Vehicle	1.40	0.35 (0.41)
CPA, 20+	0.07	11.70 (4.50)

+ Administered as two doses only
SD Standard deviation

[P25, edited to remove results from NNC 90-1170 groups]

Study outcome:

Results from male and females in each dose group were combined. The table below shows the number of peripheral blood samples evaluated at each time point. Bone marrow samples from 5 rats/sex/dose were evaluated.

Number of Samples Evaluated

NNC 90-1170 Dose (mg/kg)	Sample Day	Peripheral Blood	
		Male	Female
0	0	5	5
	28	5	5
0.1	0	5	5
	4	3	5
	7	4	4
	14	5	5
	21	4	3
	28	4	3
0.25	0	5	5
	4	4	4
	7	5	2
	14	5	5
	21	5	3
	28	4	5
1	0	3	5
	4	2	3
	7	5	3
	14	5	5
	21	5	4
	28	2	5
CPA, 20 mg/kg	4	4	4

Peripheral blood micronucleus assay results are summarized in the table labeled Appendix A and bone marrow assay results are summarized in the table labeled Appendix B. NNC 90-1170 did not affect the PCE/NCE ratio in peripheral blood or bone marrow. NNC 90-1170 did not increases the frequency of MN-PCEs in peripheral blood or bone marrow at any sample time or at any dose. The incidence of bone marrow MN-PCEs was $\leq$ 3/1000 in any rat in NNC 90-1170 dose groups (Appendix E below), within the historical control group range (see summary table of historical control group results from review of study 980192).

Appendix A: Summary of group mean data, Peripheral blood

Group mean ratio †

Treatment group	Days after start of treatment					
(mg/kg/day)	0	4	7	14	21	28
Vehicle	7.84	-	-	-	-	4.74
0.1	6.50	6.33	8.18	4.33	4.24	4.87
0.25	7.41	9.78	6.54	5.38	5.15	7.02
1	6.94	4.25	6.90	6.22	3.21	4.32
CPA, 20+	-	0.87	-	-	-	-

† Relative proportions of type I and type II reticulocytes in the reticulocyte population

Group mean frequency of micronucleated reticulocytes, per 1000 (SD)

Treatment group	Days after start of treatment					
(mg/kg/day)	0	4	7	14	21	28
Vehicle	0.20 (0.26)	-	-	-	-	0.25 (0.35)
0.1	0.20 (0.35)	0.12 (0.23)	0.19 (0.26)	0.35 (0.34)	0.43 (0.35)	0.21 (0.27)
0.25	0.25 (0.35)	0.12 (0.23)	0.21 (0.39)	0.25 (0.35)	0.12 (0.35)	0.22 (0.26)
1	0.31 (0.37)	0.40 (0.42)	0.38 (0.35)	0.15 (0.24)	0.44 (0.53)	0.29 (0.27)
Linear trend test‡	NS	NS	NS	NS	NS	NS
CPA, 20+	-	16.69 (4.00)	-	-	-	-

+ Administered as two doses only
NS Not significant
SD Standard deviation
‡ Measured against vehicle control at 0 hours

Appendix B: Summary of group mean data, Bone marrow

Treatment group (mg/kg/day)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE per 1000 (SD)
Vehicle	1.40	0.35 (0.41)
0.1	1.05	0.40 (0.52)
0.25	1.64	0.15 (0.34)
1	1.46	0.45 (0.28)
CPA, 20+	0.07	11.70 (4.50)

+ Administered as two doses only
SD Standard deviation

[P24-5]

Appendix E: Individual animal data: Bone marrow

Treatment (mg/kg/day)	Animal/ sex	PCE/NCE† counted	Ratio†	Total PCE	MN PCE	MN PCE/ 1000
Vehicle	1 M					
	2 M					
	3 M					
	4 M					
	161 M					
	41 F					
	42 F					
	43 F					
	44 F					
	46 F					
0.1	11 M					
	12 M					
	13 M					
	14 M					
	15 M					
	51 F					
	52 F					
	53 F					
	54 F					
	55 F					
0.25	21 M					
	22 M					
	23 M					
	24 M					
	25 M					
	61 F					
	62 F					
	63 F					
	64 F					
	65 F					
1.0	31 M					
	32 M					
	33 M					
	34 M					
	35 M					
	71 F					
	72 F					
	73 F					
	74 F					
	75 F					
CPA, 20	1187 M					
	1186 M					
	1185 M					
	1184 M					
	1183 M					
	1192 F					
	1191 F					
	1190 F					
	1189 F					
	1188 F					

b(4)

MN Micronucleated.
PCE Polychromatic erythrocytes
† Relative proportions of polychromatic and normochromatic erythrocytes

[P31]

2.6.6.5 Carcinogenicity

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

Mouse carcinogenicity study review attached as Appendix A.

Key study findings:

- Subcutaneously injected NNC 90-1170 (dorsal surface) was a carcinogen in male and female mice with treatment-related tumors occurring in thyroid C-cells (males and females) and dorsal skin and subcutis (males).

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	focal c-cell hyperplasia (preneoplastic)	< 1%	incidence (%)	0	0	1.5	16.4	38.0
		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	focal c-cell hyperplasia (preneoplastic)	< 1%	incidence (%)	0	0	10.4	15.2	28.9
		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.

- The NOAEL for neoplastic findings was 0.2 mg/kg/day NNC 90-1170 (human safety margin 1.8) based on increased incidence of thyroid C-cell adenomas in males and females and combined C-cell adenomas / carcinomas in females at $\geq$ 1 mg/kg/day NNC 90-1170. Focal thyroid C-cell hyperplasia, a preneoplastic lesion, 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 $\geq$ 0.2 mg/kg/day in males and females, 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.

- Between weeks 25 and 104, plasma calcitonin levels increased > 2 fold at 3 mg/kg/day in males and females.
- Dorsal skin and subcutis fibrosarcomas occurred at 3 mg/kg/day NNC 90-1170 in males. Skin and subcutis fibrosarcomas were attributed to high local drug concentrations at or near injection sites, and not systemic exposure. NNC 90-1170 concentrations in high dose drug formulation was 0.6 mg/mL, 10-times lower than the 6 mg/mL concentration in the clinical formulation.
- 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 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 comparison to controls at 3 mg/kg/day NNC 90-1170 in males. In control group females, the incidence of total sarcomas in the skin and subcutis was elevated.
- 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 $\geq$ 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 $\geq$ 0.2 mg/kg/day in males and females), liver (pigmented Kupffer cells (attributed to hemosiderin accumulation) at $\geq$ 0.03 mg/kg/day in males and at $\geq$ 0.2 mg/kg/day in females, centrilobular hypertrophy, diffuse centrilobular hepatocyte vacuolation at $\geq$ 0.03 mg/kg/day in males), spleen (hemosiderin accumulation at $\geq$ 0.03 mg/kg/day in females), femoro-tibial joint (degenerative disease at $\geq$ 0.03 mg/kg/day in males and at 0.03, 1, and 3 mg/kg/day in females), seminal vesicles (lymphocytic infiltration at $\geq$ 0.03 mg/kg/day and inflammation at 0.03 and 3 mg/kg/day in males), and thymus (tubular cystic hyperplasia at $\geq$ 0.03 mg/kg/day in males and at $\geq$ 0.2 mg/kg/day in females).
- The following protocol modifications and/or issues were identified:
    - 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 to assess anti-liraglutide antibodies (week 78/104 week group with treatment of 78 week interim sacrifice group extended to week 104) 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). Rat calcitonin was used to construct calibration curves and for assay standardization.

- 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 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, combined C-cell adenomas and 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 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, combined C-cell adenomas and carcinomas at ≥ 1 mg/kg in females, and dorsal skin and subcutis fibrosarcomas at 3 mg/kg in males.

b(4)

Study no.: 204229 (sponsor), 457274 (—

Submission, Module, and page #: N000 4.2.3.4.1.1, pages 1 - 3096

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 RPHPLC 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	390	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]

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 0.2, 2, 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 compared to humans).

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 $\geq$ 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 $\geq$ 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 commercial 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 $\geq$ 0.2 mg/kg/day in males and females. Between weeks 26 and 104, plasma calcitonin levels increased $\geq$ 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 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, seminal vesicles, and thymus. In the thyroid, inflammatory cell infiltrate occurred at 0.03 mg/kg/day NNC 90-1170 and thyroid focal C-cell hyperplasia, considered a precursor to C-cell tumors, occurred at $\geq$ 1 mg/kg/day in males and at $\geq$ 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 $\geq$ 0.03 mg/kg/day in males. Liver pigmented Kupffer cells occurred at $\geq$ 1 mg/kg/day in females. Hemosiderin accumulation in spleen occurred at $\geq$ 0.03 mg/kg/day in females. The incidence of degenerative disease in the femoro-tibial joint was above control group levels at $\geq$ 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 $\geq$ 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 (males). Equivocal findings occurred in dorsal skin and subcutis (males), injection site on the dorsal surface (males) and vasculature (males). Thyroid C-cell focal hyperplasia and tumors are rare spontaneous findings in mice. NNC 90-1170 dose-dependently increased the incidence of thyroid focal C-cell hyperplasia, a preneoplastic lesion, and dose-dependently increased the incidence of C-cell adenomas at ≥ 1 mg/kg/day in males and females, and increased the incidence of combined C-cell adenomas / carcinomas at ≥ 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 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 ($p < 0.001$). Skin and subcutis fibrosarcomas are related to high local drug concentration at or near injection sites and not systemic exposure. NNC 90-1170 concentrations in high dose drug formulation was 0.6 mg/mL, 10-times lower than the 6 mg/mL concentration in the clinical formulation. An equivocal finding of increased combined incidence of hemangiomas / hemangiosarcomas at all sites in males at 0.2 mg/kg/day NNC 90-1170, but the increased incidence was not dose related.

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

Rat carcinogenicity study review attached as Appendix B.

Key study findings:

- NNC 90-1170 was carcinogenic in male and female rats with treatment-related thyroid C-cell tumors occurring in males and females. Focal C-cell hyperplasia was considered a precursor of C-cell tumors in rats.
- Increased incidence or severity of focal thyroid C-cell hyperplasia occurred at ≥ 0.075 mg/kg/day in males (HEM ≥ 0.5) and at ≥ 0.25 mg/kg in females (HEM ≥ 2.2).
- 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 and carcinomas at ≥ 0.25 mg/kg/day in males and at ≥ 0.075 mg/kg/day in females.
- 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).
- Because the MRHD was increased from 0.6 mg/day NNC 90-1170 to 1.8 mg/day (AUC_{0-24} increased ~3-fold to 814 nM.hr) during development, the multiple of human exposure based on AUC ratio for the highest dose of 0.75 mg/kg/day (AUC_{0-24} 6,225 nM.hr) in the rat carcinogenicity study was 8.

NNC 90-1170 Tumor Findings in Rats

Result	Organ/Tissue	Neoplasm	Historical Incidence	Sex	Male				Female			
				NNC 90-1170 Dose (mg/kg/day)	0				0			
				Parameter	Trend analysis	0.075	0.25	0.75	Trend analysis	0.075	0.25	0.75
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%).

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. There are no major metabolites of lipid-labeled ^{3}H-[Pal]-liraglutide in humans, and 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 were thyroid 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 $\geq$ 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 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 / [redacted]
Submission, Module, and page #: N000 4.2.3.4.1.1, pages 1 - 1631
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 23 April 2001
GLP compliance: Yes (OECD compliance claimed, but not FDA compliance)
QA report: yes (X) no ( )

Drug, lot #, and % purity: NNC 90-1170 lots shown in the table below. Purity ranged from 97.7 – 98.4% by RP-HPLC (certificates of analysis in Appendix B, report pages 284 - 288).

Test Item

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]

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 liraglutide's peptide moiety. 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, and 8 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 ≥ 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 ≥ 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 ≥ 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 by treatment.

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 ≥ 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 ≥ 0.075 mg/kg/day and focal C-cell hyperplasia / adenomas / carcinomas above historical control group levels at ≥ 0.075 mg/kg/day in males and females.

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

Treatment-related neoplastic findings occurred in thyroid C-cells (males and females). C-cell tumors were considered a progression from focal hyperplasia to benign adenomas to malignant carcinomas. NNC 90-1170 dose-dependently caused C-cell adenomas at ≥ 0.25 mg/kg/day in males (HEM 2) and at ≥ 0.075 mg/kg/day in females (HEM 0.5), C-cell carcinomas at ≥ 0.75 mg/kg/day in males (HEM 8), and combined C-cell adenomas and carcinomas at ≥ 0.075 mg/kg/day NNC 90-1170 in males and 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 ≥ 0.075 mg/kg/day in males and at ≥ 0.25 mg/kg/day in females.

2.6.6.6 Reproductive and developmental toxicology

Combined fertility and embryofetal development

Study title: NNC 90-1170: Main segment I/II subcutaneous reproduction study in rats

Key study findings:

- In sexually mature rats, the NOAEL was 0.25 mg/kg NNC 90-1170 in females based on clinical signs of toxicity (rolling gate, hunched posture) during the first week of dosing and on days 22 - 30. The NOAEL in males was 1 mg/kg, the highest dose tested. Decreased body weight gain and food consumption in parental rats, usually occurring within the first 2 weeks of treatment, were considered pharmacologic effects of NNC 90-1170.
- The NOAEL for reproductive toxicity was 1 mg/kg NNC 90-1170 in males, the highest dose tested. Liraglutide decreased absolute weight compared to concurrent controls of seminal vesicles at ≥ 0.25 mg/kg and prostate and epididymides decreased at 1 mg/kg, but fertility was unaffected and in a 13-week study, liraglutide had no effect on the incidence of abnormalities in sperm. Decreased absolute weight of male reproductive organs was due, at least in part, to decreased body weight. The NOAEL for reproductive toxicity in females was 0.25 mg/kg NNC 90-1170, the highest dose tested.
- The incidence of early embryonic deaths was above concurrent and historical control controls at 1 mg/kg NNC 90-1170.
- The NOAEL for fetal toxicity was < 0.1 mg/kg NNC 90-1170 based on fetal abnormalities of **displaced kidney(s)**, **displaced azygous vein**, and **small additional ossified area within the cranial structure or fontanel** at ≥ 0.1 mg/kg. **Mottled liver** and **minimally kinked rib(s)** at 1 mg/kg) were above concurrent and published historical control groups at 1 mg/kg. Abnormalities considered treatment-related, but not dose-related were **misshaped oropharynx and/or narrowed opening into larynx** at 0.1 mg/kg, **umbilical hernia** at 0.1 and 0.25 mg/kg, and **narrowed azygous vein** at 0.1 and 0.25 mg/kg.
- There was a trend of more complete ossification in NNC 90-1170 groups. There was a treatment-related decrease in incomplete or unossified bones noted in pubis, sacral vertebral arches, 5th metacarpals, and skull bones (≤ 3).
- The historical control group incidence of spontaneous fetal malformations in historical control group Sprague Dawley rats in embryofetal developmental toxicity studies at ——— was submitted in an NDA amendment in June 2009 (Appendix D).

Study no.: 990284
Module and page #: 4.2.3.5.1.1, pages 1 - 175
Conducting laboratory and location: ———
Date of study initiation: 22 November 1999
GLP compliance: Yes (OECD & ———
QA reports: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch 433-991004-01 (— vials) or 433-991103-01 (— vials) (2 mg/mL aqueous solution), 98.0% by HPLC (certificate of analysis page 51).

b(4)

Methods

Doses: 0 (vehicle), 0.1, 0.25, or 1 mg/kg NNC 90-1170
Males: Starting 4 weeks prior to mating through the mating period
Females: Starting 2 weeks prior to mating through gestation day 17

Dose rationale: Doses were selected based on data from a range-finding segment I/II study in rats and based on the anticipated therapeutic dose in humans.

Species/strain: Crl:CD Sprague Dawley rats
Number/sex/group: 24/sex/dose

Group Number	Dose Level ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	
			Males	Females
1	Control	0	1-24	97-120
2	Low dose	0.1	25-48	121-144
3	Intermediate dose	0.25	49-72	145-168
4	High dose	1.0	73-96	169-192

[P17]

Route, formulation, volume: Subcutaneous injection (once a day) on dorsal surface, NNC 90-1170 solution (in aqueous 0.71 mg/mL disodium monohydrogenphosphate dihydrate, 0.62 mg/mL monosodium dihydrogenphosphate dehydrate, 38 mg/mL mannitol, 5 mg/mL phenol, pH 7.4), 1 mL/kg
Satellite groups used for toxicokinetics: None.
Study design:
Treatment

Males were dosed from 4 weeks prior to mating until termination after females most of the females were necropsied (~8 weeks). Females were dosed from 2 weeks prior to mating until gestation day 17.

Mating

Females were transferred to the cage of one male in the same dose group for up to 7 days or until mating was confirmed (in the morning) by the presence of sperm in the vaginal lavage or the presence of a copulatory plug. Mating was confirmed on gestation day 0. If mating was not confirmed after a 7 day period, the female was mated with another male. After mating was confirmed, females were housed individually. The stage of estrus determined from vaginal lavage was recorded.

Antemortem Observations

In life observations were clinical signs and viability, body weight, food consumption, water consumption (visual inspection only, not quantified), confirmation of mating and estrus staging.

Terminal Studies

Adults males were killed ~ 8 weeks after dosing started and adults females were killed on gestation day 20 by CO_2 asphyxiation and exsanguination. Necropsy was performed examining cranial, thoracic, and abdominal contents and reproductive organs were fixed in neutral buffered 10% formalin including ovaries, uterus, cervix, vagina (nonpregnant only), epididymides (weighed), seminal vesicles and coagulating gland (weighed), prostate (weighed), and pituitary gland. Testes (weighed) were preserved in Bouin's fluid.

The reproductive tract from pregnant females was dissected out, weighed, opened, and examined recording the number of corpora lutea graviditatis in ovaries and the number and position of implantation sites in the uterus. Implants were recorded as live fetuses, fetal death (ca gestation day 16), late embryonic death, or early embryonic death.

Live fetuses were killed by chilling on a metal slab at 4C for 5 minutes, examined for external anomalies, weighed, and sexed. Half the viable fetuses were fixed in methylated ethyl alcohol for dissection of thoracic and abdominal viscera followed by maceration in KOH and staining of the skeleton with Alizarin Red S. The remaining viable fetuses were fixed in Bouin's fluid for examination of soft tissue abnormalities by freehand sectioning.

Parameters and endpoints evaluated:

Mating parameters were number of nights to positive signs of mating and number passing one estrus.

Fertility parameters were male and female fertility indices.

$$\text{Fertility Index (male)} = \frac{\text{Number siring a litter}}{\text{Number paired}}$$

$$\text{Fertility Index (female)} = \frac{\text{Number pregnant}}{\text{Number paired}}$$

Pregnancy performance (total copora lutea, number implanted, preimplantation loss (%), uterus weight).
Early embryofetal development (live implant, dead implants, early embryonic deaths, late embryonic deaths, total fetal deaths)

Embryofetal development parameters were total live male fetuses, total female fetuses, fetal sex ratio, mean litter mean fetal weight, major fetal abnormalities, minor fetal abnormalities and variations, skeletal abnormalities, skeletal variations, skeletal ossification.

Results

Mortality:
There were no treatment-related unscheduled deaths.

Clinical signs:
There were no treatment-related clinical signs at 0.1 mg/kg NNC 90-1170. At $\geq$ 0.25 mg/kg, decreased fecal output occurred in the majority of male and female rats. Decreased fecal output, probably secondary to decreased food consumption, occurred within the first day of dosing and lasted for up to day 12 in males and up to day 8 in females. At 1 mg/kg, rolling gait and hunched posture occurred prior to and after mating in females.

Table 1 Group Incidence of Selected Clinical Observations and Necropsy Findings

Observation/Finding	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Males				
Total number of animals observed	24	24	24	24
Decreased faecal output	0	0	20	24
Females				
Total number of animals observed	24	24	24	24
Rolling gait	0	1	0	3
Hunched body	0	0	0	5
Decreased faecal output	0	0	14	24

[P28]

Body weight:

Decreased body weight and body weight gain compared to controls at ≥ 0.1 mg/kg in males and females with the severity and duration of the body weight changes dependent on NNC 90-1170 dose.

In males, body weight was dose-dependently reduced at ≥ 0.1 mg/kg during the first week of treatment with decreased body weight (up to 26 g body weight loss) and body weight gain (up to 620% lower than controls) occurring after the first day of dosing (week 0 to day 1). The effect was transient and body weight gain and body weight increased after the first day in all treatment groups. At 1 mg/kg, body weight gain was 26.4% below the concurrent control group over the 8 week treatment period.

Male Body Weight and Body Weight Gain (n = 24/dose)

Period		Week 0 to Day 1 (prior to mating)				Week 0 to Week 8			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
Parameter									
	N (end)								
Body weight	g, start	327	335	331	329	327	335	331	329
	g, end	332	327	313	303	520	517	506	471
	% difference from control, end	0.0	-1.5	-5.7	-8.7	0.0	-0.6	-2.7	-9.4
Body weight gain (start to end)	g	5	-8	-18	-26	193	182	175	142
	% of starting body weight	1.5	-2.4	-5.4	-7.9	59.0	54.3	52.9	43.2
	% difference from control	0.0	-260	-460	-620	0.0	-5.7	-9.3	-26.4

In females, body weight was dose-dependently decreased up to 18 g after the first dose at ≥ 0.1 mg/kg compared to controls and body weight gain was up to 500% lower (prior to mating, week 0 to day 1), but body weight in the concurrent control group also decreased 3 g after the first dose. NNC 90-1170 related decreased body weight and decreased body weight gain was transient with both parameters approaching control group levels with increased treatment-duration. During the gestation period with dosing stopped on day 17, there were no substantive differences in body weight or body weight gain between control and NNC 90-1170 groups.

Female Body Weight and Body Weight Gain, Prior to Mating and Gestation Periods (n = 24/dose)

Period		Week 0 to Day 1 (prior to mating)				Week 0 to Week 2 (prior to mating)				Gestation Days 0 to 20			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1	0	0.1	0.25	1
Parameter													
	g, start	256	263	274	262	256	263	274	262	276	281	290	268
	g, end	253	257	261	244	274	280	289	270	425	424	435	410
	% difference from control, end	0.0	1.6	3.2	-3.6	0.0	2.2	5.5	-1.5	0.0	-0.2	2.4	-3.5
Body weight gain (start to end)	g	-3	-6	-13	-18	18	17	15	8	149	143	145	142
	% of starting body weight	-1.2	-2.3	-4.7	-6.9	7.0	6.5	5.5	3.1	54.0	50.9	50.0	53.0
	% difference from control	0.0	100.0	333	500	0.0	-5.6	-16.7	-55.6	0.0	-4.0	-2.7	-4.7

Food consumption:

NNC 90-1170 transiently decreased food consumption in males and females. At ≥ 0.1 mg/kg, food consumption decreased during the first 3 days of treatment. At 0.25 mg/kg, food consumption decreased in males for the first 10 days of treatment and in females for the first 3 days. At 1 mg/kg, food consumption decreased the first 3 days of treatment in both sexes and during gestation days 0 – 13.

Table 5 Males: Group Mean Food Consumption (g.rat^{-1}.day^{-1})

Day of Treatment	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1}) 1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
-4	31.6	32.3	32.5	32.1
0	31.9	32.1	31.9	31.2
3	33.1	27.0	21.8	16.3
7	33.0	31.7	29.6	26.3
10	33.1	31.0	29.4	26.8
14	31.9	31.3	30.9	27.9
17	33.9	32.9	31.8	28.4
21	30.5	30.5	30.2	27.8
24	33.9	32.7	32.0	28.9
28	36.3	34.4	32.9	30.6
45	33.3	32.0	31.7	29.2
49	32.4	32.6	32.3	29.8
52	31.8	31.4	31.4	29.3
56	31.6	31.2	31.5	30.0

Day 0 = First day of dosing

Table 6 Females: Group Mean Food Consumption (g.rat^{-1}.day^{-1}) During the Pre-Mating Period

Day of Treatment	Group/Dose Level (mg NNC 90-1170 kg^{-1}.day^{-1}) 1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
-4	22.6	22.5	23.8	23.4
0	19.9	22.3	23.0	23.2
3	23.7	21.4	19.0	14.8
7	21.3	21.4	20.0	18.4
10	22.8	22.0	21.1	19.4
14	21.9	21.0	21.1	19.9

Day 0 = First day of dosing

Table 7 Females: Group Mean Food Consumption (g.rat^{-1}.day^{-1}) During Gestation

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1}) 1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Day of Gestation[a]				
0-6	30.2	28.8	28.2	25.5
6-13	31.9	29.5	28.9	26.8
13-20	32.4	31.5	32.8	31.4

a = Pregnant animals only

[P32-34]

Toxicokinetics:

Toxicokinetics were not performed in this study, but TK parameters were determined in a dose-range finding study using the same NNC 90-1170 doses and dosing schedule (study 980186).

Necropsy and organ weight:

There were no treatment-related macroscopic pathology findings in parental males or females.

Group mean body weight at necropsy was 9.6% lower than concurrent control at 1 mg/kg in males. Absolute weight of seminal vesicles was dose-dependently reduced up to 17.7% compared to controls at ≥ 0.25 mg/kg. At 1 mg/kg, absolute weight of epididymides was 6.2% lower than concurrent control and absolute weight of prostate was 18.6% lower. Decreased absolute weight of epididymides, prostate, and seminal vesicles in NNC 90-1170 treated groups was due, at least in part, to decrease body weight.

Male Reproductive Organ Weights (n = 23 or 24/dose)

NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1
		Value	% Difference from Control		
body (g)		510	-0.8	-2.2	-9.6
epididymides	g	1.45	-1.1	-3.7	-6.2
	% of bw	0.285	-0.3	-1.6	3.8
prostate	g	0.824	1.3	-8.5	-18.6
	% of bw	0.162	2.1	-6.5	-9.9
seminal vesicles	g	2.48	-5.7	-8.1	-17.7
	% of bw	0.487	-4.9	-6.0	-9.0
testes	g	3.72	-1.3	-3.5	-3.0
	% of bw	0.729	-0.6	-1.4	7.4

Statistically signficant differences from control are underlined ($p < 0.05$).

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

NNC 90-1170 had no effect on the fertility index in males or females (Table 8).

Table 8 Mating Performance and Fertility Indices

Number of Nights to Positive Mating Sign	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
	Number of animals (Number of these not becoming pregnant)			
1	5	2	1	3
2	6	9	10	10
3	8	6	8	7
4	5	7	4	4
5	0	0	1(1)	0
Median number of nights to positive mating sign	3	3	3	2
Number passing one oestrus	0	0	0	0
Number of males paired	24	24	24	24
Number of siring males	24	24	23	24
Male Fertility Index (%)	100	100	96	100
Number of females paired	24	24	24	24
Number pregnant	24	24	23	24
Female Fertility Index (%)	100	100	96	100

[P35]

NNC 90-1170 had no effect on the pregnancy frequency (96 – 100%) or implantation (Table 9). At 1 mg/kg, the incidence of live implants was slightly lower and the incidence of dead implants was slightly higher than concurrent controls with most of the deaths (31/32) occurring in the early embryonic stage. Six fetal deaths occurred at 0.1 mg/kg with 5/6 in 1 litter whose surviving fetuses were smaller than the control group (2.05 g/fetus versus 3.79 g/fetus for controls). In the absence of a dose-response, these fetal deaths were considered incidental. The incidence of early embryonic deaths was above the incidence in concurrent and historical control groups at 1 mg/kg.

Table 9 Pregnancy Performance and Foetal Weight

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Number of animals mated	24	24	24	24
Number pregnant	24	24	23	24
Number of premature decedents	0	0	0	0
Number pregnant at Day 20 necropsy	24	24	23	24
Pregnancy frequency as %	100	100	96	100
Total corpora lutea graviditatis	391	394	346	375
Total number of implants	373	369	345	365
Pre-implantation loss as %	5	6	0	3
Total live implants (%)	356 (95)	343 (93)	332 (96)	333 (91)
Total dead implants (%)	17 (5)	26 (7)	13 (4)	32 (9)
Total early embryonic deaths (%)	16 (4)	19 (5)	11 (3)	31 (8)
Total late embryonic deaths (%)	1 (0.3)	1 (0.3)	2 (1)	1 (0.3)
Total foetal deaths (%)	0	6 (2)	0	0
Mean corpora lutea graviditatis	16.3 ± 1.8	16.4 ± 1.7	15.7 ± 2.3	15.6 ± 1.6
Mean implants	15.5 ± 2.1	15.4 ± 2.6	15.0 ± 2.5	15.2 ± 2.2
Mean live implants	14.8 ± 2.3	14.3 ± 3.3	14.4 ± 2.7	13.9 ± 2.8
Mean dead implants	0.7 ± 0.9	1.1 ± 1.8	0.6 ± 0.8	1.3 ± 1.6
Mean early embryonic deaths	0.7 ± 0.9	0.8 ± 1.1	0.5 ± 0.7	1.3 ± 1.6
Mean late embryonic deaths	0.04 ± 0.2	0.04 ± 0.2	0.1 ± 0.3	0.04 ± 0.2
Mean foetal deaths	0	0.3 ± 1.0	0	0
Total live male foetuses (%)	185 (52)	163 (48)	174 (52)	163 (49)
Total live female foetuses (%)	171 (48)	180 (52)	158 (48)	170 (51)
Live foetal sex ratio (♂:♀)	1:1.34	1:1.24	1:0.98	1:1.16
Mean total uterus weight (g)	89 ± 11	86 ± 18	87 ± 16	83 ± 16
Mean litter mean foetal weight (g)	3.79 ± 0.25	3.75 ± 0.43	3.75 ± 0.20	3.73 ± 0.23

Means are given ± Standard Deviation

[P36]

Reviewer note: Historical control average fetal body weight is 5.51g for males (range 5.27 – 5.98) and 5.21g for females (range 4.93 – 5.78). Average fetal weight in control and liraglutide dose groups was substantially below these values.

Offspring (malformations, variations, etc.):

Visceral Abnormalities

Two major fetal abnormalities occurred, but neither was dose-related. **Misshaped oropharynx and/or narrowed opening into larynx** in 3 fetuses in 1 litter in the 0.1 mg/kg group exceeded levels in concurrent and published historical controls. The relation of misshapen oropharynx and/or narrowed larynx to treatment was equivocal because it occurred in only one low dose group litter. **Umbilical hernia** occurred in 2 pups in 2 different litters, one in the 0.1 mg/kg group and one in the 0.25 mg/kg group. There were no instances of umbilical hernia in concurrent and historical controls (Appendix D).

Minor abnormalities considered treatment-related were **narrowed azygous vein** at 0.1 and 0.25 mg/kg, **displaced azygous vein** at ≥ 0.1 mg/kg, **mottled liver** at 1 mg/kg liraglutide, and **displaced kidney(s)** at ≥ 0.1 mg/kg.

Minor abnormalities in the pituitary (small cavity at 0.1 mg/kg only), aorta (aortic arch lumen minimally misshapen, 0.25 mg/kg only), and aorta (ductus arteriosis narrowed, 0 and 1 mg/kg) were not considered treatment-related because they only occurred in a few litters, at a low incidence at or near concurrent or historical control group values, and the incidence was unrelated to dose.

Table 10 Group Incidence of Major Foetal Abnormalities

Abnormality	Group/ Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
	Incidence of Foetuses (Litters)			
Retinal split	1(1)	0	0	0
Oropharynx misshapen and/or opening into larynx narrowed	0	3(1)	0	0
Rib(s) kinked	0	0	1(1)	0
Interventricular septal defect	0	0	0	1(1)
Diaphragmatic hernia	0	1(1)	0	0
Umbilical hernia	0	1(1)	1(1)	0
Testes undescended	0	0	0	1(1)
Vertebral column terminating in sacral region; tail thread-like	1(1)	0	1(1)	0
Number with major abnormality	2(2)	4(2)	3(3)	2(2)
Total number examined	355[a](24)	343(24)	332(23)	333(24)

a = one foetus assumed lost during processing

[P37]

Skeletal Abnormalities

Small additional ossified area within the cranial structure or fontanel occurred at ≥ 0.1 mg/kg The incidence of minimally kinked rib(s) was above concurrent and published historical control groups at 1 mg/kg. There was a trend of more complete ossification in NNC 90-1170 groups. The incidence of fetuses that did not have any parameters of incomplete ossification increased with dose (51, 65, 66, and 66% in 0, 0.1, 0.25, and 1 mg/kg groups, respectively). A corresponding treatment-related decrease in incomplete or unossified bones was noted in pubis, sacral vertebral arches, 5th metacarpals, and skull bones (≤ 3).

Abnormality/Variant	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
	Incidence of Foetuses (Litters)			
Visceral				
Subcutaneous haemorrhage:				
Head	4(3)	3(2)	3(2)	1(1)
Trunk (includes haemorrhages within fat pads and muscular tissue)	4(4)	1(1)	2(1)	1(1)
Limbs	0	1(1)	0	0
Tail (annular)	1(1)	3(2)	1(1)	0
Extradural haemorrhage	1(1)	1(1)	0	1(1)
Subdural haemorrhage	0	1(1)	0	0
Lateral brain ventricles minimally dilated	1(1)	1(1)	0	0
Olfactory brain ventricles minimally dilated	1(1)	0	0	0
Pituitary gland small cavity	0	1(1)	0	0
Minimal haemorrhage aqueous chamber of eye	0	0	2(2)	0
Eye(s) enlarged	5(3)	2(2)	1(1)	0
Eye(s) reduced (in size)	1(1)	1(1)	0	0
Eye(s) oval (in shape)	2(2)	0	0	1(1)
Thyroid reduced (in size)	1(1)	0	0	0
Cervical remnant of thymus	12(8)	8(7)	14(9)	11(5)
Innominate artery absent	0	1(1)	3(3)	2(2)
Innominate artery lengthened	1(1)	0	0	0
Origins of arteries arising from aortic arch displaced	2(2)	1(1)	0	0
Aortic arch lumen minimally misshapen	0	0	1(1)	0
Ductus arteriosus narrowed	1(1)	0	0	2(2)
Azygos vein narrowed	0	2(2)	1(1)	0
Azygos vein displaced (includes right-sided)	0	1(1)	1(1)	2(2)
Heart minimal abnormal rotation	1(1)	0	1(1)	0
Small interventricular septal defect	1(1)	0	0	0
Lung lobes fused	1(1)	0	0	0
Minimal protrusion of median liver lobe with thinning diaphragm	14(10)	6(6)	3(2)	1(1)
Protrusion of median liver lobe with thinning diaphragm	13(7)	5(4)	2(2)	1(1)
Additional liver lobe within median cleft	27(17)	17(12)	12(9)	7(7)
Liver mottled	0	0	0	2(1)
Hepatic haemorrhage	0	0	1(1)	0
Intra-abdominal haemorrhage	2(2)	3(3)	1(1)	0
Spleen misshapen	0	0	0	1(1)
Kidney(s) displaced	0	1(1)	3(2)	2(2)
Renal pelvis/es dilated	4(2)	1(1)	2(1)	1(1)

[P38]

Abnormality/Variant	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
	Incidence of Foetuses (Litters)			
Ureter(s) dilated	10(6)	4(3)	10(4)	8(5)
Testis/es displaced	2(2)	0	4(4)	3(2)
Umbilical artery dilated	2(2)	1(1)	0	0
Umbilical artery left-sided	3(3)	0	2(2)	3(3)
Small foetus	1(1)	10(3)	2(2)	1(1)
Visceral - probable fixation artefact[A]				
Subdural space (brain/spinal cord) increased	3(3)	0	0	0
Subcutaneous space(s)	6(4)	1(1)	2(2)	0
Number with minor visceral abnormality/variant	89(23)	62(21)	62(20)	42(21)
Number examined by Wilson sectioning	178(24)	173(24)	166(23)	167(24)
Total number examined viscerally	356(24)	343(24)	332(23)	333(24)
Skeletal				
Small additional ossified area within cranial suture or fontanelle	0	2(1)	3(3)	1(1)
Cranial suture minimal deviation from normal course	1(1)	0	0	0
Cervical rib(s)	1(1)	1(1)	0	0
Sternebra 6 ossified area elongated	1(1)	0	0	0
Rib(s) minimally kinked	1(1)	1(1)	1(1)	8(5)
Costal cartilage asymmetrically aligned	1(1)	0	0	0
Pelvic girdle unilateral caudal displacement	1(1)	0	0	1(1)
Caudal vertebrae locally constricted	1(1)	2(2)	1(1)	0
Number with minor abnormality/variant	5(5)	6(5)	5(5)	10(7)
Total number examined skeletally	177(24)	170(24)	166(23)	166(24)
Number of ribs				
13 reduced ribs	2(1)	0	0	0
13 complete ribs	155(24)	156(24)	154(23)	150(24)
Vestigial supernumerary rib(s) on 1st lumbar vertebra	20(10)	14(9)	11(8)	16(8)
14th reduced supernumerary rib(s)	0	0	1(1)	0

[A] Excluded from number with minor visceral abnormality/variant

[P39]

Table 12 Group Incidence of Skeletal Ossification Parameters

Parameter	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
	Incidence of Foetuses (Litters)			
Incomplete ossification affecting:				
≥ 4 skull bones	6(3)	4(2)	3(2)	3(3)
≤ 3 skull bones	36(16)	21(8)	14(8)	24(13)
Cervical vertebral arch(es)	2(2)	3(2)	0	1(1)
Thoracic vertebral centrum/a	8(8)	5(4)	3(3)	3(3)
Pubis(es)	11(8)	2(2)	1(1)	0
Ischium/a	1(1)	1(1)	0	0
Sacral vertebral centrum/a	1(1)	0	0	0
Sacral vertebral arch(es)	19(12)	10(7)	4(4)	6(5)
2nd, 3rd and/or 4th metacarpal(s)	1(1)	1(1)	1(1)	0
Unossified:				
5th metacarpal(s)	59(20)	41(15)	46(14)	30(13)
5th metatarsal(s)	0(0)	3(1)	0	0
Any with incomplete ossification[a]	87(23)	59(18)	57(19)	56(20)
Ossified:				
Anterior arch of atlas	53(19)	54(19)	56(20)	50(17)
>2 cervical vertebral centra	18(8)	25(12)	12(7)	29(14)
Phalangeal elements	20(9)	54(16)	36(10)	39(15)
Mean number of caudal vertebral centra	3.8	4.0	3.9	4.0
Number of sternebrae retarded:				
0	51(18)	72(19)	73(20)	56(18)
1	81(23)	61(20)	66(22)	65(23)
2	42(16)	29(13)	26(13)	39(17)
3	1(1)	5(3)	1(1)	6(4)
>3	2(2)	3(3)	0	0
Total number examined	177(24)	170(24)	166(23)	166(24)

a = Includes unossified 5th metacarpal(s) and 5th metatarsal(s)

[P40]

Summary and Conclusions

In a dose range-finding combined fertility and embryofetal development toxicity study of subcutaneously injected 0, 0.1, 0.25, or 1 mg/kg NNC 90-1170 in Sprague Dawley rats administered to males 4 weeks prior to mating and during the mating period and administered to females from 2 weeks prior to mating to gestation day 17 (study 980186), the paternal and maternal NOAELs were < 0.1 mg/kg with clinical signs of toxicity (hunched posture, rolling gate) at ≥ 0.1 mg/kg. A high dose female (rat 79) died from an unspecified accidental injury on study day 32 with no clinical signs of toxicity prior to death. Body weight gain and food consumption were transiently decreased in all NNC 90-1170 groups compared to controls, but these were considered pharmacological effects. Body weight of 1 mg/kg males was lower than controls at the end of the study. Reproductive effects were decreased absolute weight of seminal vesicles at ≥ 0.25 mg/kg and prostate at 0.25 mg/kg, but relative weight or seminal vesicles and prostate were < 10% lower than controls. There were no effects on mating performance, fertility, or pregnancy performance or necropsy findings related to treatment. Three fetal abnormalities were noted: a dam in the 0.1 mg/kg group had a fetus with a thread-like tail and absent anus and a second fetus had an

absent tail and possible absent anus. A dam in the 0.25 mg/kg group had a fetus with subcutaneous edema, shortened head, and slightly shortened tail. Due to the low incidence and absence of a dose response, the relation to treatment of fetal abnormalities was equivocal. Toxicokinetic parameters showed there were no substantive differences between mated rats and unmated rats from a 13 week study.

Table 1 Summary of TK parameter estimates after single dose

Day	Dose (mg/kg)	C_{max} (nmol/l)	t_{max} (hr)	AUC_t (hr*nmol/l)
1	0.10	60	8	680
	0.25	158	8	1980
	1.00	612	8	9148
	Mean	-	8	-

Table 2 Summary of TK parameter estimates at steady state

Day	Dose (mg/kg)	C_{max} (nmol/l)	t_{max} (hr)	AUC_t (hr*nmol/l)
17	0.10	75	4	691
(of gestation)	0.25	214	8	2693
	1.00	1241	4	9211
	Mean	-	5	-

[P108]

In a definitive combined fertility and embryofetal development toxicity study of subcutaneously injected 0, 0.1, 0.25, or 1 mg/kg NNC 90-1170 in Sprague Dawley rats administered to males 4 weeks prior to mating and during the mating period and administered to females from 2 weeks prior to mating to gestation day 17 (study 980186), the NOAEL in sexually mature females was 0.25 mg/kg with clinical signs of toxicity (hunched posture, rolling gate) at 1 mg/kg. The NOAEL in sexually mature males was 1 mg/kg, the highest dose tested. Body weight gain and food consumption were transiently decreased in all NNC 90-1170 groups compared to controls, but these were considered pharmacological effects. Transiently decreased fecal output at $\geq$ 0.25 mg/kg was attributed to decreased food consumption. After 8 weeks of treatment, body weight was 9.6% lower in males at 1 mg/kg NNC 90-1170 compared to controls. By gestation day 20, there were no significant treatment-related differences in body weight or body weight gain of pregnant dams. There were no adverse effects of up to 1 mg/kg NNC 90-1170 in males.

The NOAEL for reproductive toxicity was 0.1 mg/kg NNC 90-1170 in males based on decreased absolute weight compared to concurrent controls of seminal vesicles at $\geq$ 0.25 mg/kg and prostate and epididymides at 1 mg/kg. Decreased absolute weight of male reproductive organs was due, at least in part, to decreased body weight. Sperm count in epididymides or testes and sperm viability were not determined in this study, but in a 13 week repeat sc dose toxicity study in SD rats, 1 mg/kg NNC 90-1170 had no effect on the incidence of abnormalities in eosin-stained sperm from the cauda epididymis (one thousand sperm/rat in study 980189). The NOAEL for reproductive toxicity in females was 0.25 mg/kg NNC 90-1170, based on an increased incidence of early embryonic deaths at 1 mg/kg, the highest dose tested. There were no other effects on mating performance, fertility, or necropsy findings related to treatment.

The NOAEL for fetal toxicity was < 0.1 mg/kg liraglutide based on fetal abnormalities of **displaced kidney(s), displaced azygous vein**, and **small additional ossified area within the cranial structure or fontanel** at $\geq$ 0.1 mg/kg. **Mottled liver** and **minimally kinked rib(s)** at 1 mg/kg, **misshaped oropharynx and/or narrowed opening into larynx** at 0.1 mg/kg and **umbilical hernia** and **narrowed azygous vein** at 0.1 and 0.25 mg/kg exceeded the incidence in concurrent and historical control groups (incidence by fetus or litter). There was a trend of more complete ossification in NNC 90-1170 groups with a treatment-related decrease in incomplete or unossified bones noted in pubis, sacral vertebral arches, 5th metacarpals, and skull bones ($\leq$ 3).

Embryofetal development

Study title: NNC 90-1170: Developmental toxicity study in rabbits

Key study findings:

- In dose-range findings studies in adult female rabbits, there were no substantive differences in pharmacologic activity of NNC 90-1170 in unmated and mated rabbits with decreased food consumption being dose-limiting at 0.4 mg/kg in unmated females.
- In a definitive embryofetal development toxicity study in New Zealand White rabbits, the NOAEL for maternal toxicity was 0.05 mg/kg NNC 90-1170, the highest dose tested.
- Decreased food consumption with corresponding transiently decreased body weight, body weight gain, and fecal output at ≥ 0.01 mg/kg in mated females were considered pharmacological effects due to GLP-1R agonist activity of NNC 90-1170.
- The NOAEL for developmental toxicity was < 0.01 mg/kg based on decreased fetal weight compared to controls, dose-related increased incidence of total major fetal abnormalities (2.1%, 3.7%, 5.7%, and 7.6% of fetuses and 18%, 30%, 35%, and 32% of litters affected by major abnormalities in 0, 0.01, 0.025, and 0.05 mg/kg NNC 90-1170 groups, respectively), and increased minor abnormalities and variations at 0.1 or ≥ 0.1 mg/kg NNC 90-1170.
- Major fetal abnormalities occurred in 5 fetuses from 1 litter in the 0.05 mg/kg NNC 90-1170 group with connected parietals bones and 2 fetuses from 2 different litters in the 0.025 mg/kg group with hydrocephaly.
- At 0.05 mg/kg, incidences of incompletely ossified or unossified superior angle of lamina (bone unspecified), slight downward shift of pelvic bones, and slight asymmetric alignment of pelvic bones were increased above control group levels.
- The historical control group incidence of spontaneous fetal malformations in New Zealand White rabbits was not included in the original submission. The reviewer referenced a 1983 publication from Stadler et al. (Fd Chem. Toxic. Vol 21 (5): 631-636) to determine the background incidence of fetal malformations. The sponsor submitted historical control group data in June 2009 (Appendix E).

Study no.: 990055
Module and page #: 4.2.3.5.1.1, pages 1 - 125 b(4)
Conducting laboratory and location: [redacted]
Date of study initiation: 10 March 1999
GLP compliance: Yes (OECD & [redacted]
QA reports: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch 433-980811-01 (2 mg/mL aqueous solution), 86.8 – 87.6% (certificate of analysis page 33).

Methods

Doses: 0 (vehicle), 0.01, 0.025, or 0.05 mg/kg NNC 90-1170 once a day to pregnant females from gestation days 6 – 18 (mated on gestation day 0).

Dose rationale: Doses were selected based on maternal findings of decreased body weight gain, reduced food consumption, and reduced fecal output at 0.1 mg/kg NNC 90-1170, and fetal findings of reduced body weight at 0.1 mg/kg in a dose-range finding reproductive toxicity study of NNC 90-1170 subcutaneously administered to pregnant rabbits during the period of organogenesis..

Species/strain: New Zealand White rabbits
Number/sex/group: 20 pregnant females / dose

Group	Treatment and Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})		Animal Numbers
1	Control:	0	1-20
2	Low Dose:	0.01	21-40
3	Intermediate Dose:	0.025	41-60
4	High Dose:	0.05	61-80

[P16]

Route, formulation, volume: Subcutaneous injection (once a day) at one of 4 dose sites (sites unspecified), NNC 90-1170 solution (in aqueous 0.71 mg/mL disodium monohydrogenphosphate dihydrate, 0.62 mg/mL monosodium dihydrogenphosphate dehydrate, 38 mg/mL mannitol, 5 mg/mL phenol, pH 7.4), 0.1 – 0.5 mL/kg
Satellite groups used for toxicokinetics: None.
Study design:
Treatment

Pregnant females were subcutaneously injected once a day from gestation days 6 – 18.

Antemortem Observations

In life observations were clinical signs and viability, body weight, and food consumption.

Terminal Studies

Adults females were killed on gestation day 29 by rapid intravenous injection of sodium pentobarbitone (150 – 200 mg/kg).

Adult females were necropsied with macroscopic examination of thoracic and abdominal cavities and representative samples of abnormal tissues fixed in neutral buffered 10% formalin. The reproductive tract was dissected out, weighed intact, and the uterus was opened and the contents were examined recording the number of corpora lutea graviditatis in ovaries and the number and position of implantation sites in the uterus. Implants were recorded as live fetuses, fetal death (ca gestation day 18), late embryonic death, or early embryonic death.

Live fetuses were euthanized by intrathoracic or intraperitoneal injection of sodium pentobarbitone, examined for external anomalies, weighed, and sexed. Eyes and cranial bones were examined after removal of skin covering these areas. Fetuses were dissected to allow examination of thoracic and abdominal viscera and the cranium was sectioned through the coronal suture to inspect the brain in that region. Eviscerated carcasses were fixed in methylated ethyl alcohol followed by maceration in KOH and staining of the skeleton with Alizarin Red S.

Parameters and endpoints evaluated:

The sponsor did not perform statistical analysis on any data.

Pregnancy performance parameters were total corpora lutea, number implanted, preimplantation loss (%), uterus weight.

Early embryofetal development parameters were live implant, dead implants, early embryonic deaths, late embryonic deaths, total fetal deaths.

Embryofetal development parameters were total live male fetuses, total live female fetuses, fetal sex ratio, litter mean fetal weight, major fetal abnormalities, minor fetal abnormalities and variations, skeletal abnormalities, skeletal variations, skeletal ossification.

Results

Mortality:

Rabbit 69 had a suspected broken leg during the acclimatization period (before treatment), so it was replaced by rabbit 81.

Clinical signs:

Increased incidence of reduced fecal output corresponding to reduced food consumption was dose-related at ≥ 0.01 mg/kg. There was a low incidence of soft and/or small feces.

Table 1 NNC 90-1170
Developmental Toxicity Study in Rabbits
Group Incidence of Clinical Observations

Observation / Finding	Group /Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (0.01)	3 (0.025)	4 (0.05)
Reduced fecal output	1	10	14	20
Soft feces	0	2	1	1
Small feces	0	1	3	3

[P22]

Body weight:

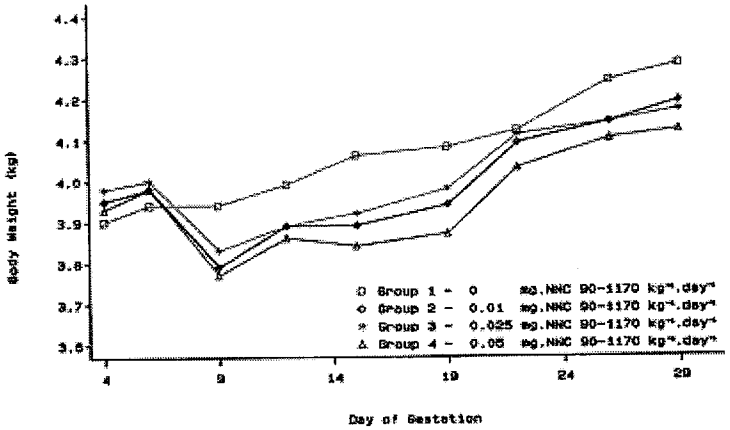
Body weigh and body weight gain of pregnant rabbits decreased compared to controls at ≥ 0.0.1 mg/kg NNC 90-1170 after the first 3 days of treatment (gestation days 6 to 9). The effect on body gain was transient at 0.01 and 0.025 mg/kg with body weight gain similar to controls from gestation days 9 – 19, but by the end of the treatment period (gestation days 6 – 18), body weight was lower than concurrent controls in all NNC 90-1170 groups (Figure 1 and summary table below). Body weight gain during the post-treatment period (gestation days 19 to 29) was similar to controls, but body weight remained lower in all NNC 90-1170 groups.

Female Body Weight and Body Weight Gain, Prior to Mating and Gestation Periods (n = 24/dose)

Period		Gestation Days 6 to 9 (first 3 doses)				Gestation Days 6 to 19 (treatment period)				Gestation Days 19 to 29 (post-treatment)			
NNC 90-1170 (mg/kg/day)		0	0.01	0.025	0.05	0	0.01	0.025	0.05	0	0.01	0.03	0.05
Parameter													
Body weight	kg, start	3.95	3.98	4.00	3.98	3.95	3.98	4.00	3.98	4.11	3.94	3.98	3.87
	kg, end	3.95	3.79	3.83	3.77	4.11	3.94	3.98	3.87	4.30	4.19	4.17	4.12
	% difference from control, end	0.0	-4.1	-3.0	-4.6	0.0	-4.1	-3.2	-5.8	0.0	-2.6	-3.0	-4.2
Body weight gain (start to end)	kg	0.00	-0.19	-0.17	-0.21	0.16	-0.04	-0.02	-0.11	0.19	0.25	0.19	0.25
	% of starting body weight	0.0	-4.8	-4.3	-5.3	4.1	-1.0	-0.5	-2.8	4.6	6.3	4.8	6.5
	% difference from control	N/C	N/C	N/C	N/C	0.0	-125.0	-112.5	-168.8	0.0	31.6	0.0	31.6

N/C = not calculated (denominator = 0)

Figure 1 NNC 90-1170
Developmental Toxicity Study in Rabbits
Group Mean Body Weight (kg)



[P30]

Food consumption:

NNC 90-1170 decreased food consumption in males and females at all doses during the treatment period from gestation days 6 to 19 (24 hours after the last dose on day 18). The magnitude of decreased food consumption was generally dose-related with the largest effect occurring during the first 3 days of treatment. During the post-treatment period (gestation days 19 – 29), food consumption in NNC 90-1170 groups was similar to or above control group levels.

Table 3 NNC 90-1170
Developmental Toxicity Study in Rabbits
Group Mean Food Consumption (g)

Day of Gestation	Group Dose Level (mg kg^{-1}.day^{-1})			
	1 (0)	2 (0.01)	3 (0.025)	4 (0.05)
4	319	322	337	330
5	343	347	352	354
6	354	339	342	351
7	334	38	18	10
8	335	25	15	8
9	338	34	38	30
10	339	67	70	57
11	359	93	83	65
12	333	84	75	62
13	325	49	61	49
14	317	55	46	35
15	323	42	46	38
16	323	57	55	48
17	340	69	58	52
18	349	74	72	61
19	338	84	87	75
20	328	137	146	123
21	317	147	149	143
22	328	144	142	159
23	352	143	141	154
24	333	134	136	145
25	334	135	133	142
26	325	122	124	121
27	315	117	114	123
28	300	108	116	108
29	307	107	100	105
Total Consumed Days 7-19	1728	769	324	588
% of Control	-	45	42	34

Note: Non-pregnant animals have been excluded from calculations

[P32-34]

Toxicokinetics:

Toxicokinetics were not performed in this study, but TK parameters in pregnant female rabbits were determined in a dose-range finding study using dose of 0, 0.01, 0.03, and 0.1 mg/kg NNC 90-1170

(study 980188) on gestation day 6 (after the first dose) and gestation day 16 (after the 11th dose). Cmax and AUC0-24 from this study are included in the Summary and Conclusions section.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

There were no treatment-related macroscopic pathology findings in adult females.

Pre-implantation loss and late embryonic deaths were increased in groups 3 and 4, but these findings are incidental because NNC 90-1170 treatment didn't start until gestation day 6. NNC 90-1170 did not affect survival or sex of the fetus. Gravid uterus weight and mean litter mean fetal weight were lower in NNC 90-1170 treated groups compared to controls. Gravid uterine weight in the 0.025 mg/kg group was significantly lower than controls ($p < 0.05$) and fetal weight was significantly lower ($p < 0.05$) at $\geq$ 0.01 mg/kg.

Table 4 NNC 90-1170
Developmental Toxicity Study in Rabbits
Pregnancy Performance and Foetal Weight

	Group/Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (0.01)	3 (0.025)	4 (0.05)
Number of animals mated	20	20	20	20
Number pregnant	17	20	17	19
Number of premature decedents	0	0	0	0
Number pregnant at Day 29 necropsy	17	20	17	19
Pregnancy frequency as %	85	100	85	95
Total corpora lutea graviditatis	170	196	168	196
Total number of implants	155	176	144	167
Pre-implantation loss as %	9	10	14	15
Total live implants (%)	138 (89)	161 (91)	122 (85)	144 (86)
Total dead implants (%)	17 (11)	15 (9)	22 (15)	23 (14)
Total early embryonic deaths (%)	9 (6)	10 (6)	8 (6)	12 (7)
Total late embryonic deaths (%)	5 (3)	1 (1)	10 (7)	10 (6)
Total foetal deaths (%)	3 (2)	4 (2)	4 (3)	1 (1)
Mean corpora lutea graviditatis	10.0 ± 2.1	9.8 ± 2.0	9.9 ± 1.5	10.3 ± 2.3
Mean implants	9.1 ± 1.8	8.8 ± 2.6	8.5 ± 1.8	8.8 ± 2.5
Mean live implants	8.1 ± 1.8	8.1 ± 2.4	7.2 ± 1.6	7.6 ± 2.3
Mean dead implants	1.0 ± 1.2	0.8 ± 0.8	1.3 ± 1.5	1.2 ± 1.3
Mean early embryonic deaths	0.5 ± 0.8	0.5 ± 0.7	0.5 ± 0.7	0.6 ± 0.8
Mean late embryonic deaths	0.3 ± 0.5	0.1 ± 0.2	0.6 ± 1.0	0.5 ± 1.0
Mean foetal deaths	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.6	0.1 ± 0.3
Total live male foetuses (%)	73 (53)	81 (50)	56 (46)	70 (49)
Total live female foetuses (%)	65 (47)	80 (50)	66 (54)	74 (51)
Live foetal sex ratio (♂:♀)	1:0.89	1:0.99	1:1.18	1:1.06
Mean total uterus weight (g)	549 ± 80	512 ± 133	482 ± 92	504 ± 100
Mean litter mean foetal weight (g)	43.9 ± 4.2	40.5 ± 4.6	41.3 ± 4.0	41.3 ± 3.8

Means are given ± Standard Deviation
Note: Premature decedents excluded below double line

[P25]

Offspring (malformations, variations, etc.):

The following table summarizes the incidence of fetal abnormalities. Historical control incidence of fetal abnormalities were not included in the original submission. A 1983 publication by Stadler et al. (Fd Chem Toxic Vol 21(5): 631-636) reported the incidence of spontaneous malformations occurring

over a 10 year period in teratogenicity studies examining over 5,500 control group New Zealand White rabbit fetuses. The sponsor submitted historical control group data from [redacted] in June 2009 (Appendix E). b(4)

Incidence (%) of Major and Minor Fetal Abnormalities in Rabbits

Fetus or Litter	Fetus				Litter				Background Incidence (% of Fetuses Affected)*
N	138	161	122	144	17	20	17	19	
NNC 90-1170 dose (mg/kg)	0	0.01	0.025	0.05	0	0.01	0.025	0.05	
Major Abnormalities Total	2.2	3.7	5.7	7.6	17.6	30.0	35.3	31.6	
hydrocephaly	0.0	0.0	1.6	0.0	0.0	0.0	11.8	0.0	0.1 (4/3185)
microphthalmia w or w/o retinal folds	0.0	0.6	0.8	0.7	0.0	5.0	5.9	5.3	0
dilated ascending aorta, narrow pulmonary trunk	0.7	0.0	0.8	1.4	5.9	0.0	5.9	10.5	NL
right kidney only small area of tissue with attached cyst	0.0	0.6	0.0	0.0	0.0	5.0	0.0	0.0	0.06 (2/3185), kidney agenesis, same incidence of polycystic kidney
hepatic duct diverticulum	0.0	0.0	0.8	0.0	0.0	0.0	5.9	0.0	NL
displaced or herniated umbilica	0.0	0.0	0.8	0.0	0.0	0.0	5.9	0.0	0 (umbilical hernia)
forelimb flexure	0.0	1.2	0.8	0.7	0.0	10.0	5.9	5.3	NL
Craniofacial bone disorganization	0.0	0.0	0.8	0.0	0.0	0.0	5.9	0.0	NL
large unossified areas of parietals	0.0	0.0	0.8	0.0	0.0	0.0	5.9	0.0	NL
connected parietals	0.0	0.0	0.0	3.5	0.0	0.0	0.0	5.3	NL
fused frontals	0.0	0.0	0.8	0.0	0.0	0.0	5.9	0.0	NL
multiple vertebral abnormalities w fused ribs	0.0	0.6	0.0	0.0	0.0	5.0	0.0	0.0	NL
brachyury	0.0	0.0	0.8	0.0	0.0	0.0	5.9	0.0	NL
split sternum	0.0	0.0	0.8	0.7	0.0	0.0	5.9	5.3	NL
curved scapula	0.0	0.6	0.0	0.0	0.0	5.0	0.0	0.0	NL
Minor Abnormalities or Variations Total	26.8	0.0 0.0 28.6	18.0	39.6	76.5	85.0	70.6	89.5	NL
corneal opacity	0.0	0.0	0.0	0.7	0.0	0.0	0.0	5.3	NL
esophageal cyst	0.0	0.0	0.0	0.7	0.0	0.0	0.0	5.3	NL
intermediate lung lobe absent	0.0	1.2	0.0	0.0	0.0	10.0	0.0	0.0	0.8 (25/3185)
bilobed or bifurcated gall bladder	0.0	3.1	4.9	3.5	0.0	20.0	35.3	26.3	NL
additional liver lobe within median cleft	0.0	0.0	0.8	0.0	0.0	0.0	5.9	0.0	NL
ovarian cyst	0.0	0.0	0.8	0.7	0.0	0.0	5.9	5.3	0.06 (2/3185)
kinked tail	0.0	0.0	0.0	0.7	0.0	0.0	0.0	5.3	0.04 (2/5592), twisted tail
jugal(s) fused to maxilla	2.2	5.6	4.9	10.4	17.6	30.0	23.5	42.1	NL
superior angle of lamina of axis incompletely ossifies	0.0	0.6	1.6	2.1	0.0	5.0	11.8	15.8	NL
slight downward pelvic shift	1.4	2.5	2.5	6.3	11.8	20.0	17.6	21.1	NL
slight asymmetric alignment of pelvic bones	0.0	0.6	0.0	2.8	0.0	5.0	0.0	21.1	NL
comma of hyoid bent inward	2.9	7.5	2.5	8.3	17.6	45.0	11.8	36.8	NL
ossification irregularity / unossified area	0.7	5.6	1.6	4.9	5.9	25.0	11.8	26.3	NL
complete supernumerary rib(s) on 13th vertebrae	21.0	32.3	48.4	42.4	64.7	80.0	94.1	84.2	NL

*Incidence of spontaneous malformations from Stadler et al.(Fd Chem. Toxic. 1983; 21(5):631 - 636). NL = not listed in as a finding in Stadler et al. (1983).

The incidence of major fetal abnormalities was higher in liraglutide treated groups compared to control with 2.1%, 3.7%, 5.7%, and 7.6% of fetuses and 18%, 30%, 35%, and 32% of litters affected in 0, 0.01, 0.025, and 0.05 mg/kg liraglutide groups, respectively. The incidence of minor abnormalities or variations exceeded concurrent control groups at 0.01 and 0.05 mg/kg for individual fetuses or litters. The incidence of major and minor abnormalities in litters from liraglutide treated groups exceeded concurrent controls for the following abnormalities:

Major and Minor Fetal Abnormalities by NNC 90-1170 Dose

0.01 mg/kg only:

Major: right kidney represented by small area of tissue with attached cyst, curved scapula

Minor: intermediate lung lobe absent

0.01 and 0.05 mg/kg groups only:

Minor: slight asymmetric alignment of pelvic bones

≥ 0.01 mg/kg:

Major: microophthalmia with or without retinal fold, forelimb flexure

Minor: bilobed or bifurcated gall bladder, jugals fused to maxilla, superior angle of lamina of axis incompletely ossified, slight downward pelvic shift,

0.025 mg/kg:

Major: hydrocephaly, hepatic duct diverticulum, displaced or herniated umbilica, brachyury, dilated pulmonary trunk with incomplete aortic arch and malrotated heart, displaced umbilicus with part of intestine adhered to umbilical vein

Minor: additional liver lobe within median cleft

≥ 0.025 mg/kg:

Major: split sternum

0.05 mg/kg:

Major: connected parietals, dilated ascending aorta and narrow pulmonary trunk

Minor: corneal opacity, esophageal cyst, kinked tail

Tables 5 and 6 show the group incidence of major and minor fetal abnormalities, respectively. Table 7 shows fetal ossification parameters.

Table 5 NNC 90-1170
Developmental Toxicity Study in Rabbits
Group Incidence of Major Foetal Abnormalities

Abnormality	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (0.01)	3 (0.025)	4 (0.05)
	Incidence of Foetuses (Litters)			
Hydrocephaly	0	0	2 (2)	0
Gross disorganisation of craniofacial bones	0	0	1 (1)	0
Large unossified area in both parietals	0	0	1 (1)	0
Fused frontals	0	0	1 (1)	0
Connected parietals	0	0	0	5 (1)
Microphthalmia with/without retinal fold	0	1 (1)	1 (1)	1 (1)
Narrow ascending aorta	1 (1)	0	0	0
Retroesophageal right subclavian artery	2 (2)	1 (1)	0	1 (1)
Dilated ascending aorta, narrow thread like pulmonary trunk	1 (1)	0	1 (1)	2 (2)
Multiple vertebral irregularities with fused ribs	0	1 (1)	0	0
Dilated pulmonary trunk, incomplete aortic arch, malrotated heart	0	0	1 (1)	0
Forelimb flexure	0	2 (2)	1 (1)	1 (1)
Curved scapula	0	1 (1)	0	0
Split sternum	0	0	1 (1)	1 (1)
Right kidney represented by small area of tissue with cyst attached	0	1 (1)	0	0
Hepatic duct diverticulum	0	0	1 (1)	0
Displaced umbilicus/umbilical hernia, part of intestine adhered to umbilical vein	0	0	1 (1)	0
Brachyury	0	0	1 (1)	0
Number with major abnormality	3 (3)	6 (6)	7 (6)	11 (6)
Total number examined	138 (17)	161 (20)	122 (17)	144 (19)

[P26]

Table 6 NNC 90-1170
Developmental Toxicity Study in Rabbits
Group Incidence of Minor Foetal Abnormalities and Variants

Abnormality/Variant	Group/Dose Level (mg kg^{-1}.day^{-1})			
	1 (0)	2 (0.01)	3 (0.025)	4 (0.05)
	Incidence of Foetuses (Litters)			
Visceral				
Dilated lateral ventricles	1 (1)	0	0	1 (1)
Corneal opacity	0	0	0	1 (1)
Cyst on right side of oesophagus	0	0	0	1 (1)
Variation in origin of minor arteries arising from aortic arch	6 (4)	4 (4)	3 (3)	3 (1)
Lungs unexpanded	1 (1)	1 (1)	0	0
Absent intermediate lung lobe	0	2 (2)	0	0
Additional lobe of liver within median cleft	0	0	1 (1)	0
Intestines and bladder distended	0	0	1 (1)	0
Bilobed or bifurcated gall bladder	0	5 (4)	6 (6)	5 (5)
Dilated ureter	0	1 (1)	0	0
Cystic ovary	0	0	1 (1)	1 (1)
Kinked tail	0	0	0	1 (1)
Number with visceral abnormality	8 (6)	13 (12)	12 (9)	11 (9)
Total number examined	138 (17)	161 (20)	122 (17)	144 (19)
Skeletal				
Sutural bone	2 (2)	5 (4)	2 (1)	2 (2)
Ossification irregularity/unossified area	1 (1)	9 (5)	2 (2)	7 (5)
Coronal of hyoid bent in/outwards	4 (3)	12 (9)	5 (2)	12 (7)
Connected/fused jugal(s) to maxilla	5 (5)	9 (6)	6 (4)	15 (8)
Additional area of ossification ventral to 1st cervical centrum	0	0	1 (1)	0
Cervical rib	12 (6)	0	1 (1)	10 (5)
Superior angle of lamina(e) of axis incompletely ossified/unossified	0	1 (1)	2 (2)	3 (3)
Hemicentric cervical centrum	0	0	1 (1)	0
Interrupted rib	0	0	1 (1)	0
Fused/connected sternebra(e) with/without slight dorsal ventral dislocation of sternum	3 (2)	3 (3)	1 (1)	2 (2)
Asymmetric alignment/floating/additional costal cartilage element	3 (3)	2 (2)	1 (1)	1 (1)
Additional sternebral centre anterior to 1st	2 (2)	0	2 (2)	2 (2)
Vertebral arch defect(s)	1 (1)	0	1(1)	2 (2)
Slight upward pelvic shift	15 (5)	6 (3)	0	7 (2)
Slight downward pelvic shift	2 (2)	4 (4)	3 (3)	9 (4)
Slight asymmetric alignment of pelvic bones	0	1 (1)	0	4 (4)
One extra pre-sacral vertebra	0	1 (1)	0	0
Misaligned caudal vertebra	0	0	0	1 (1)
Number of Ribs				
12 Complete ribs	86 (16)	71 (19)	40 (14)	46 (17)
Vestigial supernumerary rib(s) on 13th thoracic vertebra	11 (6)	18 (13)	10 (8)	20 (11)
Reduced supernumerary rib(s) on 13th thoracic vertebra	12 (8)	20 (13)	13 (12)	17 (11)
Complete supernumerary rib(s) on 13th thoracic vertebra	29 (11)	52 (16)	59 (16)	61 (16)
Number with skeletal abnormality	37 (13)	46 (17)	22 (12)	57 (17)
Total number examined	138 (17)	161 (20)	122 (17)	144 (19)

[P27-8]

Table 7 NNC 90-1170
Developmental Toxicity Study in Rabbits
Group Incidence of Skeletal Ossification Parameters

Parameter	Group/Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (0.01)	3 (0.025)	4 (0.05)
	Incidence of Foetuses (Litters)			
Overall skeletal retardation (foetuses in this category excluded from subsequent categories)	0	0	0	0
Retarded skull bone(s)	2 (1)	4 (2)	1 (1)	1 (1)
Anterior fontanelle enlarged	0	0	0	0
Hyoid retarded	0	3 (3)	1 (1)	2 (1)
One cervical centrum retarded/unossified	26 (10)	25 (8)	15 (7)	16 (9)
Pubes retarded	0	1 (1)	0	0
All 8 epiphyses of fore and hindlimbs unossified	2 (1)	1 (1)	1 (1)	2 (2)
Up to 7 epiphyses of fore and hindlimbs unossified	49 (13)	76 (18)	52 (14)	57 (16)
Unossified metacarpal and/or phalanx on pollex/pollices	3 (2)	3 (3)	0	2 (2)
Unossified metacarpal(s) on 2nd to 5th digit(s) of hindpaw	0	0	0	0
Unossified 2nd phalanx on 2nd to 5th digit(s) of forepaw(s)	22 (6)	26 (9)	19 (7)	33 (13)
Unossified 1st/2nd phalanx on 2nd to 5th digit(s) of hindpaw(s)	4 (2)	7 (5)	2 (2)	4 (3)
Astragali retarded	0	0	0	0
Olecranon(s) ossified	9 (7)	5 (4)	5 (3)	1 (1)
Number of sternebrae incompletely ossified				
0	106 (17)	123 (20)	84 (17)	102 (19)
1	28 (15)	31 (15)	34 (13)	36 (13)
2	3 (3)	7 (5)	4 (4)	6 (5)
>2	1 (1)	0	0	0
Total number examined	138 (17)	161 (20)	122 (17)	144 (19)

[P29]

Summary and Conclusions

Toxicity of NNC 90-1170 was evaluated in 2 dose range-findings studies using unmated or mated female New Zealand White rabbits. Transiently decreased food consumption and corresponding decreased fecal output, decreased body weight gain, and decreased body weight in unmated and mated female rabbits were considered pharmacological effects of NNC 90-1170, but the severity of decreased food consumption was considered dose limiting at 0.4 mg/kg in unmated females. There were no substantive differences in the toxicity of NNC 90-1170 in unmated and pregnant female rabbits.

In study 980187, unmated female New Zealand White rabbits (2/dose) were subcutaneously injected with 0.4 mg/kg NNC 90-1170 for 3 days (group 1, dose level 1), 0.1 mg/kg for 2 days (group 2), 0.02 mg/kg for 13 days (group 3), 0.01 mg/kg for 13 days (group 4), or 0.1 mg/kg for 7 days (group 1, dose level 5) (study). Study parameters were clinical signs, body weight, food consumption, and toxicokinetics (groups 3 and 4 only). Rabbits treated with 0.4 mg/kg didn't eat during the 3 day treatment period and consequently lost body weight. Five days after dosing was stopped, food consumption and

body weight gain resumed. At 0.1 mg/kg (group 2), dosing was halted after the 2nd day due to decreased food consumption and decreased body weight. At 0.02 (group 3) and 0.01 mg/kg (group 4), NNC 90-1170 transiently decreased food consumption and body weight during the 13 day treatment period with food consumption recovering after the first day of treatment and body weight remaining stable throughout the dosing period. A second group of females treated with 0.1 mg/kg NNC 90-1170 showed decreased food consumption and body weight loss were transient during the 7 day treatment period. The dose of minimal toxicity in unmated female rabbits was 0.1 mg/kg NNC 90-1170 based on the severity of decreased food consumption and decreased body weight at 0.4 mg/kg. NNC 90-1170 plasma Cmax and $AUC_{0\text{-}24h}$ increased with dose.

In a second dose-range findings study using mated females (6/dose, study 980188), New Zealand White rabbits were dosed with 0, 0.01, 0.03, or 0.1 mg/kg NNC 90-1170 on gestation days 6 – 18 (day of mating was gestation day 0). Study parameters were clinical signs, body weight, food consumption, toxicokinetics, and on gestation day 22, gross necropsy of adult females, external examination of fetuses for visible anomalies, and recording of litter and fetal weight. There were no unscheduled deaths. The duration and severity of transiently decreased food consumption with decreased fecal output and decreased body weight was NNC 90-1170 dose-related at all doses. During treatment, no body weight gain occurred at 0.1 mg/kg and only slight body weight gain occurred at ≤ 0.03 mg/kg. Mean litter mean fetal weight was 8.0% lower than concurrent controls at 0.1 mg/kg. The dose of minimal toxicity in pregnant rabbits was ≥ 0.1 mg/kg.

Table 5 NNC 90-1170
Dose Range Finding Study in Rabbits Preliminary Developmental Toxicity Study
Mated Phase: Pregnancy Performance and Foetal Weight

	Group/Dose Level (mg.kg^{-1}.day^{-1})			
	5 (0)	6 (0.01)	7 (0.03)	8 (0.1)
Number of animals mated	6	6	6	6
Number of premature decedents	0	0	0	0
Number pregnant at Day 22 necropsy	5	5	5	5
Pregnancy frequency as %	83	83	83	83
Total corpora lutea graviditatis	54	54	54	47
Total number of implants	45	47	45	39
Pre-implantation loss as %	17	13	17	17
Total live implants (%)	44 (98)	46 (98)	37 (82)	36 (92)
Total dead implants (%)	1 (2)	1 (2)	8 (18)	3 (8)
Total early embryonic deaths (%)	0	1 (2)	7 (16)	1 (3)
Total late embryonic deaths (%)	0	0	0	1 (3)
Total foetal deaths (%)	1 (2)	0	1 (2)	1 (3)
Mean corpora lutea graviditatis	10.8 ± 1.5	10.8 ± 1.8	10.8 ± 0.8	9.4 ± 1.1
Mean implants	9.0 ± 3.2	9.4 ± 1.8	9.0 ± 1.6	7.8 ± 2.9
Mean live implants	8.8 ± 2.9	9.2 ± 1.6	7.4 ± 3.4	7.2 ± 2.9
Mean dead implants	0.2 ± 0.4	0.2 ± 0.4	1.6 ± 2.5	0.6 ± 0.9
Mean early embryonic deaths	0	0.2 ± 0.4	1.4 ± 2.6	0.2 ± 0.4
Mean late embryonic deaths	0	0	0	0.2 ± 0.4
Mean foetal deaths	0.2 ± 0.4	0	0.2 ± 0.4	0.2 ± 0.4
Mean total uterus weight (g)	187 ± 60	243 ± 58	199 ± 50	179 ± 64
Mean litter mean foetal weight (g)	6.64 ± 0.43	6.55 ± 0.49	6.51 ± 0.77	6.11 ± 0.45

Means are given ± Standard Deviation

[Report 980188 P32]

Toxicokinetic parameters were determined in unmated females after the 10th daily dose of 0.01 or 0.02 mg/kg NNC 90-1170 and in mated female rabbits (pregnant) on gestation days 6 (after the first

dose) and 16 (after the 11th dose) using dose of 0, 0.01, 0.03, and 0.1 mg/kg NNC 90-1170 (studies 980187 and 980188).

Plasma Toxicokinetics

Parameter		AUC_{0-24} (nM.h)				Cmax (nM)			
NNC 90-1170 Dose (mg/kg/day)		0.01	0.02	0.03	0.1	0.01	0.02	0.03	0.1
Female Rabbits	Treatment Day[1]								
Unmated	10	140	245	--	--	10	17	--	--
Mated	1	125	--	288	571	9	--	19	36
	11	148	--	280	766	11	--	17	51

[1]Treatment days 1 and 11 correspond to gestation days 6 and 16 in mated females.

In a definitive embryofetal development toxicity study of subcutaneously injected 0, 0.01, 0.025, or 0.05 mg/kg NNC 90-1170 administered to New Zealand White rabbits from gestation days 6 to 18 in with terminal sacrifice on day 29, the NOAEL for maternal toxicity was 0.05 mg/kg NNC 90-1170, the highest dose tested. In mated females, there were no treatment-related unscheduled deaths, clinical signs of toxicity, or necropsy findings. Decreased food consumption with corresponding transiently decreased body weight, body weight gain, and fecal output at $\geq$ 0.01 mg/kg were considered pharmacological effects of NNC 90-1170.

The incidence of major fetal abnormalities was higher in liraglutide treated groups compared to control with 2.1%, 3.7%, 5.7%, and 7.6% of fetuses and 18%, 30%, 35%, and 32% of litters affected by major abnormalities in 0, 0.01, 0.025, and 0.05 mg/kg liraglutide groups, respectively. The NOAEL for fetal toxicity was < 0.01 mg/kg based on decreased fetal weight compared to controls, dose-related increased incidence of total major fetal abnormalities, major fetal abnormalities (microophthalmia with or without retinal fold, forelimb flexure, right kidney represented by small area of tissue with attached cyst, curved scapula,), and minor abnormalities and variations (bilobed or bifurcated gall bladder, jugals fused to maxilla, superior angle of lamina of axis incompletely ossified, slight downward pelvic shift, intermediate lung lobe absent, slight asymmetric alignment of pelvic bones) at 0.01 or $\geq$ 0.01 mg/kg NNC 90-1170. At 0.025 mg/kg, major fetal abnormalities were hydrocephaly in 2 fetuses from 2 different litters, hepatic duct diverticulum, displaced or herniated umbilica, brachyury, dilated pulmonary trunk with incomplete aortic arch and malrotated heart, and displaced umbilicus with part of the intestine fused to the umbilical vein and minor abnormality of an additional liver lobe within the median cleft. The major fetal abnormality of split sternum occurred at $\geq$ 0.025 mg/kg. At 0.05 mg/kg, major fetal abnormalities were connected parietal bones in 5 fetuses from 1 litter and dilated ascending aorta and narrow pulmonary trunk and minor abnormalities were corneal opacity, esophageal cyst, and kinked tail.

Prenatal and postnatal development

Study title: NNC 90-1170: Pre and post natal study in rats (subcutaneous administration)

Key study findings:

- There were no unscheduled deaths considered NNC 90-1170 related.
- The F_0 generation was subcutaneously dosed from gestation day 6 to weaning, the F_1 generation was exposed to NNC 90-1170 in utero and while nursing (NNC 90-1170 in milk from F_0), and the F_2 generation was never exposed.

- The F_0 maternal NOAEL was < 0.1 mg/kg NNC 90-1170 based on clinical signs of hunched posture, piloerection, and wet coat at ≥ 0.1 mg/kg, unkept coat at 0.1 and 1 mg/kg, and at 1 mg/kg, scabbing, rolling gait, body held low, and reduced activity.
- In F_0 dams NNC 90-1170 transiently decreased body weight and body weight gain, and food consumption were considered pharmacological effects. Decreased body weight gain was transient, but decreased body weight persisted to gestation day 20 at ≥ 0.25 mg/kg. Post partum body weights (lactation day 1) were significantly lower than controls in all NNC 90-1170 groups, but by the end of the 24 day lactation period, group mean body weight was significantly lower than controls in 1 mg/kg dams only.
- The NOAEL for F_0 reproductive toxicity was < 0.1 mg/kg liraglutide based on a dose-related increased incidence of gestation delayed to day 22 (33%, 58%, 67%, and 96% of births 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. F_0 generation reproductive parameters unaffected by treatment included gestation index, number of implant sites, number of pups/litter born and the number of pups/litter surviving to lactation day 21.
- F_0 maternal behavior or F_1 pup survival were unaffected by treatment.
- The F_1 generation NOAEL was < 0.1 mg/kg NNC 90-1170 based on significantly decreased body weight compared to controls from lactation day 7 to week 16 in males and from lactation day 7 to week 10 in females.
- Prior to weaning F_1 pups, there were no significant differences in body weight between control and treated groups (maternal F_0 treatment) on lactation day 1, but body weight was significantly and dose-dependently lower than controls in all NNC 90-1170 group F_1 pups from lactation day 7 to day 21.
- During the postweaning period for F_1 rats, body weight was significantly lower than controls in males at ≥ 0.1 mg/kg between postnatal week 4 and week 16 in males and significantly lower than controls in females at 0.1 and 1 mg/kg between weeks 4 and 10. Through most of the gestation period (days 1 – 14, but not day 20) and lactation period (days 1 – 14), body weight of pregnant females was significantly lower than controls at 0.1 and 1 mg/kg, and between lactation days 7 to 14, body weight in all NNC 90-1170 dose groups was lower than controls. Clinical signs of bleeding scab and agitated behavior occurred in males at 1 mg/kg.
- Body weight of F_2 generation male and female rats descended from F_0 dams treated with 1 mg/kg NNC 90-1170 was lower than concurrent controls, but the decrease never reached statistical significance.

Study no.: 201109
Module #, and page #: 4.2.3.5.3.1, 1 - 222
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 27 April 2001
GLP compliance: Yes (OECD & [redacted])
QA reports: yes (X) no ( )
Drug, lot #, and % purity: NNC 90-1170, batch 433-20000704-01 or 317011 (5 mg/mL aqueous solution), 98.2% by HPLC (certificate of analysis page 82).

Methods

Doses: 0 (vehicle), 0.1, 0.25, or 1 mg/kg NNC 90-1170, F_0 only from gestation day 6 to post weaning (lactation day 24). F_1 and F_2 generations were not directly dosed.

Dose rationale: Doses were selected based on data from segment I/II studies in rats and based on the anticipated therapeutic dose in humans.

Species/strain: Crl:CD (SD) IGS BR rats (Sprague Dawley)
Number/sex/group: The study design is summarized in the table below
F_0: 24 females/dose
F_1: 24/sex/dose

Group Number	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers		
			F_0 females	F_1 males	F_1 females
1	Control	0	1-24	101-124	201-224
2	Low Dose	0.1	25-29, 97, 31-48	125-148	225-248
3	Intermediate Dose	0.25	49-72	149-171	249-271
4	High Dose	1.0	73-96	173-195	273-295

[P18]

Route, formulation, volume: Subcutaneous injection (once a day) on dorsal surface, NNC 90-1170 solution (in aqueous 0.71 mg/mL disodium monohydrogenphosphate dihydrate, 0.62 mg/mL monosodium dihydrogenphosphate dehydrate, 36.9 mg/mL mannitol, 5 mg/mL phenol, pH 7.44), 1 mL/kg
Satellite groups used for toxicokinetics: Not determined.
Study design:
F_0: Mated females were treated from gestation day 6 to shortly after weaning the F_1 generation. Study observations were clinical signs, body weight, food consumption, maternal behavior, reproductive parameters (gestation index, birth index, live birth index, viability index, lactation index, overall survival index), and gross necropsy (lesions preserved). F_0 dams were terminated after F_1 rats were weaned.

Calculation of reproductive indices:
Group

$$\text{Gestation Index} = \frac{\text{Number bearing live pups}}{\text{Number pregnant}}$$

Litter and group

$$\text{Birth Index} = \frac{\text{Total number of pups born (live and dead)}}{\text{Number of implantation scars}} \qquad \text{Live Birth Index} = \frac{\text{Number of pups live on Day 0 of lactation}}{\text{Total number born (live and dead)}}$$

$$\text{Viability Index} = \frac{\text{Number of pups live on Day 4 of lactation}}{\text{Number live on Day 0}} \qquad \text{Lactation Index} = \frac{\text{Number of pups live on Day 21 of lactation}}{\text{Number live on Day 4}}$$

$$\text{Overall Survival Index} = \frac{\text{Number of pups live on Day 21 of lactation}}{\text{Total number born (live and dead)}}$$

F_1: F_1 rats were exposed to NNC 90-1170 *in utero* from gestation day 6 to delivery, then they were exposed *ex utero* during lactation from day 1 to weaning, but they were never directly dosed. Study observations were physical exam (viability, sexed, milk in stomach), clinical signs and body weight, reproductive parameters (gestation index, birth index, live birth index, viability index), and gross necropsy (lesions preserved). Two males and 2 females from each litter were selected for necropsy after weaning. One male and 1 female selected for post-weaning assessments were terminated after reproducing and prior to weaning F_2 (up to day 14 of lactation).

Prior to weaning: All pups were assessed.

Test Day(s) of Lactation	Test Parameter
1 - Criterion	Pinna Detachment
7 - Criterion	Upper Incisor Eruption
11	Negative Geotaxis
11 - Criterion	Eye Opening
16	Auditory Function
18	Visual Function

[P21]

Physical maturation (pinna detachment, upper incisor eruption, eye opening).
Sensory function (auditory, visual)

Post-weaning: One male and one female from each litter were selected for assessment on day 21 and removed from dams on day 24.

Age of Animals at Testing	Test
from 28 days	Vaginal opening
from 35 days	Balano-preputial Separation
approximately 5 weeks	Open Field
approximately 6 weeks	Rota-Rod
approximately 7 weeks	Multiple Y Water Maze
approximately 10 weeks	Reproduction Function

[P23]

Neurobehavioral assessment, including learning and memory (open field test, rota-rod test, multiple Y water maze)
Sexual maturation (vaginal opening, balano-preputial separation (males)
Reproductive function (pairing one male with one female for up to 7 days with vaginal lavage to confirm the presence of sperm or copulatory plug detected (gestation day 0) with 2 attempts at mating for unsuccessfully mated females

F_2: The F_2 generation was never directly dosed. Observations were survival and body weight and they were terminated prior to weaning (between 4 and 14 days old).

Results

F_0 in-life:

Treatment-related clinical signs of toxicity were hunched posture, piloerection, and wet coat at ≥ 0.1 mg/kg, unkept coat at 0.1 and 1 mg/kg, and at 1 mg/kg, scabbing, rolling gait, body held low, and reduced activity.

Observation/Finding	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Number of Animals with:				
Clinical Observations				
Staining	13	20	16	18
Hairloss	11	9	10	11
Scabbing	2	0	1	8
Unkempt coat	2	6	2	5
Hunched posture	1	6	13	23
Piloerection	1	13	13	23
Wet coat	0	5	4	9
Rolling gait	0	1	1	3
Body held low	0	1	0	7
Reduced activity	0	0	0	6
Total number of animals examined	24	24	24	24

[P32]

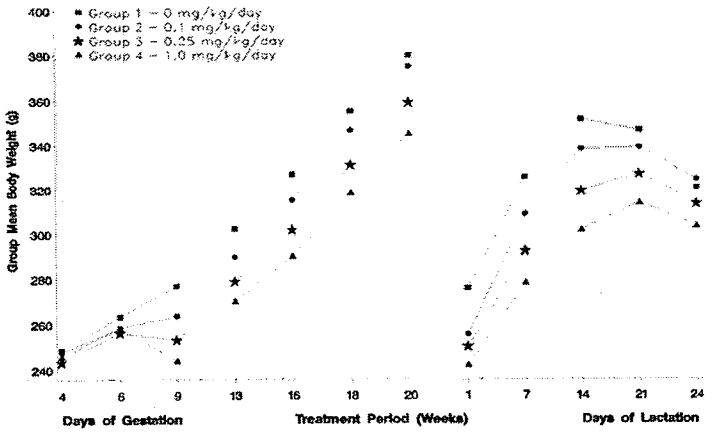
Decreased body weight and decreased body weight gain with corresponding decreased food consumption were considered pharmacologic effects of NNC 90-1170. Transient, dose-dependent, body weight loss (≥ 0.25 mg/kg) or reduced body weight gain (≥ 0.1 mg/kg) occurred between gestation days 6 – 9. Body weight gain recovered after day 9 in NNC 90-1170 groups, but by gestation day 21, body weight remained 9.2% lower than controls at ≥ 0.25 mg/kg. During lactation, body weight gain in NNC 90-1170 groups was slightly above concurrent controls, but body weight remained significantly lower than controls at ≥ 0.25 mg/kg.

F_0 Dams Body Weight and Body Weight Gain, Gestation and Lactatin Periods

Sex		Gestation (Days 4 to 20)				Gestation (Days 6 to 9)				Lactation (Days 1 to 24)			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1	0	0.1	0.25	1
Parameter													
Body weight	N (end)	24	25	22	23	24	25	22	23	24	25	22	23
	g, start	249	248	244	246	263	258	256	258	276	255	250	242
	g, end	380	375	359	345	277	264	253	244	320	324	313	303
	% difference from control, end	0.0	-1.3	-5.5	-9.2	0.0	-4.7	-8.7	-11.9	0.0	1.3	-2.2	-5.3
Body weight gain (start to end)	g	131	127	115	99	14	6	-3	-14	44	69	63	61
	% of starting body weight	52.6	51.2	47.1	40.2	5.3	2.3	-1.2	-5.4	15.9	27.1	25.2	25.2
	% difference from control	0.0	-3.1	-12.2	-24.4	0.0	-57.1	-121	-200	0.0	56.8	43.2	38.6

Statistically signifcant differences from control are underlined ($p < 0$.

F_0 Generation: Females: Group Mean Body Weights (g): During Gestation and Lactation



[P58]

The severity and duration of decreased group mean food consumption in NNC 90-1170 groups compared to controls was dose-dependent. Decreased food consumption returned to within 90% of control group levels by gestation day 12 at 0.1 mg/kg, by day 16 at 0.25 mg/kg, and by day 19 at 1 mg/kg. During lactation, food consumption was < 90% of concurrent controls at 1 mg/kg days during days 0 – 7 and at ≥ 0.25 mg/kg during days 7 – 14 (Table 3).

Table 3 F_0 Generation: Females: Group Mean Food Consumption (g) During Gestation and Lactation

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Day of Gestation				
4	23	22	23	24
5	26	25	25	25
6	25	24	26	26
7	24	11	3	1
8	24	19	16	11
9	26	23	20	17
10	27	22	22	17
11	28	23	21	20
12	30	27	25	22
13	30	28	26	26
14	30	27	26	23
15	30	28	26	22
16	31	30	28	26
17	32	32	30	27
18	31	31	30	27
19	31	31	29	28
20	26	27	26	24
Day of Lactation[b]				
0-7	284	270	257	239
7-14	519	493	451	396

b = Animals rearing young to Day 21 only

[P34]

F_0 reproduction:

In F_0 generation females, the mean duration of gestation was slightly increased from 21.3 days in controls to 22.0 days at 1 mg/kg and there was a dose-related increase in the incidence of dams continuing gestation to day 22 (33.3%, 58.3%, 66.6%, and 95.8% at 0, 0.1, 0.25, and 1 mg/kg, respectively). NNC 90-1170 had no effect on other reproductive parameters including gestation index, number of implant sites/pregnancy, number of pups born/pregnancy, or the number or pups/litter surviving up to day 24 of lactation. NNC 90-1170 had no effect on the size of litters or survival of pups.

Table 4 F_0 Generation: Duration of Gestation and Overall Litter Performance

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Number Pregnant	24	24	24	24
Duration of Gestation (Days)				
21	16	9	8	1
22	8	14	16	23
23	0	0	0	0
24	0	1	0	0
Mean Duration	21.3	21.7	21.7	22.0
Number of females producing a live litter	24	24	24	24
Gestation index as %	100	100	100	100
Mean number of implant sites* per pregnancy ± standard deviation	13.5 ± 1.9	14.4 ± 1.9	13.9 ± 2.4	13.3 ± 2.6
Mean total number of pups* born	12.6 ± 2.8	13.6 ± 1.8	13.0 ± 2.1	12.5 ± 2.5
Mean number of live pups* per litter ± standard deviation:				
Day 0 of lactation	12.6 ± 2.8	13.4 ± 1.7	12.8 ± 1.9	12.3 ± 2.6
Day 1 of lactation	12.5 ± 2.8	13.3 ± 1.7	12.2 ± 2.1	12.0 ± 3.0
Day 4 of lactation	12.3 ± 3.1	13.1 ± 1.7	12.0 ± 2.3	11.7 ± 3.1
Day 7 of lactation	12.1 ± 3.1	13.0 ± 1.7	12.0 ± 2.3	11.7 ± 3.1
Day 14 of lactation	12.1 ± 3.0	13.0 ± 1.6	12.0 ± 2.4	11.7 ± 3.1
Day 21 of lactation	12.1 ± 3.0	13.0 ± 1.6	12.0 ± 2.4	11.7 ± 3.1

* = Excludes litters where all pups died

[P35]

F_0 necropsy:

The incidence of scabbing at or near the injection site was treatment-related at 1 mg/kg NNC 90-1170. The incidence of reddened injection site was similar in control and NNC 90-1170 treated groups. There were no other treatment-related necropsy findings.

Observation/Finding	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Number of Animals with:				
Necropsy Findings				
Reddened injection site	14	12	13	14
Scabbing at or around injection site	0	0	0	3
Hairloss	3	1	2	3
Staining on coat	4	3	5	6
Uterus dilated with fluid	0	0	0	1
Total number of animals examined	24	24	24	24

[P32]

F_1 survival:

NNC 90-1170 had no effect on the size of litters or survival of pups.

Table 5 F_1 Generation: Survival Indices

		Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
		1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Birth Index	Mean Litter Index (%)	94	95	94	95
	Number Losing >2 pups	1	1	3	1
	Number of Litters	24	24	23	23
Live Birth Index	Mean Litter Index (%)	100	98	99	99
	Number Losing >1 pup	0	1	1	2
	Number of Litters	24	24	23	23
Viability Index Days 0-4	Mean Litter Index (%)	97	98	94	95
	Number Losing >3 pups	1	0	2	1
	Number of Litters	24	24	23	23
Lactation Index Days 4-21	Mean Litter Index (%)	99	99	99	100
	Number Losing >1 pup	1	1	0	0
	Number of Litters	24	24	23	23
Overall Survival Index Birth-21	Mean Litter Index (%)	95	96	89	94
	Number Losing >4 pups	0	0	3	1
	Number of Litters	24	24	24	23

[P36]

F_1 physical development:

Prior to weaning

There was a trend of decreased group mean litter weight that was apparent in all dose groups by lactation day 14 and it continued to day 21. There were no significant differences in group mean weight of pups between control and NNC 90-1170 groups on lactation day 1 (Tables 7 & 8). By lactation day 4, pup weight was slightly decreased in NNC 90-1170 groups and by day 7, the decrease was statistically significant in all NNC 90-1170 groups and it persisted to day 21

Table 6 F_1 Generation: Group Mean Litter Weight (g) ± Standard Deviation

Day of Lactation	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
LITTER				
Day 1	80 ± 17	83 ± 10	77 ± 14	74 ± 17
Day 4	114 ± 26	115 ± 18	107 ± 22	97 ± 24
Day 7	170 ± 38	168 ± 23	155 ± 31	139 ± 34
Day 14	335 ± 71	322 ± 36	289 ± 52	252 ± 63
Day 21	537 ± 115	517 ± 56	467 ± 83	400 ± 97

Means exclude litters where all pups died

[P37]

Table 7 F_1 Generation: Males: Group Mean Pup Weights (g)

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Lactation Period (Days)				
		1	4	7	14	21
1 (0)	Number	24	24	24	24	24
	Mean	6.7	9.7	14.6	28.7	46.1
	SD	0.7	1.4	1.8	3.4	6.2
2 (0.1)	Number	24	24	24	24	24
	Mean	6.5	9.1	13.3	25.6	41.1
	SD	0.6	1.2	1.7	2.6	4.5
	Prob.			*	**	**
3 (0.25)	Number	23	23	23	23	23
	Mean	6.5	9.2	13.3	25.1	40.7
	SD	0.9	1.5	2.1	3.0	5.7
	Prob.			*	***	**
4 (1.0)	Number	23	23	23	23	23
	Mean	6.5	8.7	12.5	22.6	36.2
	SD	0.8	1.9	2.6	4.2	8.0
	Prob.			***	***	***

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Means exclude litters where all pups died

Table 8 F_1 Generation: Females: Group Mean Pup Weights (g)

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Lactation Period (Days)				
		1	4	7	14	21
1 (0)	Number	24	24	24	24	24
	Mean	6.3	9.2	14.0	27.9	44.7
	SD	0.7	1.1	1.6	3.1	5.6
2 (0.1)	Number	24	24	24	24	24
	Mean	6.2	8.6	12.7	24.3	39.2
	SD	0.6	1.1	1.6	2.7	4.6
	Prob.			*	***	**
3 (0.25)	Number	23	23	23	23	23
	Mean	6.1	8.6	12.5	23.5	38.1
	SD	0.9	1.8	2.5	3.6	6.0
	Prob.			*	***	***
4 (1.0)	Number	23	23	23	23	23
	Mean	6.1	8.3	11.9	21.7	34.7
	SD	0.8	1.7	2.6	4.1	7.2
	Prob.			***	***	***

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Means exclude litters where all pups died

[P38-9]

F_1 Pups Body Weight and Body Weight Gain, Lactation Days 1 to 21

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
Parameter									
Body weight	N	24	24	23	23	24	24	23	23
	g, day 1	6.7	6.5	6.5	6.5	6.3	6.2	6.1	6.1
	g, day 21	46.1	41.1	40.7	36.2	44.7	39.2	38.1	34.7
	% difference from control, day 21	0.0	-10.8	-11.7	-21.5	0.0	-12.3	-14.8	-22.4
Body weight gain (day 1 to day 21)	g	39.4	34.6	34.2	29.7	38.4	33.0	32.0	28.6
	% of day 1 body weight	588	532	526	457	610	532	525	469
	% difference from control	0.0	-12.2	-13.2	-24.6	0.0	-14.1	-16.7	-25.5

Statistically signifcant differences from control are underlined ($p < 0.05$).

Prior to weaning F_1, there were no treatment-related effects on post-natal physical development (days to pinna detachment, upper incisor eruption, or opening eyes), functional development (negative geotaxis (turn body 90° on a 25% incline facing downward), auditory function (Pryer's reflex), or visual function (pupil response)), or sexual maturity (age at vaginal opening or balano-preputial separation).

Table 9 F_1 Generation: Assessment of Post-Natal Physical Development: Group Mean Day ± Standard Deviation

	Group/Dose Level (mg NNC 90-1170 $kg^{-1}.day^{-1}$)			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
MALES				
All Pups Reach Criterion				
Pinna Detachment	4.3 ± 0.8	4.1 ± 0.7	4.3 ± 0.9	4.1 ± 0.8
Upper Incisor Eruption	14.2 ± 1.2	14.3 ± 1.0	14.1 ± 0.9	14.0 ± 0.9
Open Eyes	15.5 ± 1.0	15.7 ± 0.8	15.7 ± 0.9	15.7 ± 0.8
Median Day to Criterion				
Pinna Detachment	3.9 ± 0.8	3.6 ± 0.6	3.7 ± 0.8	3.5 ± 0.7
Upper Incisor Eruption	13.1 ± 0.9	13.2 ± 0.9	13.4 ± 0.9	13.3 ± 0.9
Open Eyes	15.0 ± 0.9	15.1 ± 0.7	15.1 ± 0.8	15.3 ± 0.7
FEMALES				
All Pups Reach Criterion				
Pinna Detachment	4.6 ± 0.9	4.5 ± 0.9	4.5 ± 0.9	3.9 ± 0.7
Upper Incisor Eruption	14.2 ± 0.8	14.2 ± 0.9	14.2 ± 1.0	13.9 ± 1.2
Open Eyes	15.5 ± 0.8	15.6 ± 0.7	15.8 ± 0.8	15.5 ± 0.8
Median Day to Criterion				
Pinna Detachment	3.9 ± 0.6	3.6 ± 0.9	3.9 ± 1.0	3.4 ± 0.7
Upper Incisor Eruption	13.0 ± 0.6	13.0 ± 0.6	13.1 ± 1.1	13.1 ± 1.0
Open Eyes	15.0 ± 0.9	15.1 ± 0.7	15.1 ± 0.8	15.1 ± 0.7

Table 10 F_1 Generation: Assessment of Post-Natal Functional Development: Group Mean Day ± Standard Deviation

Parameter	Group/Dose Level (mg NNC 90-1170.$kg^{-1}.day^{-1}$)			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
MALES				
Negative Geotaxis	100	100	99	99
Auditory Function	100	100	100	100
Visual Function	100	100	100	100
FEMALES				
Negative Geotaxis	99	98	94	99
Auditory Function	100	100	100	100
Visual Function	98	100	100	98

[P40, 41]

Two abnormalities were recorded. A control group male (litter 14) had excessive skin on the right side of its lip and a 1 mg/kg group female (litter 79) had a small right foot on its right hind limb and was dragging the limb. Both findings were considered incidental.

Post-weaning

NNC 90-1170 had no affect on the number of days to reach sexual maturity in either males or females (Table 11).

Table 11 F_1 Generation: Assessment of Sexual Maturity

Day of Gestation	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Females				
Age (days) at vaginal opening	35.4 ± 3.0	36.4 ± 2.5	36.5 ± 2.7	35.4 ± 2.1
Weight (g) at vaginal opening	124 ± 16	120 ± 13	120 ± 16	108 ± 10
Males				
Age (days) at preputial separation	44.5 ± 1.4	45.3 ± 2.3	44.8 ± 1.9	46.4 ± 3.2
Weight (g) at preputial separation	220 ± 18	211 ± 22	206 ± 15	204 ± 21

All values given ± Standard deviation

[P42]

In weaned rats, clinical signs of bleeding scab and agitated behavior occurred in males at 1 mg/kg. There were no clinical signs considered treatment-related in females.

Observation/Finding	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
MALES				
Number of Animals with:				
Clinical Observations				
Scabbing (bleeding scab)	3	2	3	8
Agitated behaviour	0	1	1	5
Greasy coat	0	2	2	2
Swelling / opaque on eye	0	0	0	1
Total number of animals examined	24	24	23	23
FEMALES				
Number of animals with:				
Clinical Observations				
Tail damaged / missing tip / lesion	1	1	2	3
Greasy coat	4	10	5	8
Wet coat	1	2	4	0
Hairloss	6	10	8	8
Subcutaneous mass	0	0	0	1
Total number of animals examined	24	24	23	23

[Compiled from Table 15]

Exposure to NNC 90-1170 during development and prior to weaning (in maternal milk) affected body weight and body weight gain in rats. Group mean body weight of selected ,weaned F_1 generation rats was significantly lower than concurrent controls at ≥ 0.1 mg/kg NNC 90-1170 (F_0 maternal dose) from week 4 to week 16 in males and from week 4 to week 8 in females (prior to mating) and at 0.1 and 1 mg/kg during weeks 9 and 10 in females. In females, group mean body weight significantly lower than concurrent control persisted from gestation days 0 to 14 at 0.1 and 1 mg/kg NNC 90-1170, but not on gestation day 20 in any dose group. Maternal post-partum group mean body weight was lower than concurrent controls at 0.1 and 1 mg/kg NNC 90-1170 (F_0 maternal dose) on lactation day 1 and at ≥ 0.1 mg/kg from lactation days 7 to 14.

F_1 Generation Body Weight and Body Weight Gain (Weaned)

Sex		Male				Female			
NNC 90-1170 (mg/kg/day)		0	0.1	0.25	1	0	0.1	0.25	1
Parameter									
Body weight	N	24	24	23	23	24	24	23	23
	g, week 4	118	107	106	96	107	97	95	89
	g, week 16 (M) or 10(F)	531	495	497	470	254	240	245	232
	% difference from control, week 16 (M) or 10 (F)	0.0	-6.8	-6.4	-11.5	0.0	-5.5	-3.5	-8.7
Body weight gain (week 4 to week 16 (M) or 10 (F))	g	413	388	391	374	147	143	150	143
	% of week 4 body weight	350	363	369	390	137	147	158	161
	% difference from control	0.0	-6.1	-5.3	-9.4	0.0	-2.7	2.0	-2.7

Statistically signifcant differences from control are underlined ($p < 0.05$).

Table 16 F_1 Generation: Males: Group Mean Body Weight (g)

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Treatment Period (Weeks)													Body Weight Gain (g) (Week 4 - Week 16)
		4	5	6	7	8	9	10	11	12	13	14	15	16	
1 (0)	Number	24	24	24	24	24	24	24	24	24	24	24	24	24	24
	Mean	118	177	239	299	354	397	428	460	479	499	512	520	531	413
	SD	9	12	17	20	21	25	29	29	30	34	37	41	43	40
2 (0.1)	Number	24	24	24	24	24	24	24	24	24	24	24	24	24	24
	Mean	107	161	220	277	329	369	398	429	450	468	480	489	495	388
	SD	11	13	17	19	23	26	28	30	31	37	38	40	42	41
	Prob.	**	***	**	**	**	**	**	**	**	**	**	*	*	*
3 (0.25)	Number	23	23	23	23	23	23	23	23	23	23	23	23	23	23
	Mean	106	159	220	276	324	365	397	427	450	470	478	488	497	391
	SD	13	17	20	23	27	31	32	35	38	41	44	46	48	43
	Prob.	**	***	**	**	***	***	**	**	**	*	**	*	*	
4 (1.0)	Number	23	23	23	23	23	23	23	23	23	23	23	23	23	23
	Mean	96	148	204	259	308	350	378	405	424	439	451	461	470	374
	SD	16	21	27	32	35	38	42	45	45	47	51	51	54	43
	Prob.	***	***	***	***	***	***	***	***	***	***	***	***	***	**

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Table 17 F_1 Generation: Females: Group Mean Body Weight (g) During Pre-mating Period

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Pre-mating Period (Weeks)							Body Weight Gain (g) (Week 4 - Week 10)
		4	5	6	7	8	9	10	
1 (0)	Number	24	24	24	24	24	24	24	24
	Mean	107	148	180	204	226	239	254	147
	SD	6	7	10	13	17	19	21	20
2 (0.1)	Number	24	24	24	24	24	24	24	24
	Mean	97	136	167	192	209	224	240	142
	SD	9	11	14	16	18	20	24	20
	Prob.	**	***	**	**	**	*	*	
3 (0.25)	Number	23	23	23	23	23	23	23	23
	Mean	95	135	167	193	214	229	245	150
	SD	15	17	16	18	18	22	22	17
	Prob.	**	**	***	**	*			
4 (1.0)	Number	23	23	23	23	23	23	23	23
	Mean	89	127	159	183	202	219	232	143
	SD	12	13	12	15	15	18	19	15
	Prob.	***	***	***	***	***	**	***	

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Table 18 F_1 Generation: Females Group Mean Body Weight (g) During Gestation and Lactation

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Gestation Period (Days)				Lactation Period (Days)		
		0	7	14	20	1	7	14
1 (0)	Number	24	24	24	24	23	23	23
	Mean	262	300	341	416	297	347	374
	SD	21	23	28	36	23	26	27
2 (0.1)	Number	22	22	22	22	22	22	22
	Mean	244	283	325	398	279	320	349
	SD	21	24	25	30	30	26	23
	Prob.	**	*	*		*	**	**
3 (0.25)	Number	23	23	23	23	23	23	23
	Mean	250	290	330	406	285	331	355
	SD	23	25	27	36	31	30	30
	Prob.						*	*
4 (1.0)	Number	22	22	22	22	21	21	21
	Mean	241	278	317	391	274	312	337
	SD	23	22	24	30	23	24	30
	Prob.	**	**	**		**	***	***

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Day of Gestation mean values excludes non-pregnant animals
Day of Lactation mean values excludes litters where all pups died

[P49-51]

F_1 behavioral evaluation:

There were no differences in functional development of weaned F_1 rats assessed by open field (Table 12), rota-rod (Table 13), or multiple Y-maze tests (Table 14).

Table 12 F_1 Generation: Open Field Test: Group Mean Values ± Standard Deviation

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
MALES				
Time (s) to move from central sector	4.2 ± 4.3	6.7± 5.2	6.4 ± 8.6	6.8 ± 6.6
Number of sectors traversed	61.5 ± 17.6	56.9 ± 18.4	54.6 ± 22.6	51.9 ± 22.8
Number of rearings	13.8 ± 8.3	12.1 ± 7.2	12.0 ± 7.4	9.9 ± 6.5
Number of grooming episodes	1.0 ± 0.9	0.2 ± 0.4	0.8 ± 1.2	1.7 ± 1.2
Number of defecation boluses	1.5 ± 1.6	0.9 ± 1.5	1.5 ± 1.9	1.5 ± 2.2
Number of animals defecating	13	8	12	9
Incidence of abnormalities of gait or general motor activity	0	0	0	0
FEMALES				
Time (s) to move from central sector	4.1 ± 3.4	4.3 ± 3.4	3.6 ± 2.5	7.5 ± 13.9
Number of sectors traversed	71.3 ± 18.7	70.5 ± 19.1	68.6 ± 17.6	62.8 ± 21.5
Number of rearings	17.0 ± 6.8	14.4 ± 7.7	16.6 ± 7.4	12.0 ± 10.1
Number of grooming episodes	1.0 ± 1.1	1.1 ± 1.3	1.3 ± 1.5	1.3 ± 1.3
Number of defecation boluses	0.6 ± 1.1	0.4 ± 0.9	0.3 ± 0.7	0.8 ± 1.5
Number of animals defecating	5	5	3	5
Incidence of abnormalities of gait or general motor activity	0	0	0	0

[P43]

Table 13 F_1 Generation: Rota-Rod Test: Group Mean Time(s) ± Standard Deviation

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
MALES				
Maximum[c]	52.2 ± 30.6	47.7 ± 29.2	46.4 ± 30.2	49.5 ± 30.9
Mean[d]	35.0 ± 20.8	32.3 ± 20.6	33.4 ± 19.5	36.2 ± 20.6
FEMALES				
Maximum[c]	52.0 ± 27.3	50.1 ± 26.9	53.8 ± 37.0	52.0 ± 27.3
Mean[d]	36.3 ± 18.4	32.6± 14.6	33.2 ± 25.3	36.6 ± 18.2

c = Mean of the longest time achieved over the 3 trials by each animal
d = Mean of the mean length of time achieved over the 3 trials by each animal

[P44]

Table 14 F_1 Generation: Males and Females: Multiple Y Maze Test: Group Mean Values ± Standard Deviation

Males

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
TIME (s)				
Day 1, Trial 1	69.4 ± 25.2	71.8 ± 23.5	57.7 ± 26.4	63.3 ± 25.5
Day 1, Trial 2	48.3 ± 30.2	41.5 ± 23.3	49.8 ± 28.4	36.5 ± 24.7
Day 1, Trial 3	23.0 ± 19.6	24.3 ± 16.2	21.4 ± 19.3	26.7 ± 22.9
Day 3, Trial 1	25.0 ± 19.5	18.7 ± 12.9	23.1 ± 19.5	29.2 ± 19.0
Day 3, Trial 2	15.9 ± 12.3	17.7 ± 10.7	15.0 ± 12.2	19.2 ± 19.5
Day 3, Trial 3	21.7 ± 13.4	16.8 ± 11.2	13.0 ± 8.7	15.7 ± 9.6
ERRORS				
Day 1, Trial 1	4.7 ± 3.1	5.6 ± 3.3	4.7 ± 3.8	4.7 ± 3.2
Day 1, Trial 2	3.9 ± 3.7	3.0 ± 3.2	4.3 ± 3.4	2.9 ± 3.2
Day 1, Trial 3	1.3 ± 2.1	1.5 ± 2.2	1.0 ± 1.6	1.9 ± 3.3
Day 3, Trial 1	2.1 ± 3.4	0.8 ± 1.9	1.6 ± 2.3	1.8 ± 2.5
Day 3, Trial 2	0.3 ± 0.6	0.6 ± 0.6	0.8 ± 1.5	1.2 ± 3.1
Day 3, Trial 3	0.7 ± 1.3	0.6 ± 1.7	0.4 ± 1.3	0.4 ± 0.7

Females

	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
TIME (s)				
Day 1, Trial 1	69.6 ± 24.4	70.4 ± 24.7	74.7 ± 22.6	59.7 ± 27.0
Day 1, Trial 2	44.0 ± 26.2	38.1 ± 25.1	37.5 ± 27.4	40.4 ± 27.0
Day 1, Trial 3	19.8 ± 9.7	25.9 ± 18.3	17.6 ± 13.7	22.1 ± 18.4
Day 3, Trial 1	12.6 ± 4.1	19.6 ± 12.5	13.8 ± 8.2	18.6 ± 16.4
Day 3, Trial 2	13.6 ± 9.0	16.3 ± 12.1	16.4 ± 14.5	14.0 ± 6.7
Day 3, Trial 3	17.4 ± 10.1	18.3 ± 13.7	13.6 ± 8.9	11.1 ± 6.3
ERRORS				
Day 1, Trial 1	5.7 ± 3.5	6.0 ± 3.3	6.7 ± 4.1	4.7 ± 3.4
Day 1, Trial 2	4.2 ± 3.7	3.2 ± 3.3	2.8 ± 2.3	4.0 ± 3.6
Day 1, Trial 3	1.0 ± 1.1	2.3 ± 2.7	1.4 ± 2.5	1.9 ± 2.6
Day 3, Trial 1	0.5 ± 0.6	1.5 ± 2.1	0.8 ± 1.0	1.5 ± 2.6
Day 3, Trial 2	0.5 ± 0.7	0.8 ± 1.3	1.2 ± 1.8	1.1 ± 0.9
Day 3, Trial 3	1.0 ± 1.6	0.9 ± 1.2	0.8 ± 1.2	0.5 ± 0.7

A time of 90 s has been assumed for animals that had to be guided out of the maze

[P45]

F_1 reproduction:

There were no substantive differences in mating performance or fertility indices of F_1 generation rats.

Table 19 F_1 Generation: Mating Performance and Fertility Indices

Number of Nights to Positive Mating Sign	Group/Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
	Number of Animals (Number of these not becoming pregnant)			
1	6	13 (1)	10	6
2	6	5	4	4
3	5	5	6	8 (1)
4	7	1	3	3
5	0	0	0	1
11	0	0	0	1
Median number of nights to positive mating sign	2.5	1.0	2.0	3.0
Number passing one oestrus	0	0	0	0
Number of males paired	24	24	23	23
Number of siring males	24	23	23	21
Male Fertility Index (%)	100	96	100	91
Number of females paired	24	24	23	23
Number pregnant	24	23	23	22
Female Fertility Index (%)	100	96	100	96

[P52]

Table 20 F_1 Generation: Duration of Gestation and Overall Litter Performance

	Group/Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Number Pregnant	24	23	23	22
Duration of Gestation (Days)				
21	4	7	5	8
22	17	15	16	13
23	3	0	2	1
Mean Duration	22.0	21.7	21.9	21.7
Number of females producing a live litter	24	22	23	22
Gestation index as %	100	96	100	100
Mean number of implant sites* per pregnancy ± standard deviation	15.1 ± 3.2	14.5 ± 3.2	15.0 ± 1.3	15.8 ± 1.7
Mean total number of pups* born	14.0 ± 2.4	13.8 ± 3.1	13.9 ± 1.6	14.0 ± 2.4
Mean number of live pups* per litter ± standard deviation:				
Day 0 of lactation	14.0 ± 2.4	13.5 ± 2.9	13.7 ± 1.6	14.0 ± 2.4
Day 1 of lactation	13.9 ± 2.3	13.1 ± 2.8	12.9 ± 3.2	13.9 ± 2.3
Day 4 of lactation	13.6 ± 2.2	12.5 ± 3.0	12.5 ± 3.5	13.6 ± 2.2
Day 7 of lactation	13.5 ± 1.9	12.4 ± 3.0	12.5 ± 3.5	13.5 ± 1.9
Day 14 of lactation	13.4 ± 1.8	12.4 ± 3.0	12.5 ± 3.5	13.4 ± 1.8

* = Excludes litters where all pups died

[P53]

F_1 necropsy:

An increased incidence of scabs in males and females at 1 mg/kg (F_0 maternal dose) compared to controls was considered incidental because the incidence was low (9 – 13%). F_1 female 240 (F_0 maternal dose 0.1 mg/kg) was sacrificed prematurely on gestation day 27. The extended duration of gestation was attributed to a fetus lodged in the cervix / uterus discovered at necropsy, so the finding extended duration of gestation was considered incidental.

Observation/Finding	Group/Dose Level (mg NNC 90-1170.kg^{-1}.day^{-1})			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
MALES				
Number of Animals with:				
Necropsy Findings				
Scabbing	1	0	1	3
Total number of animals examined	24	24	23	23
FEMALES				
Focus on uterine horn	0	0	0	2
Total number of animals examined	24	24	23	23

[P46-8]

F_2 findings:

In the F_2 generation, there were no significant NNC 90-1170 related differences in litter survival indices including birth index, live birth index, or viability index.

Table 21 F_2 Generation: Survival Indices

		Group/Dose Level (mg.kg^{-1}.day^{-1})			
		1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
Birth Index	Mean Litter Index (%)	94	95	93	89
	Number Losing >2 pups	2	1	3	6
	Number of Litters	24	22	23	22
Live Birth Index	Mean Litter Index (%)	100	98	99	100
	Number Losing >1 pup	0	3	1	0
	Number of Litters	24	22	23	22
Viability Index Days 0-4	Mean Litter Index (%)	95	94	95	98
	Number Losing >3 pups	1	1	1	1
	Number of Litters	24	22	23	22

[P54]

Prior to weaning, group mean litter weights were not significantly different between litters descended from NNC 90-1170 or control groups (maternal F_0 treatment), however litter weight trended lower in NNC 90-1170 groups (4.9 to 10.2%).

Table 22 F_2 Generation: Group Mean Litter weight (g) ± Standard Deviation

Day of Lactation	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (0.1)	3 (0.25)	4 (1.0)
LITTER				
Day 1	97 ± 20	85 ± 15	92 ± 12	91 ± 17
Day 4	140 ± 28	119 ± 26	130 ± 25	127 ± 25
Day 7	202 ± 38	174 ± 35	190 ± 33	183 ± 31
Day 14	389 ± 69	349 ± 67	370 ± 59	359 ± 45

Means exclude litters where all pups died

[P55]

Although group mean body weight of male and female pups were consistently lower than concurrent control groups throughout the lactation period, there were no statistically significant difference between control and NNC 90-1170 groups (maternal F_0 generation doses) in F_2 generation pup weights for either sex on lactation days 1, 4, 7, or 14.

Table 23 F_2 Generation: Males: Group Mean Pup Weights (g)

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Lactation Period (Days)			
		1	4	7	14
1 (0)	Number	23	23	23	23
	Mean	7.2	10.4	15.2	29.4
	SD	0.8	1.4	2.1	4.1
2 (0.1)	Number	22	22	22	22
	Mean	6.8	9.9	14.8	29.4
	SD	0.9	1.5	2.4	4.7
	Prob.				
3 (0.25)	Number	23	23	23	23
	Mean	7.0	10.3	15.1	29.4
	SD	0.9	1.6	2.0	3.9
	Prob.				
4 (1.0)	Number	21	21	21	21
	Mean	6.6	9.6	14.0	27.5
	SD	0.9	1.4	1.7	2.7
	Prob.				

Means exclude litters where all pups died

Table 24 F_2 Generation: Females: Group Mean Pup Weights (g)

Group/Dose Level ($mg.kg^{-1}.day^{-1}$)		Lactation Period (Days)			
		1	4	7	14
1 (0)	Number	23	23	23	23
	Mean	6.8	10.0	14.6	28.4
	SD	0.8	1.3	1.9	3.8
2 (0.1)	Number	22	22	22	22
	Mean	6.4	9.5	14.2	28.6
	SD	0.8	1.5	2.5	5.0
	Prob.				
3 (0.25)	Number	23	23	23	23
	Mean	6.6	9.8	14.4	28.2
	SD	0.9	1.6	2.1	3.7
	Prob.				
4 (1.0)	Number	21	21	21	21
	Mean	6.4	9.0	13.3	26.1
	SD	0.8	1.4	1.8	2.5
	Prob.				

Means exclude litters all pups died

[P56-7]

Summary and Conclusions

In a prenatal and post natal toxicity study of 0 (vehicle), 0.1, 0.25, or 1 mg/kg NNC 90-1170 injected subcutaneously in pregnant Sprague Dawley rats from gestation day 6 to shortly after weaning F_1 litters (~ lactation day 24), the maternal NOAEL was < 0.1 mg/kg based on clinical signs of hunched posture, piloerection, and wet coat at ≥ 0.1 mg/kg, unkept coat at 0.1 and 1 mg/kg, and at 1 mg/kg, scabbing, rolling gait, body held low, and reduced activity.

NNC 90-1170 related decreased body weight, body weight gain, and food consumption were considered pharmacologic effects in F_0 dams. Between gestation days 6 and 9, body weight of pregnant F_0 rats dose-dependently decreased at ≥ 0.25 mg/kg NNC 90-1170, body weight was significantly lower than controls at all doses, and body weight gain decreased 57 – 200% compared to controls at ≥ 0.1 mg/kg. Decreased body weight gain was transient, but decreased body weight persisted to gestation day 20 at ≥ 0.25 mg/kg. Post partum body weights (lactation day 1) were significantly lower than controls in all NNC 90-1170 groups, but by the end of the 24 day lactation period, only group mean body weight of 1 mg/kg

dams was significantly lower. The magnitude and duration of decreased food consumption was dose-related with corresponding decreased body weight and body weight gain. Necropsy revealed a low incidence of scabbing at or near the injection site at 1 mg/kg in females. The NOAEL for F_0 reproductive toxicity was < 0.1 mg/kg liraglutide based on a dose-related increased incidence of continuing gestation to day 22 (33%, 58%, 67%, and 96% 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. F_0 generation reproductive parameters unaffected by treatment included gestation index, number of implant sites, number of pups/litter born and the number of pups/litter surviving to lactation day 21. Through most of the gestation period (days 1 – 14, but not day 20) and lactation period (days 1 – 14), body weight of F_0 females was significantly lower than controls at 0.1 and 1 mg/kg, and between lactation days 7 to 14, body weight in all NNC 90-1170 dose groups was lower than controls.

The F_1 generation NOAEL was < 0.1 mg/kg NNC 90-1170 based on significantly decreased body weight compared to controls from postnatal day 1 to week 16 in males and from postnatal day 1 to week 10 in females. The F_1 generation was exposed to NNC 90-1170 *in utero* from gestation day 6 to birth, and then *ex utero* via their mother's milk from lactation day 1 to the end of weaning, typically lactation days 21 to 24. NNC 90-1170 had no effect on viability of live born pups, lactation, or the overall survival of pups from birth to lactation day 21. Litter weight of all NNC 90-1170 groups were lower than controls from lactation days 14 to 21. Exposure to NNC 90-1170 during development and prior to weaning (from their mother's milk) affected body weight and body weight gain in rats. Although there were no significant differences in body weight between control and treated groups on lactation day 1, body weight was significantly and dose-dependently lower than controls in all NNC 90-1170 treated F_1 groups from lactation day 7 to day 21. Food consumption was not measured. Prior to weaning, there were no treatment-related effects on postnatal physical development or functional development. In weaned F_1 rats, NNC 90-1170 had no effect on the time to onset of sexual maturity. Clinical signs of clinical signs of bleeding scab and agitated behavior occurred in males at 1 mg/kg.. Body weight was significantly lower than controls in males at ≥ 0.1 mg/kg between postnatal week 4 and week 16 and significantly lower than controls in females at 0.1 and 1 mg/kg between weeks 4 and 10. NNC 90-1170 didn't affect functional development of weaned rats assessed by open field, rota-rod, or multiple Y-maze tests or the mating performance of mated F_1 male and female rats or the reproductive indices of females. A low incidence of scabbing and focus on the uterine horns was found at necropsy.

The F_2 generation was not exposed to NNC 90-1170. There were no differences in litter survival indices or significant differences in group mean litter weights or body weights of male or female pups, but group mean average male and female pup weights throughout the lactation period (lactation day 1 to day 14) were consistently lower in rats descended from 1 mg/kg NNC 90-1170 F_0 dams.

2.6.6.7 Local tolerance

Study title: NNC 90-1170: Local toxicity 2 and 5 days after subcutaneous injection in pigs (study 980185)

Key study findings:

- There were no substantive differences in injection site reactions between liraglutide, vehicle, and saline injections.
- Two days after injection, microscopic pathology of injection sites were consistent with subacute inflammation characterized by minimal to slight cellular infiltration (macrophages with or without granulocytes) and minimal necrosis at liraglutide, vehicle, and saline injection sites.
- Five days after dosing, minimal to slight cellular infiltrate consisting of epitheloid/giant cells and macrophages, slight formation of collagen tissue, and fat necrosis at liraglutide, vehicle, and saline injection sites.

Summary and Conclusions

Local toxicity of 200 microliters of 0 (vehicle), 0 (Protophane HM (ge)), 0 (0.9% saline), or 5 mg/mL liraglutide (batch 433-981217-02) injected subcutaneously on the dorsal surface on study days 1 and 4 was assessed in female SPF pigs (4 total, 52 – 57 kg). The vehicle for liraglutide was 0.71 mg/mL disodium monohydrogen phosphate dihydrate, 0.62 mg/mL monosodium dihydrogen phosphate dihydrate, 38 mg/mL mannitol, 5 mg/mL phenol, pH 7.4.

Injection sites were marked and injections were administered using a NovoPen (needle gauge not specified). The table below summarizes the study design.

Animal No	1		2		3		4	
Localisation	L	R	L	R	L	R	L	R
Injection at Day	1	4	1	4	1	4	1	4
Cranial	A	D	D	C	C	B	B	A
	B	C	A	B	D	A	C	D
	C	B	B	A	A	D	D	C
Caudal	D	A	C	D	B	C	A	B

L = Left R= Right

Code	Test article	Dose volume
A	NNC 90-1170	200 µl
B	NNC 90-1170 vehicle	200 µl
C	Protaphane HM (ge) 600 nmol/ml	200 µl
D	0.9% NaCl	200 µl

[P10]

Study parameters were clinical signs (including signs of hypoglycemia), body weight, and macroscopic and microscopic pathology at the injection site. On study day 6, pigs were sacrificed by exsanguination through the subclavian vein and artery after stunning, and skin samples including subcutaneous tissue were fixed in 4% formaldehyde, embedded in paraffin, and stained with hematoxylin-eosin for microscopic examination.

There were no treatment-related clinical signs, body weight changes, or macroscopic injection site findings. .

Two days after injection, microscopic pathology of injection site reactions was minimal to slight cellular infiltration (macrophages with or without granulocytes) and minimal necrosis at liraglutide, vehicle, and saline injection sites, consistent with subacute inflammation. Injection site reactions at Prothaphane HM(ge) sites was more severe with moderate to marked infiltration of macrophages associated with crystalline material. Five days after dosing, minimal to slight cellular infiltrate consisting of epitheloid/giant cells and macrophages, slight formation of collagen tissue, and fat necrosis at liraglutide, vehicle, and saline injection sites. Microscopic findings at Prothaphane HM(ge) was similar, but the severity was moderate with crystalline material in at least 1 site.

Study title: NNC 90-1170: Local toxicity 2 and 5 days after subcutaneous injection in pigs comparing a phase 2 formulation of DS campaign 2 and a phase 3 formulation of DS campaign 4A (study 203294)

Key study findings:

- There were no marked differences in local toxicity between phase 2 and phase 3 preparations of liraglutide.
- Microscopically, an inflammatory reaction was seen 2 days after treatment at all injection sites, including vehicle, and the injection site reaction persisted up to 5 days after treatment.
- On day 2, injection site reaction was characterized by cellular infiltrate and hemorrhage. On day 5, injection site reactions / inflammation were characterized by cellular infiltrate, formation of collagen tissue, and fat necrosis.

Summary and Conclusions

Local toxicity of 200 microliters of 0 (phase 2 vehicle), 0 (phase 3 vehicle), 0 (0.9% saline), 5 mg/mL liraglutide (phase 2 formulation), or 6.25 mg/mL liraglutide (phase 3 formulation) injected subcutaneously on the dorsal surface on study days 1 and 4 was assessed in female SPF pigs (5 total, 53.3 – 60.7 kg). The following preparations were injected:

A Liraglutide 5 mg/ml, Phase 2 formulation, Batch NLDP004, expiry date 30-10-2004
B Liraglutide 6.25 mg/ml, Phase 3 formulation, Batch 433-03-139, expiry date 13-05-2005
C Liraglutide 0 mg/ml, Phase 2 formulation, Batch 433-03-137, expiry date 13-11-2005
D Liraglutide 0 mg/ml, Phase 3 formulation, Batch 433-03-140, expiry date 13-11-2005
E 0.9% NaCl, Batch 03j07502, expiry date October 2006

[P10]

- Phase 2 vehicle: 0.71 mg/mL disodium monohydrogen phosphate dihydrate, 0.62 mg/mL monosodium dihydrogen phosphate dihydrate, 36.9 mg/mL mannitol, 5 mg/mL phenol, pH 7.4
- Phase 2 liraglutide: 5 mg/mL liraglutide in phase 2 vehicle, pH 7.4
- Phase 3 vehicle: 1.42 mg/mL disodium monohydrogen phosphate dihydrate, 14 mg/mL propylene glycol, 5.5 mg/mL phenol, pH—— b(4)
- Phase 3 liraglutide: 6.25 mg/mL liraglutide in phase 3 vehicle, pH 7.4

Injection sites were marked and injections were administered using a NovoPen and a 28G needle. The table below summarizes the study design.

Animal No	101		2		3		4		5	
Injection Day	1	4	1	4	1	4	1	4	1	4
Localisation	L	R	L	R	L	R	L	R	L	R
Cranial	A	B	E	D	D	C	C	E	B	A
	B	C	A	E	E	D	D	A	C	B
Caudal	C	D	B	A	A	E	E	B	D	C
	D	E	C	B	B	A	A	C	E	D

L = Left
R = Right

Code	Test article	Dose formulation concentration (mg/ml)
A	Liraglutide Phase 2 formulation	5 mg/ml, Batch NLDP004
B	Liraglutide Phase 3 formulation	6.25 mg/ml, Batch 433-03-139
C	Liraglutide Phase 2 vehicle	0, Batch 433-03-137
D	Liraglutide Phase 3 vehicle	0, Batch 433-03-140
E	0.9% NaCl	0, Batch 03j07502

[P12]

Study parameters were clinical signs (including signs of hypoglycemia), body weight, and macroscopic and microscopic pathology at the injection site. On study day 6, anesthetized pigs were sacrificed by exsanguination through the subclavian vein and artery and skin samples including subcutaneous tissue were fixed in 4% formaldehyde, embedded in paraffin, and stained with hematoxylin-eosin for microscopic examination.

On day 2, some pigs were subdued and didn't eat all of their food. Body weight of 3 pigs decreased 0.3 – 1 kg during the treatment period, and the 2 other pigs gained weight.

There were no treatment-related macroscopic injection site changes, but slight hemorrhage occurred at a few sites. Microscopically, an inflammatory reaction was seen 2 days after treatment at all injection sites, but the inflammation persisted 5 days after treatment at phase 2 vehicle or phase 3 vehicle or liraglutide groups, but not at saline only injection sites. Five days after treatment, a moderate inflammatory reaction occurred with phase 3 liraglutide and a slight inflammatory reaction occurred for phase 3 vehicle, phase 2 vehicle, and phase 2 liraglutide. On day 2, injection site reaction was characterized by cellular infiltrate and hemorrhage. On day 5, injection site reactions / inflammation was characterized by cellular infiltrate, formation of collagen tissue, and fat necrosis. There were no marked differences in local toxicity between phase 2 and phase 3 preparations of liraglutide.

Study title: NNC 90-1170: Local toxicity of three phase 3 formulations with pH 7.7, 7.9, and 8.15 two and 5 days after subcutaneous injections in pigs (study 204291)

Key study findings:

- The composition of liraglutide vehicle was not included in the report.
- Vehicle pH ranging from 7.7 – 8.15 had no effect on liraglutide injection site reactions.

Summary and Conclusions

Local toxicity of 200 microliters of 0 (vehicle), 0 (0.9% saline), or 1 of 3 formulations of 6.25 mg/mL liraglutide with pH adjusted to 7.7, 7.9, or 8.15 injected subcutaneously on the dorsal surface in female SPF pigs (5 total, 53.3 – 60.7 kg) on study days 1 and 4 was assessed on day 5. The following preparations were injected:

- **Test item(s)**
  - A Liraglutide 6.25 mg/ml, pH 7.7, batch no. PLDP002, expiry date 17 Dec 2005
  - B Liraglutide 6.25 mg/ml, pH 7.9, batch no. PLDP003, expiry date 29 Dec 2005
  - C Liraglutide 6.25 mg/ml, pH 8.15, batch no PLDP004, expiry date 01 Jan 2006

- **Control item(s)**
  - D Liraglutide 0 mg/ml, pH 7.7, batch no. PQ50299, expiry date 22 Apr 2006
  - E 0.9% NaCl, batch No 03JV10, expiry date October 2006

[P8]

The composition of vehicle was not included in the report. Injection sites were marked and injections were administered to anesthetized pigs using a NovoPen and a 28G needle. The table below summarizes the study design.

Animal No	1		2		3		4		5	
Injection Day	1	4	1	4	1	4	1	4	1	4
Localisation	L	R	L	R	L	R	L	R	L	R
Cranial	A	B	E	D	D	C	C	E	B	A
	B	C	A	E	E	D	D	A	C	B
Caudal	C	D	B	A	A	E	E	B	D	C
	D	E	C	B	B	A	A	C	E	D

L = Left
R = Right

Code	Test article/Batch number	Dose formulation concentration (mg/ml) and pH	Dose volume
A	Liraglutide / PLDP002	6.25 pH 7.7	200 µl
B	Liraglutide / PLDP003	6.25 pH 7.9	200 µl
C	Liraglutide / PLDP004	6.25 pH 8.15	200 µl
D	Liraglutide Vehicle/PQ50299	0 pH 7.7	200 µl
E	Saline	0 Not applicable	200 µl

[P14]

Study parameters were clinical signs, body weight, and macroscopic and microscopic pathology at the injection site. On study day 6, anesthetized pigs were sacrificed by exsanguination through the subclavian vein and artery and skin samples including subcutaneous tissue were fixed in 4% formaldehyde, embedded in paraffin, and stained with hematoxylin-eosin for microscopic examination. The grading system for injection site reactions is shown below

0		Normal tissue.
A		Minimal cellular infiltrate.
B		Oedema and slight cellular infiltrate.
C	a)	Oedema and slight cellular infiltrate and/or minimal necrosis.
	b)	Slight formation of collagen tissue and/or occasional fat cell necrosis.
D	a)	Moderate cellular infiltrate and/or focal necrosis.
	b)	Moderate formation of collagen tissue and/or fat cell necrosis.
E	a)	Marked widespread cellular infiltrate and/or diffuse necrosis.
	b)	Marked formation of collagen tissue and/or many fat cell necrosis.
	a)	Two days after the injection
	b)	Five days after the injection

[P16]

Clinical signs of toxicity occurred in 3 of 5 pigs including apathetic appearance (pigs 1, 2, 4 on days 1 – 3; pig 1 on days 4 – 6), trembling, lying down/tired, reduced food consumption. Except for apathetic appearance in pig 1, clinical signs resolved by study day 4. Some of the pigs were treated by offering oral glucose solutions or given iv glucose infusions (150 mL of 50 mg/mL glucose). One pig, #4, had slight body weight loose during the study, but the other gained weight. Clinical signs at the injection site were hemorrhage, erythema, and edema that were related to injection, but not liraglutide treatment.

Microscopic injection site findings occurring 2 or 5 days after injection are summarized in the tables below. On day 2, microscopic findings were minimal to slight edema, cellular infiltrate, necrosis, with or without hemorrhage in all liraglutide formulations and vehicle. Saline injection caused minimal to slight cellular infiltrate, but not necrosis.

3.5 Two days after subcutaneous injection

Dose group	A: NNC 90-1170 Phase 3 Formulation pH 7.7	B: NNC 90-1170 Phase 3 Formulation pH 7.9	C: NNC 90-1170 Phase 3 Formulation pH 8.15	D: NNC 90-1170 Phase 3 Formulation vehicle pH 7.7	E: Saline
Inj.sites/Numbers examined	4	4	4	4	4
Grade A	-	-	1	-	2
Grade B	1	-	-	1	2
Grade Ca	2	4	2	3	-
Grade Da	1	-	1	-	-
Needle canal	1	3	4	3	3

[P19]

On day 5, up to moderate cellular infiltrate, formation of collagen, and fat necrosis with or without hemorrhage occurred at most vehicle or liraglutide injection sites and at 1 saline injection site.

3.6 Five days after subcutaneous injection

Dose group	A: NNC 90-1170 Phase 3 Formulation pH 7.7	B: NNC 90-1170 Phase 3 Formulation pH 7.9	C: NNC 90-1170 Phase 3 Formulation pH 8.15	D: NNC 90-1170 Phase 3 Formulation vehicle pH 7.7	E: Saline
Inj.sites/Numbers examined	4	4	4	4	4
Grade 0	-	-	-	1	2
Grade A	-	-	-	-	1
Grade Cb	-	1	2	1	1
Grade Db	4	3	2	2	-
Needle canal	1	3	2	3	1

[P19]

There were no substantive differences in injection site reactions between liraglutide formulations with pH ranging from 7.7 – 8.15 or vehicle either 2 or 5 days after dosing.

2.6.6.8 Special toxicology studies

Mechanistic Studies of Liraglutide-Induced Rodent Thyroid C-cell Tumors

Mechanistic studies evaluating the proposed mode of action for liraglutide-induced rodent thyroid C-cell tumors were reviewed. According to this mode of action, liraglutide induces persistent calcitonin secretion and synthesis in thyroid C-cells which drives hyperplasia and hyperplasia progresses to tumors. The mode of action and supporting mechanistic studies were presented to the Executive Carcinogenicity Assessment Committee for concurrence with the reviewer's opinion that the proposed mode of action was not supported by mechanistic studies, and the Committee agreed. The complete review is attached as Appendix C. A summary of mechanistic studies, excerpted from the complete review (Appendix C), follows.

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 (GLP-1Rs) on thyroid 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 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.

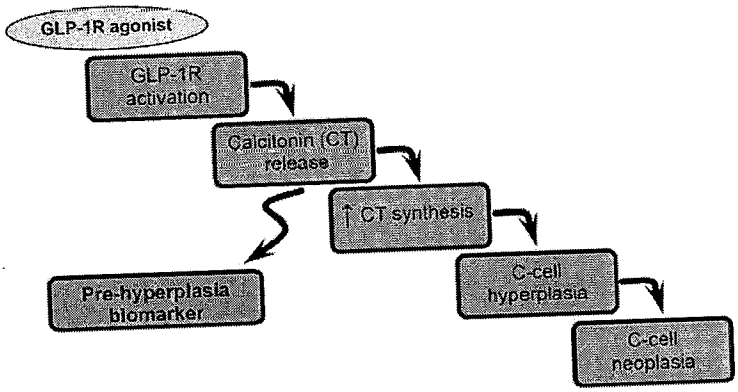


Figure 2 Key events in the process leading to rodent C-cell proliferation after long-term treatment with GLP-1 receptor (GLP-1R) agonists

[N000 4.2.3.7.3 Assessment Document P15]

The key events are 1) persistent liraglutide-induced GLP-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 spontaneous 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.

In a tissue distribution study administering a single bolus iv dose of ^{3}H-[tyr19]-liraglutide to rats, autoradiography of thyroid tissue harvested 4 hours after dosing showed radioactivity associated with thyroid blood vessel endothelium, but not C-cells. An immunohistochemical study of GLP-1R in rat thyroid tissue sections stained with anti-calcitonin antibodies to identify C-cells did not confirm the presence of the receptor on calcitonin immunoreactive cells 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.

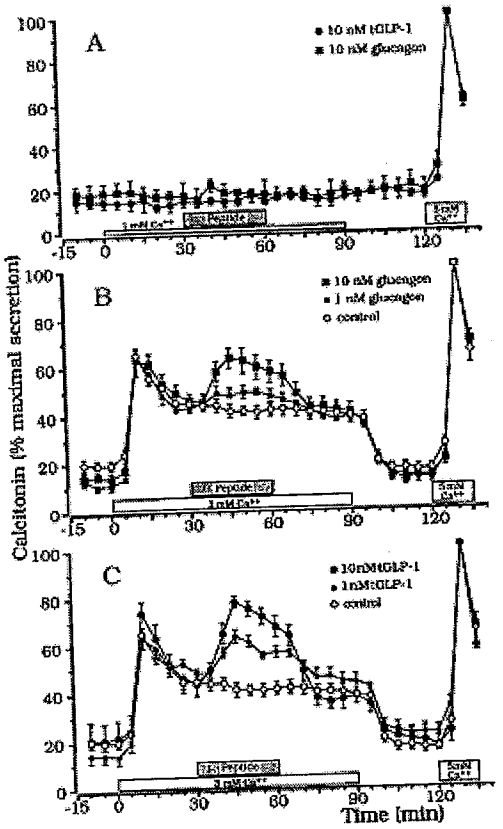


FIG. 5. Effect of glucagon (■) or tGLP-1 (●) on calcitonin secretion from perifused rat thyroid in the presence of 1 mM calcium (A) and 3 mM calcium (B and C). A stabilization period of 120 min at 0.5 mM calcium preceded the stimulation phase of 90 min at either 1 or 3 mM calcium and was followed by 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-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 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 and 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), 0.075, 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. 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 0.075, 0.25, or 0.75 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 0.75 mg/kg/day liraglutide in males and 0.25 mg/kg/day in females with 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 LOAEL for C-cell adenomas in females was lower than the LOAEL for focal hyperplasia. The incidence of C-cell carcinomas exceeded the concurrent and historical control range 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-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

I*n vitro* studies in mice 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 mouse calcitonin was

not reported. Cross reactivity of the rat calcitonin 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 to GLP-1R knockout mice suppressed elevated urinary excretion of deoxypyridinoline, a biomarker of increased bone resorption. 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) .

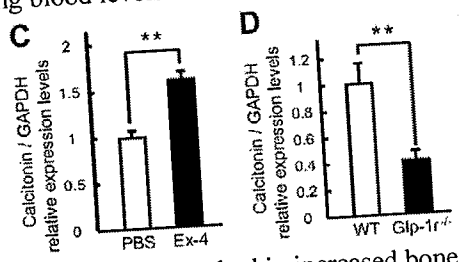


FIG. 4. Calcitonin deficiency resulted in increased bone resorption in Glp-1r$^{-/-}$ mice. **C**, Relative expression levels of calcitonin mRNA in thyroid from WT mice injected ip with PBS or 24 nmol/kg exendin-4 (Ex-4) 6 h before RNA isolation. Values are expressed as means +/- SE; n = 5 mice per group. *, 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-1R mRNA was unaffected.

Persistent C-cell stimulation 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:

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-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).

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.

Qualification of Impurities and Degradation Products

Study title: NNC 90-1170 (liraglutide): 4 week toxicity study in rats with subcutaneous administration (bridging study)

Key study findings: b(4)

- There were no substantive differences in toxicity of old formulation (drug substance from campaign 4B, pH 7.7) and new formulation (drug substance from campaign 5A, pH 8.15) at 1 mg/kg NNC 90-1170. The new formulation underwent ——— by storing it at — for —months
- NNC 90-1170 decreased body weight gain and transiently decreased food consumption in male rats, but body weight gain was only transiently decreased in females during the first week.
- NNC 90-1170 significantly increased WBC count (males and females), monocytes (males and females), neutrophils (males only, new formulation), and prothrombin time (both formulations in males, new formulation only in females).
- Absolute weight of heart, salivary glands, and ovaries were decreased by NNC 90-1170, but the effect may be due in large part to decreased body weight gain.
- Pathology findings occurred in injection sites (reddened, scab, congestion / hemorrhage, focal dermatitis, subacute inflammation, and myofiber degeneration / regeneration), kidney (inflammatory cell infiltrate, basophilic tubules), liver (inflammatory cell foci), lung (focal inflammation and focal alveolar macrophage accumulation), and lymph nodes (enlarged with correlative plasmocytosis).

Study no.: 205092
Module # and page #: 4.2.3.7.6.1, pages 1 - 355 b(4)
Conducting laboratory and location: ———

Date of study initiation: 14 June 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity, formulation:

Old formulation: batch PQ 50365 (from campaign 4B) in vehicle containing [redacted] (manufactured 9/11/04, certificate of analysis pages 77 – 78)

Analytical results:

Product batch No. PQ50365					
Analyzed	Analyzed in Dept. no.	Analysis no.	Test Method	Limits	Result

b(4)

[redacted]

Study Protocol: Interm no.:05433120

[P77-78]

New formulation: batch 433-05-020 (campaign 5A, supplier batch G1K4P025) in vehicle containing disodium [redacted]phosphate dihydrate, propylene glycol, and phenol, pH [redacted] (manufactured 9/11/04, certificate of analysis pages 77 – 78). The new formulation underwent [redacted] by storing it at [redacted] for [redacted] months. There were 2 shipments of the new formulation: [redacted] vials were received by the CRO on 16 June 2005 and [redacted] vials were received on 21 July 2005. b(4)

Analytical results:

Product batch No. 433-05-020					
Analyzed	Analyzed in Dept. no.	Analysis no.	Test Method	Limits	Result
Assay of liraglutide	*401*	*A6016-02*	*RP-HPLC*	[redacted]	
Sum of impurities	*AAI*	*N409R*	RP-HPLC	[redacted]	
Freezing point depression	*401*	*A2495-00*	Cryoscopy	[redacted]	
pH	*401*	*A2437-01*	Potentiometry, Ph.Eur, JP, USP	[redacted]	
Phenol	*401*	*A5002*	HPLC	[redacted]	
Macroscopy	*401*	*A3196-01*	Visual inspection NN	Complies[1]	Complies
Bacterial Endotoxin	*452*	*YM400*	Ph.Eur Method D	[redacted]	

Comments: [1] Complies means the sample is a colourless liquid, free from turbidity and foreign matter, and during storage, traces of a very fine sediment may be deposited.

Study Protocol: Interm no.:05433120

b(4)

Parameter	Koncentration
Liraglutide	6.0 mg/ml
Dinatrium [redacted] ostat, dihydrat	1.42 mg/ml
Propylenglykol	14.0 mg/ml
Phenol	5.5 mg/ml

pH = 8,15

[P79-80]

Analysis of dosing solutions and impurities are summarized in the table below. The level of impurities in Group 3 formulation was lower in August 2005 than in July 2005, and this may be due to differences in impurity levels of the same lot of liraglutide shipped to the CRO on different dates [redacted] vials received in June, [redacted] vials received in July). b(4)

Table 2 Analytical results of formulation analysis.

Sampling period	——— sample ID[1]	Department sample ID	Date of analysis	Label conc.of NNC 90-1170 (mg/ml)	Calc. conc.of NNC 90-1170 (mg/ml)	Sum of impurities %	Peak A %	Peak B %	Peak C %	——— impurities %	——— impurities %
24.Jun.2005	Group 1	A05739	05.Jul.2005	0.000	0.000						
24.Jun.2005	Group 2	A05740	05.Jul.2005	0.2	0.190						
24.Jun.2005	Group 3	A05741	05.Jul.2005	0.2	0.172						
22.Jul.2005	Group 1	A05863	09.Aug.2005	0.000	0.000						
22.Jul.2005	Group 2	A05864	09.Aug.2005	0.2	0.185						
22.Jul.2005	Group 3	A0565	09.Aug.2005	0.2	0.186						

b(4)

[1] Description of ——— sample ID b(4)

Group	Test article/Batch number	Dose Formulation Concentration (mg/ml)	Dose volume
1	Liraglutide / Vehicle	0,000	2 ml
2	Liraglutide / PQ 50365	0,2	2 ml
3	Liraglutide / 433-05-020	0,2	2 ml

[P79-80]

Methods

Doses: 0 (vehicle) or 1 mg/kg/day NNC 90-1170 (drug substance from campaign 4B (batch PQ 50365) compared to drug substance from campaign 5A (batch 433-05-020). Liraglutide stock solutions (6.25 or 6.0 mg/mL) were diluted to 0.2 mg/mL in their respective vehicles.

Study design:

Sprague Dawley rats (10/sex/dose main study, 6/sex/dose TK satellite, 7 weeks old at start of treatment) were subcutaneously injected once a day with vehicle, 1 mg/kg NNC 90-1170 (campaign 4B, old, 6.25 mg/mL stock), or 1 mg/kg NNC 90-1170 (campaign 5A, new, 6.25 mg/mL stock) for 4 weeks (5 mL/kg dose volume, 6 mg/mL stock). A group of 6 rats/sex were used for predose blood samples, but these rats were not treated.

Study parameters were viability, clinical signs, body weight, food consumption, water consumption, ophthalmoscopy, hematology, serum chemistry, urinalysis, anti-NNC 90-1170 antibody analysis, toxicokinetics, plasma calcitonin, organ weights, macroscopic and microscopic pathology.

Results and Conclusions

There were no unscheduled deaths, clinical signs of toxicity, or NNC 90-1170 related effects on water consumption, and ophthalmoscopy. Anti-NNC 90-1170 antibodies were not detected in plasma from treated rats using a validated RIA based on precipitation of antibody-bound [^{125}I]NNC 90-1170. Both old and new NNC 90-1170 reduced body weight gain and body weight compared to control in males, but the effect in females was equivocal (Figures 1 and 2).

Figure 1 Group Mean Body Weight (g): Main Study - Males

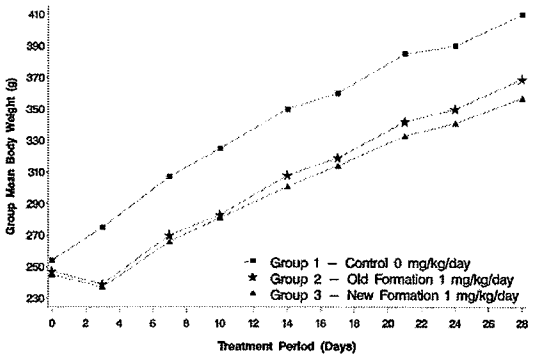
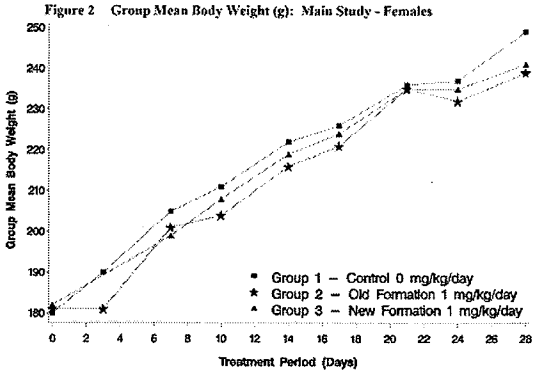


Figure 2 Group Mean Body Weight (g): Main Study - Females



[P75-6]

NNC 90-1170 transiently decreased food consumption on day 7 in males, but not in females. Both old and new NNC 90-1170 increased WBC count in males and females. The new batch significantly increased monocytes and prothrombin time in males and females and neutrophils in males.

Select Hematology & Coagulation Parameters (day 28, n = 10/group)

Sex	Male			Female		
NNC 90-1170 (mg/kg/day)	0	1 (old)	1 (new)	0	1 (old)	1 (new)
Parameter	Absolute Value	% Difference from Control		Absolute Value	% Difference from Control	
WBC (x 10^9/L)	12.50	19.7	29.4	11.45	9.8	6.1
Neut (x 10^9/L)	1.49	52.3	142.3	1.55	-1.3	-1.9
Mono (x 10^9/L)	0.49	16.3	38.8	0.28	35.7	21.4
PT (sec)	14.0	7.1	7.1	15.0	0.0	6.7

Statistically significant differences from control are underlined ($p < 0.05$)

Statistically significant serum chemistry parameter changes were increased ALT (male and females old and new), increased AST (females old), decreased Na (males new), increased Na (females old and new), decreased Cl (males new), increased chloride (females old and new), decreased total protein (male and female new), and decreased albumin (male and female old and new), and increased urea (females new). These changes were not considered toxicologically relevant because of their small magnitude and although mean group values were significantly different from controls, individual values were within a normal range. Slightly increased urine volume in females treated with new formulation NNC 90-1170 was significantly different from controls, but not considered toxicologically relevant.

Plasma calcitonin measured on days 1, 15, and 30 at 6 hours after dosing showed calcitonin levels were higher in NNC 90-1170 treated groups, but given the small number of samples in each group at each time point, the biological relevance of this finding is uncertain.

Table 7 N, mean and standard deviation (std) of calcitonin measurements

			Sex					
			F			M		
			Group			Group		
			1	2	3	1	2	3
Day	Timepoint							
1	6	N	2	2	2	2	2	2
		Mean	44.30	47.14	38.57	41.89	19.34	67.48
		Std	15.02	2.72	8.53	30.52	1.64	0.36
15	6	N	6	6	6	6	6	6
		Mean	76.90	105.67	111.84	27.38	82.82	128.53
		Std	40.14	43.61	48.71	8.65	35.43	64.76
30	6	N	2	2	2	2	2	2
		Mean	78.43	137.28	107.54	29.26	49.95	136.53
		Std	18.17	47.38	46.71	15.54	6.03	63.03

[P304]

Statistically significant changes in absolute organ weight in NNC 90-1170 treated rats were decreased heart, and salivary gland weight in males and females and decreased weight of ovaries in females. There were no substantive differences between old and new formulations. Decreased organ weight was likely due to decreased body weight, at least in part.

Table 11 Organ Weights (g) (Covariance Analysis): Main Study Group Mean Values:

		Males					Females					
Group Treatment		Body Weight (g)	Brain	Heart	Salivary Glands	Thyroid Glands		Body Weight (g)	Brain	Heart	Ovaries	Salivary Glands
1 Control (0)	Number	10	10	10	10	10	Number	10	10	10	10	10
	Mean	399	1.99	1.31	0.5277	0.0202	Mean	243	1.91	0.96	0.095	0.4442
	SE	9	0.03	0.04	0.0198	0.0013	SE	6	0.02	0.02	0.003	0.0097
2 Old Formulation (1)	Number	10	10	10	10	9	Number	10	10	10	10	10
	Mean	358	2.03	1.22	0.5727	0.0190	Mean	237	1.87	0.85	0.085	0.4077
	SE	9	0.03	0.03	0.0171	0.0012	SE	6	0.02	0.02	0.003	0.0096
	Prob.	na		ac	bc		Prob.	na		ac		bc
3 New Formulation (1)	Number	10	10	10	10	10	Number	10	10	10	10	10
	Mean	347	2.03	1.24	0.5437	0.0215	Mean	239	1.90	0.83	0.091	0.4074
	SE	9	0.03	0.03	0.0183	0.0012	SE	6	0.02	0.02	0.003	0.0096
	Prob.	na		cc	cc		Prob.	na		cc	as	cc

na = not applicable
Sexes tested combined (c) and significantly different from the control: ac P<=0.05, bc P<=0.01, cc P<=0.001.
Sexes tested separately (s) and significantly different from the control: as P<=0.05, bs P<=0.01, cs P<=0.001.
There was a significant effect (p=0.002) of treatment on the terminal kill bodyweight, therefore results should be treated with caution

[P52-3]

NNC 90-1170 treatment-related macroscopic pathology changes occurred at injection sites (reddened, scab) and mandibular lymph nodes (enlarged). Treatment-related histopathology finding occurred at injection sites, kidney, liver, lungs, and mandibular lymph nodes.

The incidence of injection site congestion / hemorrhage, focal dermatitis, subacute inflammation, and myofiber degeneration / regeneration was generally higher in NNC 90-1170 groups compared to concurrent controls. Local toxicity of liraglutide is not fully assessed in most rat toxicity studies because the liraglutide concentration in the dosing formulation for rats (0.2 mg/mL liraglutide) is ~ 30-fold lower the clinical formulation (6 mg/mL)

Kidney inflammatory cell infiltrate was increased in male and female NNC 90-1170 groups, and the incidence of basophilic tubules was only increased compared to control in females treated with the new formulation.

Liver inflammatory cell foci were increased by both old and new NNC 90-1170 formulations in males, but because of the high background incidence, not in females.

Lung findings in NNC 90-1170 treated females were increased focal inflammation and focal alveolar macrophage accumulation, but the incidence for both findings were marginally above control group levels.

Enlarged mandibular lymph nodes had correlative plasmocytosis in NNC 90-1170 treated males.

Pathology, Main Study (n = 10/sex/dose)

Organ	Finding	Severity	Sex	Male			Female		
			NNC 90-1170 (mg/kg/day)	0	1 (old)	1 (new)	0	1 (old)	1 (new)
Injection site 1	reddened	macroscopic		4	2	1	2	4	5
	scab	macroscopic		2	2	5	0	2	3
	congestion / hemorrhage	minimal / mild		5	4	1	2	4	1
	focal dermitits	minimal - moderate		2	2	5	0	2	3
	myofiber degeneration / regeneration	minimal / mild		1	3	1	2	1	0
Injection site 2	reddened	macroscopic		2	5	1	3	3	3
	congestion / hemorrhage	minimal - moderate		2	5	1	4	1	3
	subcutaneous inflammation	minimal - moderate		2	5	1	2	1	3
	myofiber degeneration / regeneration	minimal / mild		0	2	3	1	0	2
Kidney	basophilic tubules			6	3	6	2	2	6
	focal inflammatory cell infiltrate			3	7	4	1	4	4
Liver	inflammatory cell foci			5	8	6	8	7	6
Lungs	focal inflammation			2	1	1	2	4	0
	focal alveolar macrophage accumulation			3	2	1	0	1	2
Lymph node, mandibular	enlarged	macroscopic		0	3	4	3	2	0
	plasmocytosis			0	3	6	3	2	1

Day 1 and day 29 toxicokinetic parameters show NNC 90-1170 exposure from old and new formulations were similar with no substantive sex differences or accumulation with repeat dosing.

Table 1 PK parameters of liraglutide in rats (n=2/sex/group) after s.c. administration.

Period (day)	Group	Dose (mg/kg)	Gender	C_{max} (nmol/L)	C_{max}/Dose (nmol/L/nmol/kg)	t_{max} (hr)	λ_z (1/hr)	$t_{1/2}$ (hr)	$AUC_{0-tlast}$ (hr·nmol/L)	$AUC_{0-\infty}$/Dose (hr·nmol/L/nmol/kg)	$AUC_{\%Extrap}$ (%)	CL/f (L/hr/kg)	Vz/f (L/kg)	AUC (hr*nmol/L)	AUC/Dose (hr·nmol/L /nmol/kg)
1	2	1.0	F	291.3	1.3	6.0	0.09171	7.6	3925	14.7	14.0	0.05840	0.6368	4365	17.1
		1.0	M	437.7	1.6	8.0	0.07384	9.4	6159	23.1	22.8	0.03341	0.4525	7978	29.9
			Mean	364.5	1.4	7.0	0.08278	8.4*	5042	18.9	18.4	0.04591	0.5446	6272	23.5
	3	1.0	F	324.7	1.2	8.0	0.09786	7.1	4206	15.8	12.6	0.05557	0.5658	4815	18.1
		1.0	M	363.0	1.4	8.0	0.04982	13.9	5672	21.3	33.8	0.03112	0.6246	8567	32.1
			Mean	343.8	1.3	8.0	0.07384	9.4*	4939	18.5	23.2	0.04324	0.5952	6691	25.1

Period (day)	Group	Dose (mg/kg /day)	Gender	C_{max} (nmol/L)	C_{max}/Dose (nmol/L/ nmol/kg)	t_{max} (hr)	λ_z (1/hr)	$t_{1/2}$ (hr)	AUC_{tau} (hr·nmol/L)	AUC_{tau}/Dose (hr·nmol/L/ nmol/kg)	$AUC_{\%Extrap}$ (%)	CLss/f (L/hr/kg)	Vz/f (L/kg)	Rac (pred)	Rac (obs)
29	2	1.0	F	477.4	1.8	6.0	0.1301	5.3	5302	20.2	6.3	0.04944	0.3774	1.05	1.37
		1.0	M	447.7	1.7	6.0	0.1670	4.1	4807	18.0	3.1	0.05546	0.3320	1.02	0.78
			Mean	462.5	1.7	6.0	0.1490	4.7*	5099	19.1	4.7	0.05245	0.3547	1.0	1.1
	3	1.0	F	457.9	1.7	6.0	0.08909	7.8	5194	19.5	15.5	0.05132	0.5760	1.13	1.23
		1.0	M	336.2	1.3	8.0	0.1050	6.5	4374	16.4	10.0	0.06004	0.5754	1.09	0.77
			Mean	397.0	1.5	7.0	0.0975	7.1*	4784	17.9	12.7	0.05613	0.5757	1.1	1.0

Group 2: old formulation and Group 3: new formulation
a) Harmonic mean

[P256]

These results from a 4 week repeat subcutaneous dose toxicity study in rats show there were no substantive differences in toxicity of old (drug substance from campaign 4B) and new (drug substance from campaign 5A) formulations of NNC 90-1170.

2.6.7 TOXICOLOGY TABULATED SUMMARY

2.6.7.5 Single-Dose Toxicity **Test Article: Liraglutide**

Species/ Strain	Method of Administration (Vehicle/ Formulation	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Non-Lethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number (NN ref. No.)
Mouse, CD1	s.c.	0, 10	5M, 5F	10	NA	No mortality and slight toxicity were observed after single subcutaneous dose	NN980178 (4.2.3.1 Single-Dose Toxicity)
Mouse, CD1	i.v.	0, 10	5M, 5F	10	NA	No mortality and slight toxicity were observed after single intravenous dose of 10mg/kg.	NN980179 (4.2.3.1 Single-Dose Toxicity)
Rats, Sprague Dawley	s.c.	0,10	5M, 5F	10	NA	No mortality and slight toxicity were observed after single subcutaneous dose of 10mg/kg to rats.	NN980175 (4.2.3.1 Single-Dose Toxicity)
Rats, Sprague Dawley	i.v.	0, 10	5M, 5F	10	NA	No mortality and slight toxicity were observed after single intravenous dose of 10mg/kg to rats.	NN980177 (4.2.3.1 Single-Dose Toxicity)

NA- Not applicable

[N000 2.6.7 P12]

2.6.7.6 Repeat-Dose Toxicity Non-Pivotal Studies **Test Article: Liraglutide**

Species/ Strain	Method of Administration (Vehicle/ Formulation	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number (NN ref No.)
Rat/Sprague Dawley	s.c (Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	7 days	0, 0.125, 0.25, 0.4 1.0, 2.0, 10.0	5M and 5F per group	0.4	Animals given 2.0 and 10 mg/kg were killed prematurely on the second and third day of dosing due to severe body weight loss and adverse clinical signs. At 1.0 mg/kg and below, a dose-dependent decrease in food consumption and body weight gain were observed.	NN980180 (4.2.3.2 Repeat-Dose Toxicity)
Cynomolgus Monkey / *Macaca fascicularis*	s.c (Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4).	12 days (3 days at each dose level followed by a 4-day dose free period)	0.1, 0.5, 2.5, 5.0	2M and 2F receiveing increasing doses of liraglutide	5 mg/kg	Slight body weight loss in females and poor body weight gain in males. A glucose tolerance test showed an enhanced decline of artificially elevated glucose levels confirming the pharmacological responsiveness in this species.	NN970455 (4.2.3.2 Repeat-Dose Toxicity)
Cynomolgus Monkey / *Macaca fascicularis*	s.c (Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	14 days	4 mg/kg	2M and 2F	4 mg/kg	Transient decreased food consumption in the first week of dosing, slight decrease in body weight on day 14. Subcutaneous reddening and thickening at the injection site.	NN980181 (4.2.3.2 Repeat-Dose Toxicity)

[N000 2.6.7 P13]

2.6.7.7 Repeat-Dose Toxicity Pivotal Studies **Test Article: Liraglutide**

2.6.7.7.A 4 Week Toxicity Study in Mice with Subcutaneous Administration NN203261

Report Title: NNC 90-1170:
4 Week Toxicity Study in Mice with Subcutaneous Administration

Study No. NN203261/204288

Species/Strain: Mice/CD-1 (Crl: CD-1$^{(ICR)}$(ICR)BR)	**Duration of Dosing:** 4 week	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 7 weeks	**Duration of Postdose:** 0 weeks	
Date of First Dose: 27 October 2003	**Method of Administration:** Subcutaneous	**GLP Compliance:** Yes
	Vehicle/Formulation: Disodium hydrogen phosphate, propylene glycol, phenol and water (pH 7.75)	

Parameters collected:
Body Weight (g), Food Consumption (g), Water Consumption (visual), Clinical Observations, Ophthalmoscopy, Haematology, Clinical Chemistry, Urinalysis, Organ Weights (g),Gross Pathology, Histopathology

Special Features: None

No Observed Adverse-Effect Level: NOAEL was 5 mg/kg/day

Brief conclusion:
In males and females, pharmacological effects on body weight and food consumption were observed at nearly all dose levels and changes in urine parameters, also considered to be a pharmacological effect of treatment. Changes in organ weight, haematology and clinical chemistry findings, was seen in both sexes, and considered to be of equivocal significance.

Daily Dose (mg/kg)	**0 (Control)**		**0.1**		**0.5**		**1.0**		**5.0**	
Number of Animals + (satellite animals)	M: 10 (16)	F: 10 (16)	M: 10 (16)	F: 10 (16)	M: 10 (16)	F: 10 (16)	M: 10 (16)	F: 10 (16)	M: 10 (16)	F: 10 (16)

Daily Dose (mg/kg)	**0 (Control)**		**0.1**		**0.5**		**1.0**		**5.0**	
Toxicokinetics:										
AUC (h*nmol/L)		-								
Day 1	-	-	1155	990	6891	4888	15554	12345	100125	64378
Day 28	-		992	632	5462	3411	12758	6509	71417	43801
C_{max} (nmol/L)										
Day 1		-								
Day 28	-	-	75.07	74.74	522.50	666.50	1246.00	1132.00	6505.50	5145.50
T_{max} (h)	-		72.36	58.60	410.40	307.30	1308.05	552.73	5712.50	4340.00
Day 1		-								
Day 28	-	-	8	4	6	4	4	4	4	6
	-		4	8	8	8	6	8	4	4
Noteworthy Findings										
Died or Sacrificed Moribund	0 (1)	0 (0)	0 (0)	0 (1)			1 (0)	0 (0)	0 ()	0 (0)
Body weight (g)^{a}										
Day 1	35.9		35.8		35.5**		35.0*		33.3**	
Day 2	35.6		35.2		35.4		34.4**		33.3**	
Day 3	35.9		35.9		35.9		34.8**		34.3**	
Day 4	35.9		36.4		36.5		35.5*		34.5**	
Day 5, 6, 7 ....28.	*	*	*	*	*	*	*	*	*	*
Body weight gain (Day 0-28)	*	*	*	*	*	*	*	*	*	*
Food Consumption (g/animal/day)^{a}										
Day 3	6.08	5.01	5.24**	4.57**	5.03***	3.40***	5.01***	5.28***	4.23***	3.29***
Day 7	6.10	5.01	5.63	5.26	5.47	4.74	5.55*	4.52*	5.60*	4.23*
Day 10, 14, 7, 21, 24 and 28	*	*	*	*	*	*	*	*	*	*
Water consumption	-	-	-	-	-	-	-	-	-	-
Opthalmoscopy	-	-	-	-	-	-	-	-	-	-
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Haematology										
Haemoglobin (g/dL)	14.0	14.9	14.4	14.5	14.3	13.8	13.3*	14.0*	14.2	13.7
Red Blood Cell Count (x10^{12}/L)	9.29	9.39	9.25	9.21	9.16	8.60	8.65*	8.84*	9.28	8.60
Haematocrit (L/L)	0.471	0.484	0.475	0.478	0.474	0.444	0.441*	0.457*	0.471	0.448
Clinical chemistry										

Daily Dose (mg/kg)	0 (Control)		0.1		0.5		1.0		5.0	
Aspartate Aminotransferase iu/L	68	54	48*	71	41**	52	51	55	49*	55
Alanine Aminotransferase iu/L	46	29	30*	38	26**	31	32	27	31	28
Total protein g/L	51	49	51	49	51	50	52	51	53**	52**
Albumin g/L	32	33	32	34	32	34	33*	35*	35***	37***
Cholesterol mmol/L	3.3	2.2	2.6**	2.2	2.7*	2.2	3.0	2.0	2.6*	2.0
Glutamate Dehydrogenase	47.5	11.2	10.7*	25.3	9.2**	12.8	11.1**	12.3	15.3	9.6
Urinanalysis										
Urine Volumen (ml)	0.3	0.6	0.7	0.8	0.5	0.7	0.7*	0.9*	1.0*	0.6*
Urinary pH	8.5	8.5	7.6	7.4*	8.4	7.4*	7.8	7.7	7.6	7.1**
Urinary Chloride (mmol/L)	98.2	137.1	120.2	115.7	103.9	100.9	88.6*	93.4*	92.0*	88.3*
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Organ weights										
Spleen	0.110	0.110	0.089**	0.125	0.088**	0.107	0.092*	0.111	0.098	0.114
Histopathology	-	-	-	-	-	-	-	-	-	-
Thyroid gland										
No. abnormality detected	10	10	10	9	10	9	9	9	9	8
C-cells, unilateral, perithyroidal, focal							1			2
Ultimobranchial cyst				1		1	1	1		1
Only one examined		1								1
Injection/Treatment site										
No abnormality detected	5	3							7	3
Epidermal hyperplasia, focal	0	0							1	0
Exudate, inflammatory, emidemal	2	0							0	0
Dermatitis, ulcerative focal	1	0							0	0
Regeneration, subcutaneous, myofibre	2	3							2	1
Inflammatory cell infiltration, focal	3	4							2	6
Inflammation, focal, panniculitis unsele	1	0							0	1
Pigmented macrophages	1	2							0	0
Only one examined	0	1							0	0

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

a - Adjusted weight group mean value

[N000 2.6.7 P14 - 16]

2.6.7.7.B 13 Week Toxicity Study in Mice with Subcutaneous Administration NN204082

Report Title: 13 Week Toxicity Study in Mice with Subcutaneous Administration **Study No. NN204082**

Species/Strain: Mice/CD-1 (Crl: CD-1^{TM}(ICR)BR)	**Duration of Dosing:** 13 weeks	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 7 weeks	**Duration of Postdose:** 0 weeks	**GLP Compliance:** Yes
Date of First Dose: 15 March 2004	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium hydrogen phosphate, propylene glycol, phenol and water (pH 7.7)	

Parameters collected: Body Weight (g), Food Consumption (g), Water Consumption (visual), Clinical Observations, Ophthalmoscopy, Haematology, Clinical Chemistry, Urinalysis, Organ Weights (g),Gross Pathology, Histopathology

Special Features: Determination of antibodies was conducted in 15 male and female animals (control) and 5 male and 5 females for each dose.

No Observed Adverse-Effect Level: NA

Brief conclusion:

In males and females, pharmacological effects on body weight and food consumption were observed at nearly all dose levels and changes in urine parameters, also considered to be a pharmacological effect of treatment, were apparent at all dose levels. Changes in organ weight, haematology and clinical chemistry findings, seen in both sexes, were considered to be of equivocal significance. Changes in C-cells of the thyroid gland were seen at 0.2 mg/kg/day and above in males and females. A No Observed Effect Level (NOEL) was not identified for the C-cell findings. However, none of the other related findings were considered to be of a dose limiting nature.

Daily Dose (mg/kg)	0 (Control)		0.2		1.0		5.0	
Number of Animals +(Satellite animals)	M: 10 (28)	F: 10 (28)	M: 10 (28)	F: 10 (28)	M: 10 (28)	F: 10 (28)	M: 10 (28)	F: 10 (28)
Toxicokinetics:								
AUC (h*nmol/L)								
Day 1			2229	1636	13549	11298	73336	74765
Week 13			1876	2042	11854	18592	74212	62857
C_{max} (nmol/L)								
Day 1			142	179	975	1017	4670	7127
Week 13			145	247	1012	1320	5927	6289
T_{max}(h)								
Day 1			4.0	6.0	4.0	4.0	8.0	4.0
Week 13			4.0	8.0	1.0	6.0	4.0	4.0

Daily Dose (mg/kg)	0 (Control)		0.2		1.0		5.0	
Noteworthy Findings								
Died or Sacrificed Moribund	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Body weight (g)[a]								
Day 1	35.1	27.5	34.0**	27.4	33.5***	27.7	33.2***	25.8***
Day 2	35.9	28.0	34.9*	27.9	34.6**	27.4	33.7***	26.8*
Day 3	35.3	28.4	35.8	28.5	34.2**	27.8	34.4*	28.1
Food Consumption (g/animal/day)[a]								
Day 7	5.66	4.78	5.66	4.80	5.02**	4.34**	4.72**	3.91***
Clinical Observations	-	-	-	-	-	-	-	-
Water consumption	-	-	-	-	-	-	-	-
Opthalmoscopy	-	-	-	-	-	-	-	-
Haematology (% of control)								
Red Blood Cell Count			94*	97*	88***	94***	95**	94**
Haematocrit			95*	98*	90***	97***	95*	97*
Mean Cell Haemoglobin			103*	103*	104**	103**	101*	104*
Red Cell Distribution			102	93*	106	94	101	93*
Clinical chemistry (% of control)								
Urea			113	119	134*	127*	105	104
Total protein			102	100	96	100	100	103*
Cholesterol			88	100	70**	110	79*	105
Creatinine			108	107	100	107	112**	114**
Triglycerides			87	106	59**	138	60**	83
Glutamate Dehydrogenase			73	46	70	50	76	52
Urinanalysis (% of control)								
Day 1								
Urine specific gravity							99***	99***
Urine Volume							337***	400***
Urinary pH							101	99
Urinary Magnesium							54***	59***
Urinary Sodium							468***	230***
Urinary Potassium							64***	67***
Urinary Chloride							248***	168***
Urinary Calcium							67***	37***
Urinary Phosphate							119	144
Week 13								
Urine specific gravity			100	101	101	100	100	100
Urine Volume			244	200	100	283	133	217
Urinary pH			95	103	85	98	94	97
Urinary Magnesium			96	77	175	92	156	79
Urinary Sodium			134	108	136	109	119	108
Urinary Potassium			94	79	108	91	103	66
Urinary Chloride			112	100	106	110	104	81
Urinary Calcium			88	168	86	158	102	124
Urinary Phosphate			256	45	468***	72	246	82
Gross Pathology	-	-	-	-	-	-	-	-
Organ weights (% of control)								
Kidneys			98	98	98	102	95	100
Salivary Glands			92	94	90*	90*	93*	91*
Histopathology								
Thyroid gland								
No. examined	23	23	24	23	24	24	24	24
No. abnormality detected	21	17	9***	10	9***	12	4***	8**
Ultimobranchial cyst	2	6	10*	12	12**	8	17***	11
C-cell hyperplasia								
Minimal	0	0	9**	8**	8**	10***	6*	8**
Mild	0	0	0	0	0	0	4	5*
Injection/Treatment site								
No. abnormality detected	2	3					2	1
Inflammation	7	7					7	9
Inflammation and necrosis, needle track	1	1					1	0
Squamous cell hyperplasia	2	0					1	1
Pigmented macrophages, localised	0	1					0	0
Fibrosis, dermis, localised	2	0					0	0
Antibody determination								
No. of positive	0	0	0	0	0	0	0	0

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

a - Adjusted mean (Covariance Analysis)

[N000 2.6.7 P17 - 20]

2.6.7.7.C 28 Day Subcutaneous Toxicity Study in Rats NN980183

Report Title: 28 Day Subcutaneous Toxicity Study in Rats		**Study No. NN980183**
Species/Strain: Sprague Dawley rats (Crl:CD®BR)	**Duration of Dosing:** 4 weeks	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 5 weeks	**Duration of Postdose:** 0 weeks	**GLP Compliance:** Yes
Date of First Dose: 17 November 1998	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:
Body Weight (g), Food Consumption (g), Water Consumption (visual), Clinical Observations, Ophthalmoscopy, Haematology, Clinical Chemistry, Urinalysis, Organ Weights (g),Gross Pathology, Histopathology

Special Features: Samples for measurement of antibodies were obtained from samples originally collected for determination of antigen for toxicokinetic analysis

No Observed Adverse-Effect Level: 0.25 mg/kg

Brief conclusion:
Dose levels of 0.1, 0.25 or 1.0 mg/kg/day for 28 days via the subcutaneous route resulted in a reduced body weight gain in the male High dose group and increased levels of alanine aminotransferase (ALT) in all groups (in both sexes).

Daily Dose (mg/kg)	**0 (Control)**		**0.1**		**0.25**		**1.0**	
Number of Animals + (Satellite animals)	M: 10 (10)	F: 10 (10)	M: 10 (10)	F: 10 (10)	M: 10 (10)	F: 10 (10)	M: 10 (10)	F: 10 (10)
Toxicokinetics:								
AUC (h*nmol/L)								
Day 1			523	440	1498	2127	9070	7325
Day 28			549	460	1915	2485	11995	6155
C_{max} (nmol/L)								
Day 1			37	39	93	136	1195	413
Day 28			44	32	155	173	783	371
T_{max} (h)								
Day 1			6	9	8	8	4	4
Day 28			4	8	8	6	8	12
Daily Dose (mg/kg)	**0 (Control)**		**0.1**		**0.25**		**1.0**	
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body weight (g)[a]								
Day 1	231	187	217***	182*	203***	176***	197***	172***
Day 2	238	190	230	190	217***	183*	207***	179**
Day 3	245	194	238***	195	226***	190	212***	189*
Day 4	253	198	243**	199	231***	194	215***	189***
Day 5	265	200	253	204	243***	197	226***	188***
Day 6	272	201	265	205	253**	200	233***	199
Day 7	278	204	270	210	259**	208	244***	204
Day 14	320	248	312	224	307	222	281***	219
Day 21	351	233	349	240	349	238	324*	242
Day 28	*	*	*	*	*	*	*	*
Body weight gain (Day 0-28)	*	*	*	*	*	*	*	*
Food Consumption (g/animal/day)								
Day 3	31	22	27*	21	19***	17***	14***	14***
Day 7	31	22	29	22	27*	21	25**	20
Day 10	30	22	30	22	30	22	27**	20**
Day 14	31	22	31	22	31	21	28**	20**
Day 24	29	21	28	22	27	21	27*	20*
Day 28	-	-	-	-	-	-	-	-
Clinical Observations								
Faecal output[b]	-	-	-	-	10	10	10	10
Rolling gait	-	-	-	-	-	-	10	10
Hunched posture	-	-	-	-	-	-	7	9
Piloerection	-	-	-	-	-	-	6	4
Unkempt	-	-	-	-	-	-	6	2
Coat staining	-	-	-	1	-	1	2	2
High stepping gait	-	-	-	-	-	-	1	3
Partial hair loss	-	-	1	-	-	-	2	3
Water consumption	-	-	-	-	-	-	-	-

Daily Dose (mg/kg)	0 (Control)		0.1		0.25		1.0	
Opthalmoscopy	-	-	-	-	-	-	-	-
Clinical Observations								
Haematology								
Red Blood Cell Count ($x10^{12}$/L)	7.76	7.85	7.54***	7.48***	7.52**	7.56**	7.47***	7.50***
Haematocrit (L/L)	0.439	0.420	0.423**	0.409**	0.428**	0.406**	0.420***	0.403***
Red Cell Distribution Width ($x10^{9}$/L)	12.3	11.3	12.4	11.4	12.6	11.6	12.8**	11.9**
Monocyte (x 10^{9}/L)	0.21	0.13	0.29	0.13	0.23	0.15	0.35*	0.19*
Clinical chemistry								
Urea (mmol/L)	5.2	5.3	6.0	6.0	5.7	6.0	5.7*	6.5*
Aspartate Aminotransferase (u/L)	79	84	93	86	100***	92***	93*	85*
Alanine Aminotransferase iu/L	82	76	108*	83*	113***	91***	116***	97***
Alkaline Phosphatase (iu/L)	574	374	592	389	642*	464*	639	452
Potassium (mmol/L)	4.7	4.2	4.8	4.3	5.0**	4.6**	4.6	4.3
Total protein (g/L)	64	68	62**	66	62	67	63	67
Globulin (g/L)	33	35	32**	33**	31*	34*	32*	34*
Calcium (mmol/L)	2.83	2.73	2.85	2.76	2.87**	0.07**	2.88*	0.07*
Inorganic Phosphokinase (iu/L)	2.13	1.76	2.38	1.76	2.11	1.94	2.23**	0.15**
Creatine Phosphokinase (u/L)	159	111	182	141	274*	369**	210*	167**
Urinanalysis	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Organ weights[a]								
Heart	1.38	0.99	1.30	0.93	1.25***	0.88***	1.25**	0.93**
Histopathology[b]	-	-	-	-	-	-	-	-
Injection site								
No abnormality detected	8	8					6	7
Haemorrhage, subcutaneous								
Minimal	0	0					0	1
Mild	0	0					0	0
Panniculitis								
Minimal	2	1					1	1
Mild	0	1					1	2

Daily Dose (mg/kg)	0 (Control)		0.1		0.25		1.0	
Antibody determination								
No. of positive	0	0	0	0	0	0	0	0

- No noteworthy findings

[a] Adjusted weight group mean value

[b] Only apparent for the first week of dosing

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

[N000 2.6.7 P21 - 24]

2.6.7.7.D 13 Week Subcutaneous Toxicity Study in Rats with Recovery Period NN980189

Report Title: 13 Week Subcutaneous Toxicity Study in Rats with Recovery Period **Study No. NN980189**

Species/Strain: Rat/(Crl:CD®(SD)IGSBR)	**Duration of Dosing:** 13 weeks	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 4-6 weeks	**Duration of Postdose:** 4 weeks	
Date of First Dose: 22 March 1999	**Method of Administration:** Subcutaneous	**GLP Compliance:** Yes
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:
Body Weight (g), Food Consumption (g), Water Consumption (visual), Clinical Observations, Ophthalmoscopy, Haematology, Clinical Chemistry, Urinalysis, Sperm analysis, Organ Weights (g), Gross Pathology, Histopathology

Special Features: Samples for measurement of antibodies were obtained from all recovery animals prior to Day 1 of dosing, 24 hours after last dose and on the day of necropsy.

No Observed Adverse-Effect Level: The NOEL is below 0.01 mg/kg. The NOAEL based on clinical signs is 0.25 mg/kg.

Brief conclusion: In males and females, pharmacological effects on body weight and food consumption were observed at nearly all dose levels and changes in urine parameters, also considered to be a pharmacological effect of treatment, were apparent at all dose levels. Changes in organ weight, haematology and clinical chemistry findings, seen in both sexes, were considered to be of equivocal significance. Changes in C-cells of the thyroid gland were seen at 0.2 mg/kg/day and above in males and females. a No Observed Effect Level (NOEL) was not identified for the C-cell findings. However, none of the other related findings were considered to be of a dose limiting nature.

Daily Dose (mg/kg)	**0 (Control)**		**0.1**		**0.25**		**1.0**	
Number of Animals + (satellite animals)	M: 10 (5)	F: 10 (5)	M: 10 (5)	F: 10 (5)	M: 10 (5)	F: 10 (5)	M: 10 (5)	F: 10 (5)
Toxicokinetics:								
AUC (h*nmol/L)								
Day 1	-	-	776	844	2721	2582	11948	13536
Week 13	-	-	643	865	2445	3731	9188	12208
C_{max} (nmol/L)								
Day 1	-	-	62	76	227	307	920	1159
Week 13	-	-	39	53	144	368	358	768
Daily Dose (mg/kg)	**0 (Control)**		**0.1**		**0.25**		**1.0**	
T_{max} (h)								
Day 1	-	-	4	6	8	6	4	4
Week 13	-	-	4	2	8	4	12	6
Noteworthy Findings								
Died or Sacrificed Moribund	2		1				1	
Body weight gain Time 0-Day 91 (g)	368	133	332	145	318*	155	291***	140
Body weight (g/animal/day)								
Day 1	200	154	195***	154	183***	148***	175***	142***
Day 2	208	158	206	159	194***	152**	186***	147***
Day 3	215	160	213	163	203**	160	190***	155
Day 4	224	163	222	166	210***	163	196***	157*
Day 5	236	167	232	173	219***	168	203***	161*
Day 6	244	171	242	176	229***	173	214***	168
Day 7	247	172	248	179*	236***	178	221***	175
Food Consumption (g/animal/day)								
Day 3	29.7	22.1	24.2***	20.1	20.2***	16.4***	14.9***	13.2***
Day 7	31.8	20.6	30.5	21.9	29.2**	22.3*	26.3***	20.3
Day 10	30.9	20.5	31.0	21.4	30.2	20.3	27.9**	20.1**
Clinical Observations[a]								
Rolling gait	-	-	-	-	-	-	10	10
Piloerection	-	-	-	-	-	-	10	10
High stepping gait	-	-	-	-	-	-	9	5
Hunched posture	-	-	-	-	-	-	5	5
Thin/weight loss	-	-	-	-	-	-	4	0
Water consumption	-	-	-	-	-	-	-	-
Opthalmoscopy	-	-	-	-	-	-	-	-
Haematology								
White blood Cell Count (x 10^9/L)	10.41	6.26	12.11	9.89**	11.72	7.75	13.20	10.17***
Neutrophil (x 10^9/L)	1.31	0.67	1.33	0.84	1.35	0.75	2.19**	1.17***
Lymphocyte (x 10^9/L)	8.64	5.33	10.25	7.91**	9.86	6.68	10.38	8.57**
Monocyte (x 10^9/L)	0.15	0.09	0.21*	0.11*	0.18	0.11	0.23***	0.17***
Large Unclassified Cells (x 10^9/L)	0.09	0.05	0.12	0.03	0.13	0.03	0.13**	0.03**

Daily Dose (mg/kg)	0 (Control)		0.1		0.25		1.0	
Clinical chemistry								
Urea (mmol/L)	5.7	5.6	6.0*	6.6*	6.4*	6.3*	6.1**	6.9**
Alanine Aminotransferase (iu/L)	67	67	70	73	85*	69	90**	71
Alkaline Phosphatase (iu/L)	334	212	366*	316*	403*	289*	398***	359***
Total protein (g/L)	75	77	72**	75**	73**	75**	74**	74**
Albumin (g/L)	32	36	32	34*	31	34**	32	33***
Albumin/Globulin Ratio	0.7	0.9	0.8	0.8	0.8	0.8	0.8	0.8*
Inorganic Phosphokinase (iu/L)	1.85	1.56	2.02	1.87**	1.92*	1.89*	2.04***	1.91***
Creatine Phosphokinase (iu/L)	105	144	275	178	127	222**	135*	182
Urinanalysis								
Urine specific gravity	1.057	1.057	1.056	1.040	1.064	1.044	1.045*	1.038*
Gross Pathology	-	-	-	-	-	-	-	-
Organ weights[b]								
Heart	1.65	1.05	1.57	1.03	1.49	0.99	1.56	1.01
Sperm analysis	-						-	
Histopathology	-	-	-	-	-	-	-	-
Injection/Treatment side								
No. abnormality detected	7	6					9	8
Fibrosis subcutaneous								
Minimal	0	2					1	0
Mild	1	1					0	1
Fibrosis, dermal (needle track) mild	0	0					0	1
Haemorrhage, subcutaneous								
Minimal	0	1					0	0
Mild	0	1					0	0
Panniculitis								
Minimal	2	2					1	1
Mild	1	0					0	0
Postdose Evaluation:	5	5	5	5	5	5	5	5
Number Evaluated								
Died or Sacrificed Moribund							1	
Body weight gain Day 91-119 (g)	36	25					80*	27

Daily Dose (mg/kg)	0 (Control)		0.1		0.25		1.0	
Haematology								
Neutrophil (x 10^9/L)	1.53	1.13					0.30*	0.28*
Clinical chemistry								
Total protein (g/L)	72	76					67*	78
Globulin (g/L)	41	40					37*	41
Organ weights (g)								
Spleen	0.99	0.60					0.74*	0.56*
Antibody determination								
No. of positive	0	0	0	0	0	0	0	0

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

a- clinical signs were only seen during the first two weeks.

b- Adjusted group mean value.

[N000 2.6.7 P25 - 28]

2.6.7.7.E 26 Week Subcutaneous Toxicity Study in Rats NN200239

Report Title: 26 Week Subcutaneous Toxicity Study in Rats		**Study No. NN200239**
Species/Strain: Rat/(Crl:CD®(SD)IGSBR)	**Duration of Dosing:** 26 weeks	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 4 weeks	**Duration of Postdose:** 0 weeks	
Date of First Dose: 11 December 2000	**Method of Administration:** Subcutaneous	**GLP Compliance:** Yes
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:
Body Weight (g), Food Consumption (g), Water Consumption (visual), Clinical Observations, Ophthalmoscopy, Haematology, Clinical Chemistry, Urinalysis, Organ Weights, Gross Pathology, Histopathology

Special Features: None

No Observed Adverse-Effect Level: 1.0 mg/kg/day

Brief conclusion:
Pharmacological effects on food consumption and body weight were seen at all dose levels in both sexes. Treatment did not cause any signs of toxicity at dose levels of 0.1, 0.25 or 1.0 mg.kg-1.day-1. Minimally decreased heart weight was seen at all dose levels in both sexes and was considered to be of equivocal toxicological significance.

Daily Dose (mg/kg)	**0 (Control)**		**0.1**		**0.25**		**1.0**	
Number of Animals	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Toxicokinetics:								
AUC (h*nmol/L)								
Day 1			868	875	2130	2130	9460	7290
Week 26			379	583	1270	1900	5640	6840
C_{max} (nmol/L)								
Day 1			69.7	84.3	180	196	675	560
Week 26			24.8	44.5	85.2	128	468	668
T_{max} (h)								
Day 1			6.0	6.0	4.0	6.0	6.0	8.0
Week 26			8.0	6.0	12.0	6.0	8.0	6.0
Noteworthy Findings								
Died or Sacrificed Moribund[a]	1	0	1	0	0	0	0	1
Body weight gain Time 0-Day 181 (g)[b]	445.8	214.8	393.1	201.9	380.4***	196.3	360.6***	196.4
Food Consumption (g/animal/day)[b]								
Day 6	28.6	20.8	24.5**	19.7	21.2***	17.5**	17.5***	15.7***
Day 13	30.6	22.4	30.0	22.0	27.5	19.8	24.9*	20.9*
Clinical Observations	-	-	-	-	-	-	-	-
Water consumption	-	-	-	-	-	-	-	-
Opthalmoscopy	-	-	-	-	-	-	-	-
Haematology								
Week 13	-	-	-	-	-	-	-	-
Week 25	2.9	2.2	2.3*	2.1*	2.2**	2.0**	2.5*	1.9*
Reticulocyte Count (%)								
Clinical chemistry								
Week 13								
Lactate Dehydrogenase (iu/L)	282	288	265	346	314	552*	250	367**
Chloride (mmol/L)	102	105	103	104	100	106	104*	103*
Creatinine (µmol/L)	47	52	45	53	44	54	44*	48*
Inorganic Phosphate (mmol/L)	1.73	1.37	1.79	1.56	1.80	1.49	1.76	1.71***
Triglycerides (mmol/L)	1.25	0.77	1.16	0.72	1.15	0.45	0.79*	0.88
Non-esterified Fatty Acids (mmol/L)	0.60	0.52	0.50	0.44	0.42***	0.56	0.40***	0.43
Week 25								
Chloride (mmol/L)	98	103	100	101	98	101	100**	101
Total Bilirubin (µmol/L)	1.8	2.1	1.3**	1.9**	1.4*	1.9*	1.5*	1.8*
Urinanalysis								
Week 13								
Urine pH	8.7	7.1	8.2	6.4	7.8*	5.9*	7.9	7.4
Week 25	*	*	*	*	*	*	*	*
Gross Pathology	-	-	-	-	-	-	-	-
Organ weights (% of controls)[c]								
Heart			95*	94*	91***	91***	96*	90*

Daily Dose (mg/kg)	0 (Control)		0.1		0.25		1.0	
Histopathology	-	-	-	-	-	-	-	-
Injection/Treatment site								
No. abnormality detected	1	1					1	5
Crusting, epithelia (mild)	0	0					0	1
Dermatitis, focal								
minimal	1	1					2	3
mild	0	1					2	0
Fibrosis, subcutaneous								
minimal	4	4					4	3
mild	1	0					1	0
Haemorrhage, subcutaneous								
minimal	2	2					0	1
mild	2	5					7	3
moderate	0	0					1	0
Myositis (mild)	0	0					1	0
Panniculitis, focal								
minimal	4	8					5	6
mild	6	2					4	1

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

a - None of the deaths were related to treatment

b - Adjusted (covariance analysis)

c- p-values is from covariance analysis of organ weights

[N000 2.6.7 P29 - 31]

2.6.7.7.F 28 Day Subcutaneous Toxicity Study in Cynomolgus Monkeys NN980184

Report Title: 28 Day Subcutaneous Toxicity Study in Cynomolgus Monkeys **Study No. NN980184**

Species/Strain: Cynomolgus Monkey (*Macaca fascicularis*)	**Duration of Dosing:** 4 weeks	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 14-18 months	**Duration of Postdose:** 0 weeks	**GLP Compliance:** Yes
Date of First Dose: 12 November 1998	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:

Body Weight (g), Food Consumption (g), Water Consumption (ml), Clinical Observations, Ophthalmoscopy, Haematology, Clinical Chemistry, Urinalysis, Faecal samples, Organ Weights (g),Gross Pathology, Histopathology

Special Features: Determination of antibodies from day 1 and the final day of dosing, pre-dose and 24 h post dose.

No Observed Adverse-Effect Level: 5.0 mg/kg

Brief conclusion:

The reduction in bodyweight gain or body weight loss in animals dosed with 0.5 or 5 mg/kg/day is considered to be due to treatment with NNC 90-1170. The reduction in food consumption over the initial few days of dosing in animals receiving 0.05, 0.5, and 5 mg/kg/day is also considered to be due to treatment with NNC 90-1170. There was a dose related increase (not significant) in pancreas in males only.

Daily Dose (mg/kg)	0 (Control)		0.05		0.5		5	
Number of Animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Toxicokinetics:								
AUC_{0-24h} (h*nmol/L)								
Day 1			257	210	2755	2615	32031	32829
Day 28			233	170	2285	1430	35381	14958
C_{max} (nmol/L)								
Day 1			16	13	191	183	1903	2293
Day 28			15	11	157	127	2679	945
T_{max}(h)								

Daily Dose (mg/kg)	0 (Control)		0.05		0.5		5	
Day 1			12	11	8	8	8	11
Day 28			11	6	7	6	7	7
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body weight [a]								
Day 7	2.0	1.9	1.9	1.8	1.7***	1.7***	1.8**	1.7**
Day 14	2.0	1.9	2.0	1.8	1.7**	1.8**	1.8**	1.7**
Day 21	1.9	2.0	1.9	1.9	1.7*	1.8*	1.8	1.8
Day 28	2.0	2.0	2.0	1.9	1.7*	1.9*	1.8*	1.7*
Body weight gain (Time 0-Day 28)	0.1	0.1	0.1	0.1	-0.3*	0.0*	-0.1*	-0.1*
Food Consumption (g/animal/day)								
Day -1	137	127	133	123	137	150	133	143
Day 1	147	140	120	117	113	117	103	100
Day 2	130	127	113	107	107	103	107	103
Day 3	127	123	110	110	113	113	110	113
Day 4	130	133	123	117	117	117	107	113
Day 5	127	140	113	120	120	127	110	117
Day 6	123	127	123	120	123	127	123	130
Day 7	143	133	130	130	127	133	113	127
Clinical Observations	*	*	*	*	*	*	*	*
Injection site (incidences per week)								
Subcutaneous thickening								
Week 3	18	13	13	14	20	14	12	21
Week 4	21	15	21	21	21	21	21	21
Slight bruising								
Week 1	0	2	0	0	0	0	0	2
Week 2	0	0	0	0	2	0	0	0
Reddened area (week 1)	0	0	0	0	1	1	0	1
Water consumption	-	-	-	-	-	-	-	-
Opthalmoscopy	*	*	*	*	*	*	*	*
Haematology	-	-	-	-	-	-	-	-
Clinical chemistry	-	-	-	-	-	-	-	-

Daily Dose (mg/kg)	0 (Control)		0.05		0.5		5	
Urinanalysis incl. faecal samples	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Organ weights (g) [b]								
Pancreas	2.80	3.51	3.65	3.08	3.70	2.64	3.66	3.63
Histopathology	*	*	*	*	*	*	*	*
Injection/Treatment site								
Fasciitis								
minimal	0	1	1	1	0	1	1	0
mild	1	1	1	1	3	1	1	1
moderate	2	0	1	0	0	1	1	2
Pigmented macrophages								
minimal	1	0	2	0	1	1	1	3
mild	2	0	0	1	1	1	0	0
moderate	0	0	0	1	0	0	0	0
Necrosis, needle track, unilateral								
minimal	0	0	0	1	0	0	0	0
Haemorrhage								
minimal	0	1	0	1	0	0	1	1
mild	1	2	2	2	2	2	2	0
moderate	2	0	1	0	1	1	0	2
Granuloma, unilateral.								
mild	0	0	0	1	0	0	0	1
Antibody determination								
No. of positive		0	0	0	0	0	0	0

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

a - Adjusted group mean values

b – Adjusted weight (covariance analysis)

[N000 2.6.7 P32 - 34]

2.6.7.7.G 13 Week SC Toxicity Study Cynomolgus Monkeys with a Recovery NN990191

Report Title: 13 Week Subcutaneous Toxicity Study in the Cynomolgus Monkeys with a Recovery Period **Study No. NN990191**

Species/Strain: Cynomolgus Monkey (*Macaca fascicularis*)	**Duration of Dosing:** 13 weeks	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 11-19 months	**Duration of Postdose:** 2 weeks	
Date of First Dose: 10 August 1999	**Method of Administration:** Subcutaneous	**GLP Compliance:** Yes
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:
Body Weight (g), Food Consumption (g), Water Consumption (visual), Clinical Observations, Ophthalmoscopy, Haematology, Clinical Chemistry, Urinalysis, Faeces samples, Organ Weights (g),Gross Pathology, Histopathology

Special Features: Samples for measurement of antibodies were obtained from all animals once pretrial, week 6 and week 13 and towards the end of the recovery period.

No Observed Adverse-Effect Level: 0.05 mg/kg

Brief conclusion:
Treatment related reduction in bodyweight was observed in animals dosed with 0.5 or 5 mg/kg/day and reduction in food consumption in animals receiving 0.05, 0.5, and 5 mg/kg/day . This is believed to be due to the pharmacological action of the test material. Haematology revealed an increase in eosinophil levels in male animals receiving 5 mg/kg/day and in all female animals receiving the test article, which correlated with the histology findings. A slight decrease in alkaline phosphatase levels in male and female animals receiving 0.5 or 5 mg/kg/day is of unknown toxicological significance.

Daily Dose (mg/kg)	**0 (Control)**		**0.05**		**0.5**		**5.0**	
Number of Animals	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Toxicokinetics: [a]								
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body weight [b]								
Body weight gain (Time 0-Week 13)	0.2	0.3	0.2	0.3	0.2	0.1	0.0**	0.2**
Daily Dose (mg/kg)	**0 (Control)**		**0.05**		**0.5**		**5.0**	
Food Consumption (g/animal/day)								
Week -1	135	133	134	137	134	136	135	133
Day 1	135	133	130	131	127	127	113	116
Clinical Observations [c]	-	-	-	-	-	-	-	-
Water consumption	-	-	-	-	-	-	-	-
Opthalmoscopy	-	-	-	-	-	-	-	-
Haematology [d]								
Week 6								
Eosinophil ($x10^9$/L)	0.04	0.05	0.09	0.11	0.00	0.13	0.24**	0.21**
Week 13								
Haemoglobin (g/dL)	13.8	13.5	13.5	13.6	13.0**	12.9**	13.0***	12.8***
Red Blood Cell Count ($x10^{12}$/L)	7.13	6.99	7.02	6.97	6.78	6.84	6.66**	6.63**
Haematocrit (L/L)	0.466	0.460	0.457	0.454	0.452	0.438	0.440	0.435**
Eosinophil ($x10^9$/L)	0.09	0.01	0.15	0.18*	0.26	0.24**	0.55*	0.33***
Clinical chemistry								
Week 6								
Alkaline Phosphatase (u/L)	1384	1535	1360	1423	1077**	1281**	1058***	963***
Cholesterol (mmol/L)	3.2	2.8	2.9	2.6	2.4	2.8	2.5*	2.6*
Phosphate (mmol/L)	2.05	2.15	1.95	1.94	1.90	1.83	1.87*	1.77**
Week 13								
Alkaline Phosphatase (u/L)	1408	1335	1374	1446	1087	1142	980**	1043**
Urinanalysis incl. faeces samples	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-
Organ weights (g) [b]	-	-	-	-	-	-	-	-
Pancreas	4.41	3.46	4.73	4.11	3.63	4.46	4.31	4.06
Histopathology	-	-	-	-	-	-	-	-
Injection/treatment site								
Fasciitis, chronic								
mild	0	1	1	0	1	0	1	0
moderate	4	3	2	1	3	0	0*	0
marked	0	0	0	0	0	1	0	0
severe	0	0	1	0	0	0	0	0

Daily Dose (mg/kg)	0 (Control)		0.05		0.5		5.0	
Fasciitis, chronic active								
moderate	0	0	0	2	0	3	0	2
marked	0	0	0	1	0	0	3	2
Pigmented macrophages	3	3	1	3	2	4	3	4
Haemorrhage	4	4	4	4	4	4	4	4
Granuloma	1	0	0	0	0	0	1	0
Antibody determination								
No. of positive	0	0	0	0	0	0	0	0
Postdose Evaluations:	2	2					2	2
Number Evaluated								
Noteworthy Findings	-	-	-	-	-	-	-	-

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

a - Drug exposure were assessed after 8 hours pre-dose, day 1, day 42 and day 91. The test article was detectable in plasma samples from all treated animals. No accumulation was observed during the 13 weeks of dosing. Results are in agreement with the 4 week toxicity study.

b - Adjusted weight (Covariance analysis).

[N000 2.6.7 P35 - 37]

2.6.7.7.H 52 Week Subcutaneous Toxicity Study in Cynomolgus Monkeys with a 4 week Recovery Period NN200241

Report Title: 52 Week Subcutaneous Toxicity Study in Cynomolgus Monkeys with a 4 week Recovery Period **Study No. NN200241**

Species/Strain: Cynomolgus Monkey (*Macaca fascicularis*)	**Duration of Dosing:** 52 weeks	**Location in CTD:** 4.2.3.2 Repeat-Dose Toxicity
Initial Age: 12-17 months	**Duration of Postdose:** 4 weeks	**GLP Compliance:** Yes
Date of First Dose: 20 February 2001	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol and water	

Parameters collected:
Body Weight (g), Food Consumption (g), Clinical Observations, Opthalmoscopy, Electrocardiography, Haematology, Clinical Chemistry, Bone Marrow Smears, Urinalysis, Organ Weights (g),Gross Pathology, Histopathology

Special Features: Samples for measurement of antibodies were obtained from all animals at week 13, 26, 39 and 52.

No Observed Adverse-Effect Level: 0.05 mg/kg

Brief conclusion:
Liraglutide was well tolerated when given to cynomolgus monkey for 52 weeks. No signs of systemic toxicity were observed at dose levels of 0.05, 0.5 or 5.0mg/kg. Slight changes clinical pathological parameters and increased pancreas weights were observed without corresponding pathological organ changes, and were considered to be of equivocal toxicological importance. Pharmacological changes on body weight and food consumption were seen in both sexes in animals given 0.5 and 5.0mg/kg.

Daily Dose (mg/kg)	0 (Control)		0.05		0.5		5.0	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Toxicokinetics:								
AUC (h*nmol/L)								
Day 1			407	560	6550	7870	87800	118000
Week 52			523	1110	7610	6430	62100	56300
C_{max} (nmol/L)								
Day 1			24.5	35.1	384	450	4900	5160

Daily Dose (mg/kg)	0 (Control)		0.05		0.5		5.0	
Week 52			29.9	62.4	439	406	3680	3570
T_{max} (h)								
Day 1			7.0	7.5	7.5	7.0	8.0	9.0
Week 52			8.5	7.5	7.0	7.5	4.7	5.7
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body weight , group mean (kg)								
Week 1	2.1	2.1	2.1	2.0	2.0	2.0	1.9***	1.8***
Week 2	2.1	2.2	2.1	2.1	2.0*	2.0	1.9***	1.9***
Week 3	2.1	2.2	2.0	2.0	2.0*	2.0	1.9***	1.9***
Week 4	2.1	2.2	2.1	2.1	2.0**	2.0	1.9***	2.0***
Week 5	2.1	2.2	2.1	2.1	2.0	2.0	2.0*	2.0
Weight gain (kg)	0.8	0.5	0.9	0.7	0.3	0.6	0.4	0.5
Food Consumption	-	-	-	-	-	-	-	-
Clinical Observations								
Subcutaneous thickening of injection site (%)								
Week 1-4	0	0	0	0	0	0	0	0
Week 5-8	19	37	31	13	31	37	69	81
Week 9-12	19	60	56	31	31	94	100	100
Week 13-16	65	86	75	75	96	71	100	100
Week 17-20	79	100	78	75	100	75	100	100
Week 21-24	81	100	100	98	100	75	100	100
Week 25-28	83	100	100	100	98	97	99	100
Week 29-40	83	100	100	100	100	100	100	100
Week 41-59	100	100	100	100	100	100	100	100
Water consumption	-	-	-	-	-	-	-	-
Opthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Haematology								
Week 26								
Haemoglobin (g/dL)	13.2	13.2	12.8	13.3	12.3	12.4**	11.9***	12.2***
Red Blood Cell Count ($x10^{12}$/L)	6.81	6.80	6.32	6.85	6.15	6.47*	6.15***	6.32***
Daily Dose (mg/kg)	**0 (Control)**		**0.05**		**0.5**		**5.0**	
Haematocrit (L/L)	0.428	0.414	0.384	0.424	0.388	0.403*	0.382***	0.391***
Lymphocytes ($x10^9$/L)	5.83	5.16	4.91	4.39	3.62	4.51	3.41**	3.73**
Week 39								
Haemoglobin (g/dL)	13.0	12.8	12.6	12.9	12.4	12.3	12.3*	12.2*
Red Blood Cell Count ($x10^{12}$/L)	6.67	6.59	6.65	6.83	6.20	6.50	6.31*	6.30*
Week 52								
Haemoglobin (g/dL)	13.8	13.2	12.8	12.9	12.3**	12.8**	12.4***	12.5***
Red Blood Cell Count ($x10^{12}$/L)	7.09	6.73	6.75	6.67	6.27*	6.65*	6.36***	6.17***
Haematocrit (L/L)	0.470	0.434	0.427	0.434	0.417*	0.439*	0.420***	0.412***
Reticulocytes (%)	0.5	0.7	0.5	0.8	0.5	0.6	0.7*	1.1*
Clinical chemistry								
Week 13								
Total Bilirubin	2.5	3.5	3.0	3.1	2.9	3.8	3.5**	4.3**
Week 26								
Urea (mmol/L)	7.3	7.1	7.0	5.8	6.4	6.1	6.2*	6.0*
Alkaline Phosphatase (iu/L)	1643	-	1241	-	1254	-	1027**	-
Phosphate (mmol/L)	1.92	-	1.80	-	2.01	-	1.67**	-
Week 39								
urea	7.7	8.3	8.1	6.7	6.0*	7.2*	6.7*	6.6*
Total Bilirubin (µmol/L)	-	2.4	-	2.3	-	2.2	-	3.5**
Week 52								
Alkaline Phosphatase (iu/L)	2236	1671	1750	1882	1539	2214	1456**	1464**
Globulin (g/L)	31	30	31	30	30	30	33*	32*
Total Bilirubin (µmol/L)	2.8	4.0	2.5	3.3	4.1	3.8	5.0**	5.1**
Urinanalysis	-	-	-	-	-	-	-	-
Gross Pathology								
Number of animals necropsied	4	4	4	4	4	4	4	4
Injection site/Treatment site								
Thickened	1	1	2	3	3	3	4	4
Reddened	4	4	4	4	4	4	4	4
Organ weights (absolute (g))								
Pancreas	3.712	3.569	5.319	4.307	5.755**	5.358**	6.026***	6.499***

Histopathology								
Injection site/Treatment site								
Fibrosis, subcutaneous								
minimal	0	2	0	0	0	0	0	0
mild	4	2	1	4	4	4	0*	2
moderate	0	0	3	0	0	0	1	1
Sclerosis, subcutaneous								
minimal	0	0	-	-	-	-	1	1
mild	0	0	-	-	-	-	1	0
marked	0	0	-	-	-	-	1	0
Pigmented macrophages, subcutaneous								
minimal	2	3	2	2	1	1	2	2
mild	2	1	2	2	1	2	2	2
moderate	0	0	0	0	2	0	0	0
Giant cells, foreign body, subcutaneous								
Minimal	0	0	-	-	-	-	1	0
mild	0	0	-	-	-	-	1	3
moderate	0	0	-	-	-	-	1	0
Inflammatory cell infiltration, subcutaneous								
minimal	1	0	0	0	1	0	1	2
mild	0	1	1	3	2	0	1	1
moderate	0	0	1	0	0	2	1	0
Foreign material, subcutaneous	-	-	-	-	-	-	1	2
Haemorrhage, subcutaneous	3	4	4	4	4	4	4	4
Masson's trichrome stain examined	0	0	-	-	-	-	4*	0
Antibody determination (week 52)								
No. of positive	0	0					1	0
Postdose Evaluation:								
Number Evaluated	2	2					2	2
Noteworthy Findings								
Injection site/Treatment site								
Dark	-	-					1	1

Daily Dose (mg/kg)	**0 (Control)**		**0.05**		**0.5**		**5.0**	
Thickened	-	-					1	1
Histopathology								
Injection site/Treatment site								
Fibrosis								
minimal	2	1					0	0
Sclerosis, subcutaneous								
moderate	0	0					2	2
Pigmented macrophages, subcutaneous								
minimal	0	1					2	2
mild	2	1					0	0
Giant cells, foreign body, subcutaneous								
Minimal	0	0					1	1
moderate	0	0					1	1
Inflammatory cell infiltration, subcutaneous								
minimal	0	0					1	0
mild	0	0					0	1
Antibody determination (recovery)								
No. of positive	0	0					1	1

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

[N000 2.6.7 P38 - 42]

2.6.7.8 Genotoxicity: *In-Vitro* **Test Article: Liraglutide**

2.6.7.8.A Reverse Mutation in Four Histidine-requiring strains of Salmonella typhimurium and Two Tryptophan-requiring strains of *Escherichia coli* NN980191

Report Title: Reverse Mutation in Four Histidine-requiring strains of *Salmonella typhimurium* and Two Tryptophan-requiring strains of *Escherichia coli* **Study No. NN980191**

Test for Induction of: Reverse mutation in bacterial cells		**No. of Independent Assays:** 2	**Location in CTD:** 4.2.3.3 Genotoxicity
Strains: S. typhimurium and E. Coli		**No. of Replicate Cultures:** 3	
Metabolizing System: Aroclor-induced rat liver S9, 10%		**No. of Cells Analyzed/Culture:** -	**GLP Compliance:** Yes
Vehicles:	**For Test Article:** 4 mM phosphate buffer	**For Positive Controls:** 4 mM phosphate buffer	
Treatments: Treat and plate assay			**Date of Treatment:** 24 September 1998
Cytotoxic Effects: None			
Genotoxic Effects: None			
Brief conclusion: The test compound did not induce mutation in four strains of S. thyphimurium and two strains of E. coli when tested in the presence or absence of S-9			

Metabolic Activation	Test Article	Dose Level (µg/ml)	Assay # 1 Revertant colonies count					
			TA 98	TA 100	TA 1535	TA 1537	WP2	WP2 uvrA
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Without Activation	Phosphate buffer	1000 µl	17 ± 5	113 ± 13	15 ± 4	5 ± 3	35 ± 11	110 ± 8
	NNC 90-1170	8	26 ± 3	105 ± 7	16 ± 3	8 ± 6	33 ± 3	111 ± 22
		40	17 ± 2	106 ± 10	13 ± 1	7 ± 1	29 ± 3	126 ± 11
		200	24 ± 2	107 ± 15	13 ± 1	4± 2	32 ±3	119 ± 12
		100	21 ± 4	104 ± 12	12 ± 3	4 ± 3	28 ± 7	118 ± 7
		5000	58 ± 5	95 ± 14	18 ± 6	6 ± 1	36 ± 12	111 ± 9
	Positive controls	Compound	2 NF	NQO	MNNG	ICR-191	MNNG	MNNG
		Dose level	25 µg/ml	1 µg/ml	2.5 µg/ml	0.5 µg/ml	7.5 µg/ml	7.5 µg/ml
		Mean ± SD	264 ± 23	272 ± 18	1077 ± 65	217 ± 25	210 ± 26	306 ± 17

Metabolic Activation	Test Article	Dose Level (µg/ml)	Assay # 1 cont. Revertant colonies count					
			TA 98	TA 100	TA 1535	TA 1537	WP2	WP2 uvrA
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
With Activation	Phosphate buffer	750 µl	21 ± 5	98 ± 16	14 ± 5	4 ± 1	30 ± 4	133 ± 6
	NNC 90-1170	6	46 ± 3	122 ± 15	17 ± 5	5 ± 1	33 ± 6	132 ± 9
		30	26 ± 9	102 ± 9	15 ± 3	9 ± 3	27 ± 5	131 ± 3
		150	24 ± 6	109 ± 12	14 ± 3	9 ± 2	30 ± 5	119 ± 22
		750	21 ± 3	121 ± 10	13 ± 7	5± 2	33 ± 2	117 ± 14
		3750	24 ± 3	113 ± 13	14 ± 4	4± 2	29 ± 4	105 ± 12
	Positive controls	Compound	AAN	AAN	AAN	AAN	-	AAN
		Dose level	2.5 µg/ml	2.5 µg/ml	2.5 µg/ml	2.5 µg/ml	-	2.5 µg/ml
		Mean ± SD	83 ± 60	326 ± 75	71 ± 7	8 ± 4	-	447 ± 35

Metabolic Activation	Test Article	Dose Level (µg/ml)	Assay # 2 Revertant colonies count					
			TA 98	TA 100	TA 1535	TA 1537	WP2	WP2 uvrA
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Without Activation	Phosphate buffer	1000 µl	20 ± 5	88 ± 7	10 ± 4	3 ± 1	34 ± 8	93 ± 12
	NNC 90-1170	50	20 ± 11	87 ± 7	14 ± 6	3 ± 2	31 ± 6	117 ± 10
		158.1	13 ± 5	90 ± 11	11 ± 2	5 ± 2	28 ± 5	102 ± 9
		500	11 ± 3	91 ± 15	15 ± 3	4 ± 2	25 ± 4	102 ± 16
		1581	14 ± 4	99 ± 11	12 ± 2	5 ± 2	23 ± 3	116 ± 5
		5000	16 ± 1	81 ± 7	15 ± 2	6 ± 2	27 ± 6	107 ± 11
	Positive controls	Compound	2 NF	NQO	MNNG	ICR-191	MNNG	MNNG
		Dose level	25 µg/ml	1 µg/ml	2.5 µg/ml	0.5 µg/ml	7.5 µg/ml	7.5 µg/ml
		Mean ± SD	262 ± 110	280 ± 14	1152 ± 46	128 ± 46	288 ± 32	204 ± 56

Metabolic Activation	Test Article	Dose Level (µg/ml)	Assay # 2 cont. Revertant colonies count					
			TA 98	TA 100	TA 1535	TA 1537	WP2	WP2 uvrA
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
With Activation	Phosphate buffer	750 µl	13 ± 4	95 ± 10	12 ± 3	7 ± 2	18 ± 1	84 ± 15
	NNC 90-1170	6	19 ± 4	-	-	-	-	-
		37.5	16 ± 4	75 ± 10	11 ± 5	8 ± 3	18 ± 5	81 ± 15
		118.6	17 ± 3	84 ± 8	10 ± 3	5 ± 1	21 ± 7	76 ± 4
		375	19 ± 4	91 ± 16	11 ± 4	5 ± 3	20 ± 6	76 ± 13
		1186	18 ± 2	90 ± 4	11 ± 3	10 ± 3	21 ± 4	87 ± 2
		3750	14 ± 5	86 ± 20	10 ± 4	7 ± 1	24 ± 4	85 ± 13
	Positive controls	Compound	AAN	AAN	AAN	AAN	-	AAN
		Dose level	2.5 µg/ml	5 µg/ml	2.5 µg/ml	2.5 µg/ml	-	20µg/ml
		Mean ± SD	156 ± 34	198 ± 9	27 ± 5	14 ± 5	-	615 ± 37

[N000 2.6.7 P43 - 45]

2.6.7.8.B Induction of chromosome aberrations in cultured human peripheral blood lymphocytes NN203114

Report Title: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes **Study No. NN203114**

Test for Induction of: Induction of chromosome aberrations		**No. of Independent Assays: 2**	**Location in CTD:** 4.2.3.3 Genotoxicity
Strains: Human lymphocytes from female donors		**No. of Replicate Cultures: 2-4**	
Metabolizing System: Aroclor-induced rat liver S9, 2 %		**No. of Cells Analyzed/Culture: 100**	**GLP Compliance: Yes**
Vehicles:	**For Test Article:** Sterile purified water	**For Positive Controls:** DMSO	
Treatment: Continuous treatment 20 hour + 0 hour recovery without S9; 3 hour pulse treatment +17 hour recovery +/- S9.			**Date of Treatment:** 07 July 2003
Cytotoxic Effects: None			
Genotoxic Effects: None			

Brief conclusion: Treatment of cultures in the absence and presence of S-9 resulted in frequencies of cells with structural aberrations which were similar to those in concurrent negative controls. Numbers of aberrant cells (excluding gaps) in all treated cultures fell within historical negative control ranges. No increases in the frequency of cells with numerical aberrations, which exceeded the historical negative control range, were observed in cultures treated with the test compound in the absence and presence of S-9.

3 hour treatment, 17 hours recovery

Metabolic Activation	Test Article	Concentration or Dose Level (µg/mL)	Replicate	Cells Scored	Cells with aberrations incl. gaps	Cells with aberrations excl. gaps	Mitotic Index (mean)
Without Activation	Purified water	-	A	100	1	1	7.3
		-	B	100	1	1	11.1
		-	C	ND	ND	ND	8.8
		-	D	ND	ND	ND	8.1
			Totals	200	2	2	(8.8)
	NNC 90-1170	2048	A	100	0	0	9.2
			B	100	1	1	8.3

3 hour treatment, 17 hours recovery							
			Totals	200	1	1	(8.8)
		3200	A	100	0	0	8.1
			B	100	1	1	8.6
			Totals	200	1	1	(8.4)
		5000	A	100	0	0	10.1
			B	100	0	0	8.0
			Totals	200	0	0	(9.1)
	4-Nitroquinoline 1-dioxide	5.495	A	100	17[a]	16[a]	
			B	100	15[a]	14[a]	
			Totals	200	32	30***	
With Activation	Purified water	-	A	100	0	0	10.1
		-	B	100	0	0	9.8
		-	C	ND	ND	ND	9.4
		-	D	ND	ND	ND	10.4
			Totals	200	0	0	(9.9)
	NNC 90-1170	2048	A	100	1	0	10.5
			B	100	0	0	12.2
			Totals	200	1	0	(11.4)
		3200	A	100	2	1	9.0
			B	100	1	0	9.5
			Totals	200	3	1	(9.3)
		5000	A	100	1	0	8.7
			B	100	0	0	8.7
			Totals	200	1	0	(8.7)
3 hour treatment, 17 hours recovery							
	Cyclophosphamide	6.868	A	100	31[a]	30[a]	
			B	100	22[a]	21[a]	
			Totals	200	53	51***	

20 hour treatment, 0 hours recovery

Metabolic Activation	Test Article	Concentration or Dose Level (µg/mL)	Replicate	Cells Scored	Cells with aberrations, incl. gaps	Cells with aberrations, excl. gaps	Mitotic Index (mean)
Without Activation	Purified water	-	A	100	2	1	5.4
		-	B	100	1	0	6.2
		-	C	ND	ND	ND	6.5
		-	D	ND	ND	ND	6.7
			Totals	200	3	1	(6.2)
	NNC 90-1170	3200	A	100	2	0	5.9
			B	100	1	0	6.5
			Totals	200	3	0	(6.2)
		4000	A	100	0	0	6.0
			B	100	1	1	5.4
			Totals	200	1	1	(5.7)
		5000	A	100	0	0	6.1
			B	100	2	1	6.7
			Totals	200	2	1	(6.4)

* = p<0.05 ** = p<0.01 *** = p<0.001

ND Not determined

[a] Exceed historical negative control range

[N000 2.6.7 P46 - 48]

2.6.7.9 Genotoxicity: *In-Vivo* **Test Article: Liraglutide**

2.6.7.9.A Induction of Micronuclei in the Bone Marrow of Treated Rats NN980192

Report Title: Induction of Micronuclei in the Bone Marrow of Treated Rats		**Study No. NN980192**
Test for Induction of: Bone-marrow micronuclei	**Treatment Schedule:** Four daily doses	**Location in CTD:** 4.2.3.3 Genotoxicity
Species/Strain: rats/ Crl:CD®BR(CD)	**Sampling Time:** 30 October 1998	**GLP Compliance:** Yes
Age: NA	**Method of Administration:** Subcutaneous	
Cells Evaluated: Polychromatic erythrocytes	**Vehicle/Formulation:** Phosphate buffer	**Date of Treatment:** 27-30 October 1998
No. of Cells Analyzed/Animal: 2000		
Special Features: None		
Toxic/Cytotoxic Effects: Reduction in weight gain was observed at all dose levels compared to controls. . Reduction in the ratio between polychromatic and normochromatic erythrocytes as compared with concurrent negative controls, at all dose levels.		
Genotoxic Effects: None		
Evidence of Exposure: Clinical signs at and bone marrow toxicity at all doses.		
Brief conclusion: The test compound did not induce micronuclei in polychromatic erythrocytes of the bone marrow of rats treated up to 30 mg/kg/day, a dose at which bone marrow toxicity were seen.		

Test Article	Dose (mg/kg)	No. of Animals	Mean ratio PEC/NCE ± sd	Group mean frequency of micronucleated PCE (per 1000 ± sd)
Vehicle control	-	7M	0.98 ± 0.44	0.14 ± 0.24
Untreated control	-	7M	1.16 ± 0.39	0.14 ± 0.24
NNC 90-1170	7.5	7M	0.44 ± 0.27	0.50 ± 0.58
	15	7M	0.33 ± 0.22	0.21 ± 0.39
	30	7M	0.42 ± 0.16	0.29 ± 0.57
Cyclophoschamide[b]	40[a]	7M	0.58 ± 0.74	4.50 ± 2.90***

2x2 Contingency chi square * - p<0.05 ** - p<0.01 *** - p<0.001

a - administrated as a single dose

b- positive control

[N000 2.6.7 P49]

2.6.7.9.B Genotoxicity: *In-Vivo* NN990072

Report Title: Assessment of Micronucleus Frequencies on Microscope Slide Preparations from Rats		**Study No. NN990072**
Test for Induction of: Bone-marrow micronucleus	**Treatment Schedule:** 28 daily doses	**Location in CTD:** 4.2.3.3 Genotoxicity
Species/Strain: Sprague Dawley rats/ Crl:CD®BR(CD)	**Sampling Time:** Day 28	**GLP Compliance:** Yes
Age: 9-10 weeks	**Method of Administration:** Subcutaneous	
Cells Evaluated: Type I and type II reticulocytes and PCE	**Vehicle/Formulation:** NNC 90-1170 Vehicle	**Date of Treatment:** 4 December 1998
No. of Cells Analyzed/Animal: 2000		
Special Features: Bone marrow smears and slides were prepared from a 28 toxicity study in rats (NN980183)		
Toxic/Cytotoxic Effects: None		
Genotoxic Effects: None		
Evidence of Exposure: Exposure was demonstrated in all rats. The elimination half-life was calculated to be in the range of 5-10 hours (NN980183)		
Brief conclusion: There was no effect on the frequency of micronucleated reticulocytes in the peripheral blood nor on the frequency of micronucleated polychromatic erythrocytes in the bone borrow.		

Test Article	Dose (mg/kg)	No. of Animals	Mean ratio PEC/NCE	Group mean frequency of micronucleated PCE (per 1000 ± sd)
Vehicle control	-	5M + 5F	1.40	0.55 ± 0.41
NNC 90-1170	0.1	5M + 5F	1.05	0.40 ± 0.52
	0.25	5M + 5F	1.64	0.15 ± 0.34
	1	5M + 5F	1.46	0.45 ± 0.28
Cyclophoschamide[a]	20	5M + 5F	0.07	11.70 ± 4.50***

2x2 Contingency chi square * = p<0.05 ** = p<0.01 *** = p<0.001

a - administrated as two doses only

[N000 2.6.7 P50]

2.6.7.10 Carcinogenicity **Test Article: Liraglutide**

2.6.7.10.A 104 Week Carcinogenicity Study in Mice with SC Adm. NN204229

Report Title:104 Week Carcinogenicity Study in Mice with Subcutaneous Administration **Study No. NN204229**

Species/Strain: CD-1 Mice (Crl:CD-1™(ICR) BR)	**Duration of Dosing:** 104 weeks	**Location in CTD:** 4.2.3.4.1 Long-term Studies
Initial Age: 4 weeks	**Method of Administration:** Subcutaneous	**GLP Compliance:** Yes
Date of First Dose: 08 December 2004	**Vehicle/Formulation:** NNC 90-1170 Vehicle	
	Treatment of Controls: NNC 90-1170 Vehicle	

Parameters collected:
Body Weight (%), Food Consumption (%), Water consumption (visual), Clinical observations, Antibody analysis, Haematology, Plasma calcitonin Gross Pathology, Histopathology

Basis for High-Dose Selection: 13 week toxicity study in mice with subcutaneous administration (NN ref No. 2040082)

Special Features: None

Brief conclusion: Administration of NNC 90-1170 to mice was associated with adenoma and carcinoma of C-cells in the thyroid gland at dose levels of 1.0 mg/kg/day and above, and focal hyperplasia of C-cells in the thyroid gland at dose levels of 0.2 mg/kg/day and above.

Daily Dose (mg/kg)	**0 (control)**		**0.03**		**0.2**		**1.0**		**3.0**	
Gender: Number Evaluated	M: 79	F: 75	M: 66	F: 66	M: 65	F: 67	M: 67	F: 66	M: 79	76
Toxicokinetics:										
Week 26										
AUC (h*nmol/L)			133	322	1636	1685	10490	7973	26290	24480
C_{max} (nmol/L)			14	39	123	127	739	1024	2289	1938
T_{max} (h)			6	12	6	4	6	4	6	12
Week 52										
AUC (h*nmol/l)			80	102	1011	434	11660	17050	38420	26190
C_{max} (nmol/L)			8	6	62	58	962	2313	2572	1762
T_{max} (h)			6	12	6	6	6	6	4	12
Week 104										
Daily Dose (mg/kg)	**0 (control)**		**0.03**		**0.2**		**1.0**		**3.0**	
AUC (h*nmol/l)			203	168	1587	1415	10090	6215	36380	37280
C_{max} (nmol/L)			22	18	145	137	1244	941	3373	3673
T_{max} (h)			6	6	12	4	6	4	6	6
Number of Animals[a]	M	F	M	F	M	F	M	F	M	F
At Start	79	79	67	67	67	67	67	67	79	79
Died/Sacrificed Moribund	40	55	33	53	30	23	29	43	46	51
Terminal Sacrifice	39	24	34	14	37	44	38	24	33	28
Survival (%)	49	30	51	21	55	66	57	36	42	35
Noteworthy Findings:										
Body weight (% from control)[b]										
Week 0			101	92	103	101	99	105	100	100
Week 13			100	104	100	105	101	103	99	103
Week 26			101	104	100	104	100	102	98	101
Week 52			103	103	101	100	102	98	100	99
Week 78			103	100	100	102	100	100	100	100
Week 104			96	98	97	100	100	101	99	105
Food Consumption (% from control)[b]										
Week 1			95*	98	87***	90***	80***	84***	74***	76***
Week 13			108**	102	97	102	105**	98	98	98
Week 28			103	102	103	96	105	98	100	93
Week 52			105	96	105	96	105	94	100	88
Week 80			102	100	100	98	100	94	95	96
Week 104			106	102	104	95	106	89	100	80
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Water consumption	-	-	-	-	-	-	-	-	-	-
Opthalmoscopy	-	-	-	-	-	-	-	-	-	-
Haematology										
Red Blood Cells (10^{12}/l)	9.05	8.10	8.39	7.60	8.29*	7.49*	7.99**	7.82**	7.81**	7.77**
Antibody analysis in plasma	-	-	-	-	-	-	-	-	-	-
Plasma calcitonin levels										
Week 26	17.9	76.01	22.66	33.46	65.10***	125.63***	120.21***	152.07***	119.02***	133.72***
Week 52	8.58	63.32	25.05*	52.57*	66.43***	107.46***	70.37***	129.56***	211.37***	191.36***

Daily Dose (mg/kg)	0 (control)		0.03		0.2		1.0		3.0	
Week 104	9.67	13.33	20.92*	39.06*	102.16***	61.43***	228.47***	98.65***	453.94***	383.51***
Gross Pathology										
Mass in thyroid gland									1	
Masses in bone						1			1	1
Masses in heart									1	
Masses in duodenum										
Masses in caecum				1			1	1		
Histopathology										
No. examined	79	79	67	67	67	66	67	65	79	78
Thyroid gland										
C-cell carcinoma	0	0	0	0	0	0	0	0	0	2
C-cell adenoma	0	0	0	0	0	0	9***	4*	15***	15***
Focal C-cell hyperplasia	0	0	0	0	1	7**	11***	10***	30***	22***
Pigmented kupffer cells in the liver	3	13	7	11	6	19	10*	27**	6	35***

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * - p<0.05 ** - p<0.01 *** - p<0.001

a – Combined data from main study and week 78 animals

b – only main study animals

[N000 2.6.7 P51 - 53]

2.6.7.10.B 104 Week Carcinogenicity Study in Rats with SC Adm. NN200240

Report Title:104 Week Carcinogenicity Study in Rats with Subcutaneous Administration **Study No. NN200240**

Species/Strain: Sprague-Dawley Rats (Crl:CD®(SD)IGS BR)	**Duration of Dosing:** 104 weeks	**Location in CTD:** 4.2.3.4.1 Long-term Studies
Initial Age: 4 weeks	**Method of Administration:** Subcutaneous	
Date of First Dose: 07 May 2001	**Vehicle/Formulation:** NNC 90-1170 Vehicle	**GLP Compliance:** Yes
	Treatment of Controls: NNC 90-1170 Vehicle	

Parameters collected:

Body Weight (%), Food Consumption (%), Water consumption (visual), Clinical observations, Ophthalmoscopy, Haematology, Gross Pathology, Histopathology

Basis for High-Dose Selection: 13 week toxicity study in rats with subcutaneous administration (NN ref No. 980189)

Special Features: None

Brief conclusion:

Lower body weight gain was seen at all levels in both sexes, as well as lower food consumption at 0.25 and 0.75 mg.kg^{-1}.day^{-1}.

There was no other evidence of toxicity or carcinogenicity at any dose level. Administration of the test item to rats at levels of 0.075 mg.kg^{-1}.day^{-1} and above for a 104 week period was associated with an increase in hyperplasia and neoplasia of the C-cells in the thyroid gland of males and females.

Daily Dose (mg/kg)	0 (Control)		0.075		0.25		0.75	
Gender: Number Evaluated	M: 50	F: 50	M: 50	F: 50	M: 50	F: 50	M: 50	F: 50
Toxicokinetics:								
Day 1								
AUC (h*nmol/L)			304	373	1320	1320	5230	4830
C_{max} (nmol/L)			32.7	31	115	109	361	338
T_{max} (h)			8.0	4.0	8.0	8.0	8.0	6.0
Week 53								
AUC (h*nmol/L)			360	579	1700	2910	580	8380
C_{max} (nmol/L)			24.1	41.1	104	154	287	453
T_{max} (h)			8.0	8.0	8.0	8.0	4.0	8.0
Week 104								
AUC (h*nmol/L)			361	485	2190	2450	6420	8820
C_{max} (nmol/L)			25.8	35.5	137	164	394	401

Daily Dose (mg/kg)	0 (Control)		0.075		0.25		0.75	
T_{max} (h)			8.0	8.0	8.0	8.0	8.0	8.0
Number of Animals								
At Start	50	50	50	50	50	50	50	50
Died/Sacrificed Moribund	27	22	26	25	21	29	24	21
Terminal Sacrifice	23	28	24	25	29	21	26	29
Survival (%)	46	56	48	50	58	42	52	58
Noteworthy Findings:								
Body weight (% from control)								
Week 1			97***	102**	91***	99	84***	95***
Week 13			97	101	93**	99	87***	101
Week 26			94*	101	89***	97	85***	97
Week 50			95	99	89***	92***	85***	91***
Week 78			95*	95*	87***	84***	81***	83***
Week 104			94*	92*	87***	79***	81***	77***
Food Consumption (% from control)[a]								
Week 1			94***	105	76***	89***	63***	78***
Week 13			99	102	96*	103	93**	104
Week 26			97	100	94**	98	90***	98
Week 50			95	100	89***	98***	89***	93***
Week 78			97	106	91**	93**	88**	97*
Week 104			96	97	88	100	84	92*
Clinical Observations								
Hunched posture								
Incidence (out of 50 per sex)	12	23	12	18	18	32	20	26
Observations (No. of occasions observed)	168	195	75	108	180	419	148	352
Shining on fur								
Incidence (out of 50 per sex)	28	39	39	43	41	44	36	47
Observations (No. of occasions observed)	549	1231	1015	2565	1328	2917	1275	3120
Fibrosection								
Incidence (out of 50 per sex)	15	24	17	26	18	36	19	39
Observations (No. of occasions observed)	156	162	304	175	177	513	165	617
Water consumption	-	-	-	-	-	-	-	-
Daily Dose (mg/kg)	**0 (Control)**		**0.075**		**0.25**		**0.75**	
Opthalmoscopy	*	*	*	*	*	*	*	*
Haematology								
Lymphocytes ($x10^9.l^{-1}$)								
Week 52	7.17	3.93	7.14	4.24	6.98	4.41	7.42*	4.82*
Week 78	5.52	3.32	6.02	3.66	5.58	3.76	6.08**	4.02**
Week 103	4.57	3.19	10.10	3.36	4.74	3.40	5.04	3.84
Gross Pathology								
Mass(es), one/both	-	-	1	-	-	-	1	1
Enlarged, one/both	*	*	*	*	5	1	7	2
Organ weights, adjusted group mean (g)								
Thyroid Glands	0.0371	0.0309	0.0446	0.0336	0.0471	0.0309	0.0602	0.0427
Histopathology								
No. examined	50	50	49	49	50	49	50	50
Thyroid gland								
C-cell carcinoma	1	0	4	0	3	2	7	3
C-cell adenoma	6	5	8	13*	21**	16**	23***	28***
Focal C-cell hyperplasia	11	14	14	14	20	27**	24*	24
Diffuse C-cell hyperplasia	3	6	6	8	3	3	7	3
Injection/Treatment site								

- No noteworthy findings

ANCOVA, Kruskall Wallis one way ANOVA or Dunnett's test: * = p≤0.05 ** = p≤0.01 *** = p≤0.001

a – p-values is from adjusted group mean values

[N000 2.6.7 P54 - 56]

2.6.7.12 Reproductive and Developmental Toxicity Fertility and Early Embryonic Development to Implantation Pivotal **Test Article: Liraglutide**

2.6.7.12.A Prelim. Segment I/II Subcutaneous Reproduction Study in Rats NN980186

Report Title: Preliminary Segment I/II Subcutaneous Reproduction Study in Rats		**Study No. NN980186**
Design similar to ICH S5 Paragraph 4.1.1: No (combined 4.1.1 and 4.1.3)		**Location in CTD:** 4.2.3.5.1 Fertility and Early Embryonic Development
Species/Strain: Rats/Sprague Dawley	**Duration of Dosing: M:** 8 weeks (from 4 weeks prior mating) **F:** 2 weeks prior mating – GD 17	
	Day of Mating: Day 0	**GLP Compliance:** Yes
Initial Age: 6 weeks	**Day of C-Section:** GD 20	
Date of First Dose: 29 March (males) and 12 April (females)	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:
Males: Clinical Observations, . Body Weight (g), Food Consumption (g), Organ weights (g),Necropsy Observations Mean No. Days Prior to Mating, No. of Males that Mated, No. of Fertile Males
Females: Clinical Observations, Premating Body Weight (g), Gestation Body Weight (g), Premating Food Consumption (g), Gestation Food Consumption (g), Median number of nights to positive mating sign, Number passing one oestrus, No. of Females Parred, No. of Pregnant Females, Pregnancy Performance, Mean total uterus weight, Mean litter Fetal Body Weight (g),

Special Features: None

No Observed Adverse-Effect Level
F0 Males: NA
F0 Females: NA
F1 Litters: NA

Brief conclusion: No parental no-effect level was demonstrated in this study, although the body weight and food consumption effects were considered to be expected from the pharmacological nature of the test material. Reproductive effects were confined to equivocal effects on the seminal vesicle weights at 0.25 and 1.0 mg/kg/day.

Daily Dose (mg/kg)	**0 (Control)**	**0.1**	**0.25**	**1.0**
Males:				
No. Evaluated	10	10	10	10
No. Died or Sacrificed Moribund	0	0	0	0
Noteworthy findings				
Bodyweight (g ± SD)				
Overall weight gain ((g)Day 0-week 8)	205	208	154**	176*
Food Consumption (g/rat/day)				
Day 0	27.2	28.8	27.5	28.0
Day 3	30.9	28.6	20.7	15.3
Day 7	27.6	28.3	24.2	23.4
Clinical Observations				
Hunched posture	0	1	5	8
Rollin gait	0	2	5	10
Decreased faecal output	0	0	6	10
Piloerection	0	0	8	10
Organ weights (g) (covariance analysis)				
Prostate	0.773	0.762	0.592**	0.734
Seminal Vesicles	2.4575	2.3338	1.9617**	2.0808*
Necropsy findings	-	-	-	-
Females:				
No. Evaluated (satellites)	10	10 (6)	10 (6)	10 (6)
No. Died or Sacrificed Moribund	0	0	0	1#
Toxicokinetics:				
AUC_T (h*nmol/L)				
Day 1		680	1980	9148
Day 17		691	2603	9211
C_{max} (nmol/L)				
Day 1		69	158	612
Day 17		75	214	1241
T_{max} (h)				
Day 1		8	8	8
Day 17		4	8	4

Daily Dose (mg/kg)	0 (Control)	0.1	0.25	1.0
Noteworthy findings				
Bodyweight GD 0-17 (% of control)		98	100	91
Food Consumption (g/rat/day)				
Day 0	20.4	21.1	20.7	20.4
Day 3	21.8	19.3	18.1	14.8
Day 7	20.4	20.4	18.9	17.9
G 0-6	23.6	25.5	25.6	24.8
G 6-13	27.5	27.2	26.0	24.5
G 13-20	28.4	30.1	31.5	27.8
Clinical Observations				
Piloerection	0	1	10	10
Hunched posture	0	0	0	10
Rollin gait	0	0	10	10
Decreased faecal output	0	0	0	1
Necropsy findings				
Mating performance				
Median number of nights to positive mating sign	3.5	1	2	3.0
Number passing one oestrus	0	0	0	1
Fertility				
Number of males paired	10	10	10	10
Number of siring males	10	10	10	10
Male fertility Index (%)	100	100	100	100
Number females paired	10	10	10	10
Number pregnant	10	10	10	10
Female fertility index(%)	100	100	100	100
Pregnancy Performance				
Pregnancy frequency as %	100	100	100	100
Total corpora lutea gravidatis	152	146	148	127
Total number of implants	151	141	145	125
Pre-implantation loss as %	1	3	2	2
Total live implants (%)	148 (98)	138 (98)	138 (95)	119 (95)
Daily Dose (mg/kg)	**0 (Control)**	**0.1**	**0.25**	**1.0**
Total dead implants (%)	3 (2)	3 (2)	7 (5)	6 (5)
Total early embryonic deaths (%)	2 (1)	3 (2)	6 (4)	5 (4)
Total late embryonic deaths (%)	0	0	1 (1)	1 (1)
Total foetal deaths (%)	1 (1)	0	0	0
Mean corpora lutea gridiatis	15.2 ± 1.9	14.6 ± 2.1	14.8 ± 2.0	14.1 ± 1.4
Mean implants	15.1± 1.9	14.1± 2.0	14.5 ± 2.0	13.9 ± 1.5
Mean live implants	14.8 ± 1.9	13.8 ± 1.9	13.8 ± 1.8	13.2 ± 1.7
Mean dead implants	0.3 ± 0.5	0.3 ± 0.5	0.7 ± 0.8	0.7 ± 1.0
Mean early embryonic deaths	0.2 ± 0.4	0.3 ± 0.5	0.6 ± 0.7	0.6 ± 0.9
Mean late embryonic deaths	0	0	0.1 ± 0.3	0.1 ± 0.3
Mean foetal deaths	0.1 ± 0.3	0	0	0
Mean total uterus weight (g)	87 ± 8	82 ± 10	81 ± 9	74 ± 7
Mean liter mean foetal weight (g)	3.61 ± 0.32	3.68 ± 0.15	3.61 ± 0.22	3.50 ± 0.18

a- Died following accidental injury on Day 32
ANCOVA, ANOVA: * - p<0.05 ** - p<0.01 *** - p<0.001

[N000 2.6.7 P57 - 61]

2.6.7.12.B Main Segment I/II Subcutaneous Reproduction Study in Rats NN990284

Report Title: Main Segment I/II Subcutaneous Reproduction Study in Rats		**Study No. NN990284**
Design similar to ICH S5 Paragraph 4.1.1: No (combined 4.1.1. and 4.1.3 study)		**Location in CTD:** 4.2.3.5.1 Fertility and Early Embyonic Development
Species/Strain: Rats/Sprague-Dawley	**Duration of Dosing: M:** 8 weeks (from 4 weeks prior mating) **Fe** 2 weeks prior mating -GI7	
	Day of Mating: Day 0	**GLP Compliance:** Yes
Initial Age: 6 weeks	**Day of C-Section:** G 20	
Date of First Dose: 13 (males) and 27 (females) December 1999	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:

Males: Clinical Observations, , Body Weight (g), Food Consumption (g), Water Consumption (visual) Organ weights (g),Necropsy Observations Mean No. Days Prior to Mating, No. of Males that Mated, No. of Fertile Males

Females: Clinical Observations. Premating Body Weight (g), Gestation Body Weight (g), Premating Food Consumption (g), Gestation Food Consumption (g), Median number of nights to positive mating sign, Number passing one oestrus, No. of Females Furred, No. of Pregnant Females, Pregnancy Performance.

Litters, Foetal survival, Mean total uterus weight, Mean liter Fetal Body Weight (g), Fetal Sex Ratios, Major Abnormities, Visceral Abnormities, Skeletal Abnormities

Special Features: None

No Observed Adverse-Effect Level

F0 Males: NA

F0 Females: NA

F1 Litters: 0.25 mg/kg/day

Brief conclusion:

There were no effects on mating performance or fertility. A slightly increased number of early embryonic deaths were recorded on the high dose Group. Foetal abnormalities were confined to an equivocal increase in the incidence of minimally kinked ribs in the high dose groups. No parental No Adverse Effect Level (NOAEL) was demonstrated in this study. Pharmacological effects on body weight and food consumption were seen in all treated animals. Reproductive effects were confined to decreased weight of the seminal vesicles in the intermediate and high dose males and decreased prostate weight in the high dose males.

Daily Dose (mg/kg)	**0 (Control)**	**0.1**	**0.25**	**1.0**
Males:				
No. Evaluated	24	24	24	24
No. Died or Sacrificed Moribund	0	0	0	0
Noteworthy findings				
Bodyweight (g ± SD)				
Pretrial	263 ± 21	266 ± 28	264 ± 21	265 ± 26
Day 1	332 ± 24	327 ± 31	313 ± 25	303 ± 29
Day 2	342 ± 25	334 ± 33	320 ± 24	309 ± 30
Day 3	348 ± 24	346 ± 34	331 ± 24	314 ± 30
Overall weight gain ((g)Day 0-week 8)	193	182	175	142
Food Consumption (g/rat/day)				
Day 0	31.0	32.1	31.9	31.2
Day 3	33.1	27.0	21.8	16.4
Day 7	33.0	31.7	29.6	26.3
Clinical Observations				
Decreased faecal output	0	0	200	24
Organ weights (g) (covariance analysis)				
Epididymis	1.4418	1.4277	1.3944	1.3809
Prostate	0.839	0.831	0.752	0.681**
Seminal Vesicles	2.4454	2.3140	2.2699*	2.1188***
Testes	3.71	3.66	3.59	3.65
Necropsy findings	-	-	-	-
Females:				
No. Evaluated	24	24	24	24
No. Died or Sacrificed Moribund	0	0	0	0
Noteworthy findings				
Bodyweight (g± SD)				
Pretrial	232 ± 22	238 ± 20	250 ± 24	239± 23
Day 1	256 ± 28	257 ± 24	261 ± 26	244 ± 26
Day 2	257 ± 26	260 ± 24	264 ± 27	248 ± 27
Day 3	256 ± 26	263 ± 25	269 ± 29	254 ± 26
G 0	276 ± 26	281 ± 28	290 ± 27	268 ± 28

Daily Dose (mg/kg)	0 (Control)	0.1	0.25	1.0
G 6	310 ± 27	310 ± 30	314 ± 33	289 ± 31
G 9	325 ± 29	323 ± 28	325 ± 29	300 ± 32
G 13	346 ± 30	345 ± 30	346 ± 30	318 ± 32
G 17	389 ± 34	384 ± 33	386 ± 37	355 ± 36
G 20	425 ± 38	424 ± 44	435 ± 40	410 ± 52
Food Consumption (g/rat/day)				
Day 0	19.9	22.3	23.0	23.2
Day 3	23.7	23.4	19.0	14.8
Day 7	21.3	21.4	20.0	18.4
Day 10	22.8	22.0	21.1	19.4
Day 14	21.9	21.0	21.1	19.9
G 0-6	30.2	28.8	28.2	25.5
G 6-13	31.9	29.5	28.9	26.8
G 13-20	32.4	31.5	32.8	31.4
Clinical Observations				
Decrease faecal output	0	0	14	24
Rolling gate	0	1	0	3
Hunched body	0	0	0	5
Necropsy findings	-	-	-	-
Mating performance				
Median number of nights to positive mating sign	3	3	3	2
Number passing one oestrus	0	0	0	0
Fertility				
Number of males paired	24	24	24	24
Number of siring males	24	24	23	24
Male fertility Index (%)	100	100	96	100
Number females paired	24	24	24	24
Number pregnant	24	24	23	24
Female fertility index(%)	100	100	96	100
Pregnancy Performance				
Number of premature decendents	0	0	0	0
Daily Dose (mg/kg)	**0 (Control)**	**0.1**	**0.25**	**1.0**
Number pregnant at Day 29 necropsy	24	24	23	24
Pregnancy frequency as %	100	100	96	100
Total corpora lutea graviditatis	394	394	346	375
Total number of implants	373	369	345	365
Pre-implantation loss as %	5	6	0	3
Total live implants (%)	356 (95)	343 (93)	332 (96)	333 (91)
Total dead implants (%)	17 (5)	26 (7)	13 (4)	32 (9)
Total early embryonic deaths (%)	16 (4)	19 (5)	11 (3)	31 (8)
Total late embryonic deaths (%)	1 (0.3)	1 (0.3)	2 (1)	1 (0.3)
Total foetal deaths (%)	0	6 (2)	0	0
Mean corpora lutea graviditatis	16.3 ± 1.8	16.4 ± 1.7	15.7 ± 2.3	15.6 ± 1.6
Mean implants	15.5 ± 2.1	15.4 ± 2.6	15.0 ± 2.5	15.2 ± 2.2
Mean live implants	14.8 ± 2.3	14.3 ± 3.3	14.4 ± 2.7	13.9 ± 2.8
Mean dead implants	0.7 ± 0.9	1.1 ± 1.8	0.6 ± 0.8	1.3 ± 1.6
Mean early embryonic deaths	0.7 ± 0.9	0.8 ± 1.1	0.5 ± 0.7	1.3 ± 1.6
Mean late embryonic deaths	0.04 ± 0.2	0.04 ± 0.2	0.1 ± 0.3	0.04 ± 0.2
Mean foetal deaths	0	0.3 ± 1.0	0	0
Litters:				
No. Live male foetuses (%)	185 (52)	165 (48)	174 (52)	163 (49)
No. Live female foetuses (%)	171 (48)	180 (52)	158 (48)	170 (51)
Live foetal sex ratio	1:1.34	1:1.24	1:0.98	1:1.16
Noteworthy findings				
Foetal weight				
Mean foetal uterus weight (g)	89 ± 11	86 ± 18	87 ± 16	83 ± 16
Mean litter mean foetal weight (g)	3.79 ± 0.25	3.75 ± .043	3.75 ± 0.20	3.73 ± 0.23
Major Abnormalities	-	-	-	-
Minor Visceral Abnormalities and Variants	-	-	-	-
Minor Skeletal Abnormalities and Variants	-	-	-	-

- Noteworthy findings
ANCOVA, ANOVA, Student's T-test: * - p<0.05 ** - p<0.01 *** - p<0.001
G= Gestation day

[N000 2.6.7 P62 - 65]

2.6.7.13 Reproductive and Developmental Toxicity Effects on Embryo-Fetal Development Test Article: Liraglutide

2.6.7.13.A Dose Range Finding Study in Rabbits Preliminary to developmental Toxicity Study NN980188/ NN980187

Report Title: Dose Range Finding Study in Rabbits Preliminary to developmental Toxicity Study		**Study No. NN980188&NN980187**
Design similar to ICH S5 Paragraph 4.1.3: Yes		**Location in CTD:** 4.2.3.5.2 Embryo-foetal development
Species/Strain: Rabbits/New Zealand White	**Duration of Dosing: F:** G6-G18	
	Day of Mating: Day 0	
Initial Age: 4-5 months	**Day of C-Section:** G29	**GLP Compliance: Yes**
Date of First Dose: 23 November 1998	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:
Dams: Clinical Observations. , Body Weight (kg), Food Consumption (g), Pregnancy performance, Foetal survival, Mean total uterus weight, Mean litter Fetal Weight (g).

Special Features: None

No Observed Adverse-Effect Level
F0 Females: NA

Brief conclusion: Maternal effects were demonstrated at 0.01, 0.03 and 0.1 mg.kg^{-1}.day^{-1} NNC 90-1170 by reduced food consumption. No overall group mean body weight gain during treatment was noted at 0.1 mg.kg^{-1}.day^{-1} and little overall group mean body weight gain throughout the treatment period was noted at 0.03 and 0.01 mg.kg^{-1}.day^{-1}. Reduced faecal output and small faeces were noted and were considered to be secondary to the effects on body weight and food consumption.

Daily Dose (mg/kg)	**0 (Control)**	**0.01**	**0.03**	**0.1**
Number of animals mated	6	6	6	6
Toxicokinetics:				
AUC$_{0-24}$ (h*nmol/L)				
Day 6		125	288	571
Week 16		148	280	766
Daily Dose (mg/kg)	**0 (Control)**	**0.01**	**0.03**	**0.1**
C$_{max}$ (nmol/L)				
Day 6		9	19	36
Week 16		13	17	51
T$_{\frac{1}{2}}$ (h)				
Day 6		6.4	8.1	-
Week 16		6.8	11.7	11
No. Died or Sacrificed Moribund	0	0	0	0
Noteworthy findings				
Body weight (kg ± SD)				
Body weight G 6-19 (kg)	0.12 ± 0.05	0.06 ± 0.14	0.03 ± 0.10	-0.01 ± 0.11
Food consumption (g)				
Total consumed Days 7-19	1679	939	849	675
% of control	-	56	51	40
Clinical observations				
Reduced faecal output	-	2	3	5
Soft faeces	-	1	-	1
Small faeces	-	1	1	4
Pregnancy Performance				
Number of animals mated	6	6	6	6
Number pregnant at Day 22 necropsy	5	5	5	5
Pregnancy frequency as %	83	83	83	83
Total corpora lutea graviditatis	54	54	54	47
Total number of implants	45	47	45	39
Pre-implantation loss as %	17	13	17	17
Total live implants (%)	44 (98)	46 (98)	37 (82)	36 (92)
Total dead implants (%)	1 (2)	1 (2)	8 (18)	3 (8)
Total early embryonic deaths (%)	0	1 (2)	7(16)	1 (3)
Total late embryonic deaths (%)	0	0	0	1 (3)
Total foetal deaths (%)	1 (2)	0	1 (2)	1 (3)
Mean corpora lutea graviditatis	10.8± 1.5	10.8 ± 1.8	10.8 ± 0.8	9.4 ± 1.1
Mean implants	9.0 ± 3.2	9.4 ± 1.8	9.0 ± 1.6	7.8 ± 2.9
Mean live implants	8.8 ± 2.9	9.2 ± 1.6	7.4 ± 3.4	7.2 ± 2.9
Daily Dose (mg/kg)	**0 (Control)**	**0.01**	**0.03**	**0.1**
Mean dead implants	0.2 ± 0.4	0.2 ± 0.4	1.6 ± 2.5	0.6 ± 0.9
Mean early embryonic deaths	0	0.2 ±0.4	1.4 ± 2.6	0.2 ± 0.4
Mean late embryonic deaths	0	0	0	0.2 ± 0.4
Mean foetal deaths	0.2 ± 0.4	0	0.2 ± 0.4	0.2 ± 0.4
Mean total uterus weight (g)	187 ± 60	243 ± 50	199 ± 50	179 ± 64
Mean litter mean foetal weight (g)	6.64 ± 0.43	6.55 ± 0.49	6.58 ± 0.77	6.11 ± 0.45

- No noteworthy findings.

[N000 2.6.7 P66 - 68]

2.6.7.13.B Developmental Toxicity Study in Rabbits NN990055

Report Title: Developmental Toxicity Study in Rabbits		**Study No. NN990055**
Design similar to ICH S5 Paragraph 4.1.3: Yes		**Location in CTD:** 4.2.3.5.2 Embryo-foetal development
Species/Strain: Rabbits/New Zealand White	**Duration of Dosing:** F: G6-G18	
	Day of Mating: Day 0	
Initial Age: 5 months	**Day of C-Section:** G29	**GLP Compliance:** Yes
Date of First Dose: 15 March 1999	**Method of Administration:** Subcutaneous	
	Vehicle/Formulation: Disodium monohydrogenphosphate, Monosodium dihydrogenphosphate, Mannitol, Phenol, and water (pH 7.4)	

Parameters collected:
Dams: Clinical Observations, , Body Weight (kg), Food Consumption (g), Pregnancy performance.
Litters: Foetal survival, Mean total uterus weight, Mean litter Fetal Body Weight (g), Fetal Sex Ratios, Major Anomalies, Visceral Anomalies, Skeletal Anomalies.

Special Features: None

No Observed Adverse-Effect Level
F0 Females: NA
F1 Litters: 0.01 mg/kg/day

Brief conclusion:
No maternal no-effect level was demonstrated in this study, although the maternal effects were considered to be expected from the pharmacological nature of the material. The dose level of 0.01 mg/kg/day was considered to have been without embryo-foetal effects.

Daily Dose (mg/kg)	**0 (Control)**	**0.01**	**0.025**	**0.05**
Number of animals mated	20	20	20	20
No. Died or Sacrificed Moribund	0	0	0	0
Noteworthy findings				
Body weight (kg ± SD)				
G 4	3.91 ± 0.17	3.95 ± 0.39	3.98 ± 0.29	3.93 ± 0.35
G 6	3.95 ± 0.18	3.98 ± 0.33	4.00 ± 0.31	3.98 ± 0.35
G 9	3.95 ± 0.18	3.79 ± 0.32	3.83 ± 0.28	3.77 ± 0.33
G19	4.11 ± 0.15	3.94 ± 0.26	3.98 ± 0.21	3.87 ± 0.28
Daily Dose (mg/kg)	**0 (Control)**	**0.01**	**0.025**	**0.05**
G 22	4.14 ± 0.16	4.09 ± 0.29	4.11 ±0.23	4.03 ± 0.29
Body weight G 4-29 (kg)	0.39	0.24	0.19	0.19
Food consumption (g)				
G 6	154	139	142	151
G 7	134	38	18	10
G 8	135	25	15	8
G 9	135	34	38	30
G 10	139	67	70	57
Total consumed Days 7-19	1728	769	724	586
% of control		45	42	34
Clinical observations				
Reduced faecal output	1	10	14	20
Soft faeces	0	2	1	1
Small faeces	0	1	5	3
Pregnancy Performance				
Number of animals mated	20	20	20	20
Number pregnant	17	20	17	19
Number of premature descendents	0	0	0	0
Number pregnant at Day 29 necropsy	17	20	17	19
Pregnancy frequency as %	85	100	85	95
Total corpora lutea gravidiatis	170	196	168	196
Total number of implasas	155	176	144	162
Pre-implantation loss as %	9	10	14	15
Total live implants (%)	138 (89)	161 (91)	122 (85)	144 (86)
Total dead implants (%)	17 (11)	15 (9)	22 (15)	23 (14)
Total early embryonic deaths (%)	9 (6)	10 (6)	8 (6)	12 (7)
Total late embryonic deaths (%)	5 (3)	1 (1)	10 (7)	10 (6)
Total foetal deaths (%)	3 (2)	4 (2)	4 (3)	1 (1)
Mean corpora lutea gridiatis	10.0 ± 2.1	9.8 ± 2.0	9.9 ± 1.5	10.3 ± 2.3
Mean implants	9.1 ± 1.8	8.8 ± 2.6	8.5 ± 1.8	8.8 ± 2.5
Mean live implants	8.1 ± 1.8	8.1 ± 2.4	7.2 ± 1.6	7.6 ± 2.3
Mean dead implants	1.0 ± 1.2	0.8 ± 0.8	1.3 ± 1.5	1.2 ± 1.3

Daily Dose (mg/kg)	0 (Control)	0.01	0.025	0.05
Mean early embryonic deaths	0.5 ± 0.8	0.5 ± 0.7	0.5 ± 0.7	0.6 ± 0.8
Mean late embryonic deaths	0.3 ± 0.5	0.1 ± 0.2	0.6 ± 1.0	0.5 ± 1.0
Mean foetal deaths	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.6	0.1 ± 0.3
Litters:				
No. Litters Evaluated	17	20	20	20
No. Live male fetuses (%)	73 (53)	81 (50)	56 (46)	70 (49)
No. Live female fetuses (%)	65 (47)	80 (50)	66 (54)	74 (51)
Live foetal sex ratio	1:0.89	1:0.99	1:1.18	1:1.06
Mean total uterus weight (g)	549 ±80	512 ±133	482 ± 92	504 ± 100
Mean litter mean foetal weight (g)	43.9 ± 4.2	40.5 ± 4.6	41.3 ± 4.0	41.3 ± 3.8
Group Incidences (foetuses (Litters))				
Number examined	138 (17)	161 (20)	122 (17)	144 (19)
Major Abnormalities				
Number with major abnormality	3 (3)	6 (6)	7 (6)	11 (6)
Connected Parietals	0	0	0	5 (1)
Minor Visceral Abnormalities and Variants				
Number with visceral abnormality	8 (6)	13 (12)	12 (9)	11 (9)
Minor Skeletal Abnormalities and Variants				
Number with skeletal abnormality	37 (13)	46 (17)	22 (12)	57 (17)
Connected/fused Jungal(s) to maxilla	3(3)	9(6)	6(4)	15(8)
Number of Ribs				
12 complete Ribs	86 (16)	71 (19)	40 (14)	46 (17)
Vestigal supernumerary rib (s) on 13^{th} thoracic vertebra	11 (6)	18 (13)	10 (8)	20 (11)
Reduced supernumerary rib (s) on 13^{th} thoracic vertebra	12 (8)	20 (13)	13 (12)	17 (11)
Complete supernumerary rib (s) on 13^{th} thoracic vertebra	29 (11)	52 (16)	59 (16)	61 (16)

- No noteworthy findings

G= Gestation day No formal statistical analyses were performed

[N000 2.6.7 P69 - 71]

2.6.7.14 Reproductive and Developmental Toxicity Effects on Pre- and Postnatal Development, Including Maternal Function **Test Article: Liraglutide**

Report Title: Pre and Post Natal Study in Rats		**Study No. NN201109**
Design similar to ICH S5 Paragraph 4.1.2: Yes		**Location in CTD:** 4.2.3.5.3 Prenatal and postnatal development
Species/Strain: Rat/ Sprague-Dawley	**Duration of Dosing:** G6-termination after weaning of F_1	
	Day of Mating: Day 0	
Initial Age: 9 weeks	**Method of Administration:** Subcutaneous	**GLP Compliance:** Yes
Date of First Dose: 07 May 2001	**Vehicle/Formulation:** NNC 90-1170 Vehicle	
	Litters Culled/Not Culled: Not culled	

Special Features: None

No Observed Adverse-Effect Level
F0 Females: N/A
F1 Males: N/A
F1 Females: N/A

Brief conclusion:
Pharmacological effects on food consumption and body weight were seen at all dose levels in the F0 generation. The body weight effect persisted into the pre-weaning period in the F1 generation at all dose levels and during the post-weaning period in the high dose group. Group mean litter weight was reduced in the high dose F2 generation.

Daily Dose (mg/kg)		0 (Control)	0.1	0.25	1.0
F0 Females:	**No. Pregnant**	24	24	24	24
	No. Died or Sacrificed Moribund	0	0	0	0
	Females producing a live litter	24	24	24	24
	Mean Duration of gestation	23.3	21.7	21.7	22.0
	Clinical Observations				
	Hunched posture	1	6	13	23
	Piloerection	1	13	13	23

Daily Dose (mg/kg)		0 (Control)	0.1	0.25	1.0
	Wet coat	0	5	4	9
	Body held low	0	1	0	7
	Reduced activity	0	0	0	6
	Necropsy Observations				
	Reddened injection site	14	12	13	14
	Scrabbling at or around injection site	0	0	0	3
	Gestation Body Weight (g)				
	G 6	263	258	256	258
	G 9	277	264*	253**	244***
	G 13	302	290	279**	270***
	G 20	380	375	359*	345**
	Lactation Body Weight (g)				
	L 1	276	255**	250***	242***
	L 7	325	309*	292***	278***
	L 24	320	324	313	303*
	Gestation Food Consumption (g)				
	G 6	25	24	26	26
	G 7	24	11	3	1
	G 8	24	19	16	13
	G 12	30	27	25	22
	G 20	26	27	26	24
	Lactation Food Consumption (g)				
	L 0-7	284	270	257	239
	L 7-14	519	493	451	396
F1 Litters: (Preweaning)	**No. Litters Evaluated**	24	24	23	23
	Litter size	-	-	-	-
	Litter survival	-	-	-	-
	Group mean Litter Weights (g)[a]	537 ± 115	517 ± 56	467 ± 83	400 ± 97
	Mean Pup Body Weights, Males (g)[a]	46.1	41.1**	40.7**	36.2***
	Mean Pup Body Weights, Females (g)[a]	44.7	39.2**	38.1***	34.7***
	Physical and Functional Development	-	-	-	-
	Pup Clinical Signs	-	-	-	-
	Pup Necropsy Obs.	-	-	-	-

Daily Dose (mg/kg)		0 (Control)	0.1	0.25	1.0
	Wet coat	0	5	4	9
	Body held low	0	1	0	7
	Reduced activity	0	0	0	6
	Necropsy Observations				
	Reddened injection site	14	12	13	14
	Scrabbling at or around injection site	0	0	0	3
	Gestation Body Weight (g)				
	G 6	263	258	256	258
	G 9	277	264*	253**	244***
	G 13	302	290	279**	270***
	G 20	380	375	359*	345**
	Lactation Body Weight (g)				
	L 1	276	255**	250***	242***
	L 7	325	309*	292***	278***
	L 24	320	324	313	303*
	Gestation Food Consumption (g)				
	G 6	25	24	26	26
	G 7	24	11	3	1
	G 8	24	19	16	13
	G 12	30	27	25	22
	G 20	26	27	26	24
	Lactation Food Consumption (g)				
	L 0-7	284	270	257	239
	L 7-14	519	493	451	396
F1 Litters: (Preweaning)	**No. Litters Evaluated**	24	24	23	23
	Litter size	-	-	-	-
	Litter survival	-	-	-	-
	Group mean Litter Weights (g)[a]	537 ± 115	517 ± 56	467 ± 83	400 ± 97
	Mean Pup Body Weights, Males (g)[a]	46.1	41.1**	40.7**	36.2***
	Mean Pup Body Weights, Females (g)[a]	44.7	39.2**	38.1***	34.7***
	Physical and Functional Development	-	-	-	-
	Pup Clinical Signs	-	-	-	-
	Pup Necropsy Obs.	-	-	-	-

Daily Dose (mg/kg)		0 (Control)	0.1	0.25	1.0
F1 Generation	Mating Performance and gestation				
	Median Number of nights to positive mating sign	2.5	1.0	2.0	3.0
	Number passing one estrus	0	0	0	0
	Females producing a live litter	24	22	23	22
	Mean Duration of gestation	22.0	21.7	21.9	21.7
F2 Litters:	No. Litters Evaluated	24	22	23	22
	Litter size	-	-	-	-
	Litter survival	-	-	-	-
	Group mean Litter Weights (g)[a]	389 ± 69	349 ± 67	370 ± 59	359 ± 45
	Mean Pup Body Weights, Males (g)[b]	29.4	29.4	29.4	27.5
	Mean Pup Body Weights, Females (g)[b]	28.4	28.6	28.2	26.1

- = No noteworthy findings
Anova, Student's t-test, Kruskal-Wallis non-parametric ANOVA, z-test: *=p<0.05 ** = p<0.01 ***= p<0.001 ***
G=Gestation day
L= Lactation day
a = end of gestation period
b = L 14

[N000 2.6.7 P72 - 75]

2.6.7.15 Local Tolerance **Test Article: Liraglutide**

Species/ Strain	Method of Administration	Doses (200 µl/injection site)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref. No.)
Crossbred SPF pigs (50% Duroc, 25% Danish Landrace, 25% Yorkshire)	Single subcutaneous injection 2 or 5 days before sacrifice	Liraglutide: [redacted]	4 females (4 injection sites/ test compound/day)	Single subcutaneous injection of Liraglutide or Liraglutide vehicle caused a mild subacute inflammation in the injection site tissue. At day 2, the changes were similar to those caused by 0.9% NaCl, but milder than those caused by Protaphane HM(ge). At day 5, no differences in severity were observed between the tested compounds.	NN980E85 (4.2.3.6 Local tolerance)

b(4)

Species/ Strain	Method of Administration	Doses (200 µl/injection site)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref. No.)
SPF pigs	Single subcutaneous injection 2 or 5 days before sacrifice	Liraglutide Phase 2 formulation: [redacted]	5 females (4 injection sites/ test compound/day)	At day 2, a minimal inflammatory reaction was seen for all treatments, but NaCl where no reaction was seen. At day 5, a minimal to slight inflammatory reaction was seen for Liraglutide Phase 2 and for the phase 2 and 3 vehicles, whereas no reaction was seen for NaCl. For Liraglutide Phase 3 minimal to moderate inflammatory reaction was seen. There was no statistically difference between the reactions caused by the Phase 2 and 3 Liraglutide formulations two or five days after a single subcutaneous injection.	NN203294 (4.2.3.6 Local tolerance)

Species/ Strain	Method of Administration	Doses (200 µl/injection site)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref. No.)
SPF pigs	Single subcutaneous injection 2 or 5 days before sacrifice	Liraglutide Phase 3 formulation: 6.25mg/ml, pH 7.7 (batch No PLD002 with propylenglycol); Liraglutide Phase 3 formulation: 6.25mg/ml, pH 7.9 (batch No PLD003 with propylenglycol); Liraglutide Phase 3 formulation: 6.25mg/ml, pH 8.15 (batch No PLD004 with propylenglycol); Liraglutide Phase 3 vehicle: pH 7.7 (batch No PQ50299 with propylenglycol); 0.9% NaCl (batch No 03JV10)	5 females (4 injection sites/ test compound/day)	At day 2, a minimal to moderate inflammatory reaction was seen for all treatments, but NaCl where a minimal to slight reaction was observed. At day 5, a moderate inflammatory reaction was seen in all sites treated with Liraglutide, pH 7.7. This severity was also seen in two or three of the sites treated with Liraglutide, pH 7.9 and 8.15 and vehicle. A minimal to slight reaction was seen at the remaining sites. The tissue reaction to the Liraglutide formulation was slightly more intense than the reaction seen after 0.9% NaCl. When applying the proportional odds regression model, the response both day 2 and 5 had the following pattern in increasing order of severity: 0.9% NaCl, vehicle pH 7.7, Liraglutide pH 8.15, Liraglutide 7.9, Liraglutide 7.7.	NN204291 (4.2.3.6 Local tolerance)

[N000 2.6.7 P76 - 78]

2.6.7.16 Other Toxicity Studies **Test Article: Liraglutide**

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
In vitro studies						
Mechanistic Studies to evaluate rodent thyroid C-cell tumour finding						
GLP-1 receptor expression and function						
Thyroid specimens from CD-1 mice, GLP-1 receptor knock-out mice (CD-1 background), Sprague-Dawley rats, Cynomolgus monkeys (*Macaca fascicularis*) and human subjects.	N/A	N/A	N/A	N/A	The GLP-1 receptor is expressed in thyroid C-cells in all species tested. Quantification was not possible using this technique.	NN204370 (4.2.3.7.3 Mechanistic Studies)
Thyroid specimens from NMRI mice, Sprague-Dawley rats, Cynomolgus monkeys (*Macaca fascicularis*) and human subjects.	N/A	N/A	N/A	N/A	GLP-1 receptor mRNA is expressed in low quantities in mice and rat thyroid C-cells, but cannot be detected in monkey and human C-cells, despite other positive control mRNA expression	NN2004051SPR4 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Rat (rMTC 6-23 and CA77) and human (TT) thyroid C-cell lines. Rat pancreatic beta-cell line INS 1E	N/A	N/A	N/A	N/A	The GLP-1 receptor is expressed in rat thyroid C-cell lines and well as rat pancreatic beta-cell line, but only in very small numbers on a human thyroid C-cell line (measured by saturation binding).	NN14725-006 (4.2.3.7.3 Mechanistic Studies)
Rat (rMTC 6-23 and CA77) and human (TT) thyroid C-cell lines	N/A	N/A	N/A	N/A	The GLP-1 receptor is expressed in rat thyroid C-cell lines, but not in a human thyroid C-cell line (measured by flow cytometry receptor binding).	NN205088 (4.2.3.7.3 Mechanistic Studies)
Rat (rMTC 6-23 and CA77) and human (TT) thyroid C-cell lines	N/A	N/A	N/A	N/A	The GLP-1 receptor is expressed in rat thyroid C-cell lines, but not in a human thyroid C-cell line (measured by western blotting).	NN205218 (4.2.3.7.3 Mechanistic Studies)
Rat (rMTC 6-23 and CA77) and human (TT) thyroid C-cell lines	N/A	N/A	N/A	N/A	GLP-1 receptor mRNA is expressed at relatively low numbers in human thyroid C-cell line, and high in rat thyroid C-cell lines	NN204415 (4.2.3.7.3 Mechanistic Studies)
Rat (rMTC 6-23 and CA77) and human (TT) thyroid C-cell lines. Rat pancreatic beta-cell line RIN2A18	N/A	N/A	N/A	N/A	Rat thyroid C-cell lines express a functional GLP-1 receptor that respond to GLP-1 analogues by cAMP accumulation and calcitonin secretion. The human cell line has a poor cAMP response to GLP-1 and no calcitonin release. A positive control did release calcitonin.	13737-025 (4.2.3.7.3 Mechanistic Studies)
Human (SNU, SHER-1, MTC-SK) C-cell lines	N/A	N/A	N/A	N/A	These C-cell lines are not valid models of human C-cells but still, they do not express functional GLP-1 receptors.	14725-062 (4.2.3.7.3 Mechanistic Studies)
Rat (rMTC 6-23 and CA77) and human (TT) thyroid C-cell lines. Rat pancreatic beta-cell line INS 1E	N/A	N/A	N/A	N/A	GLP-1 or liraglutide did not stimulate proliferation in rat or human thyroid C-cell lines, but did in positive control, rat pancreatic beta-cell line.	NN205295 (4.2.3.7.3 Mechanistic Studies)
Tissue/Species/ Strain	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)**	**Gender and No. per Group**	**Noteworthy Findings**	**Study Number (NN ref No.)**
Receptor binding to other receptors						
Human calcitonin receptor expressed in BHK cells	N/A	N/A	N/A	N/A	GLP-1 or liraglutide did not bind to the human calcitonin receptor.	14718-007 (4.2.3.7.3 Mechanistic Studies)
Rat gastrin/bombesin receptors in cell line AR42J	N/A	N/A	N/A	N/A	Liraglutide has no cross-reactivity to gastrin (CCK2) or bombesin (BB2) receptors.	13736-092 (4.2.3.7.3 Mechanistic Studies)
In vivo studies						
Impurities						
4 Week Subcutaneous Toxicity Study in Rats (bridging study)	Subcutaneous	4 weeks	0, 1	10 males and 10 females	Pharmacologically mediated effects on food consumption and body weight were recorded. These findings were consistent with the effects observed in previous rat studies. No differences were recorded between the old formulation and the new forcedly degraded formulation.	NN205092 (4.2.3.7.6 Impurities)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Mechanistic studies						
Assessment of beta and non-beta cell mass in pancreatic islets of cynomolgus monkeys treated with liraglutide for 52 weeks (in-life phase NN2000241)	Subcutaneous	52 weeks	0, 5mg/kg	4 males and 4 females	The relative volume fraction of the beta and non-beta cells was decreased by the liraglutide 5 mg/kg/day treatment. The absolute beta- and non-beta-cell mass was not influenced by the treatment. The relative volume fractions of duct (CK-7+ cells) and exocrine cells were not changed, but the absolute duct cell mass was 67% higher and that of exocrine cells 64% in the liraglutide group than in the vehicle group.	CGo040301 (4.2.3.7.3 Mechanistic Studies)
Mechanistic Studies to evaluate rodent thyroid C-cell tumour finding						
Studies with liraglutide (NNC 90-0000-1170)						
Mouse studies						
Liraglutide (NNC 90-1170): Single Dose Study in Mice with Subcutaneous Administration	Subcutaneous	Single dose	0, 0.03, 0.2, 1.0, 3.0	24 males and 24 females	A significant treatment-related and dose-dependent increase in plasma calcitonin was observed at doses of 0.2 mg/kg and above.	NN2053106 (4.2.3.7.3 Mechanistic Studies)
Tissue/Species/ Strain	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)**	**Gender and No. per Group**	**Noteworthy Findings**	**Study Number (NN ref No.)**
Liraglutide (NNC 90-1170): 4 Week toxicity study in mice with subcutaneous administration. Calcitonin determinations in mouse plasma	Subcutaneous	4 weeks	0, 0.1, 0.5, 1.0, 5.0	14 males and 14 females	Plasma calcitonin levels were higher in all liraglutide treated groups, when compared to the vehicle group, in both female and male mice	NN2003261/ NN2004288 (4.2.3.2 Repeat-Dose Toxicity
Liraglutide (NNC 90-1170): A 9 week exploratory study with reversibility in mice - Combined evaluation of the in life phase, calcitonin determination, molecular analysis and C-cell pathology of the thyroid gland	Subcutaneous	2 or 9 weeks, 6 or 15 weeks recovery	0, 0.2 and 5	80 males and 80 females	Both two and nine weeks of treatment with liraglutide resulted in a dose-dependent increase in plasma calcitonin. In line with this, there was a dose-dependent increase in the transcriptional activity of CT (mRNA) after two weeks of treatment. At this time-point no treatment-related C-cell changes were observed by enhanced histopathological techniques. The plasma CT increase was reversible upon cessation of treatment as 6 and 15 weeks of recovery resulted in CT levels reverting to normal. Reversibility was also observed for the C-cell pathology data. Thus, treatment-related minimal to mild C-cell hyperplasia was observed after 9 weeks of treatment with evidence of reversibility shown after 15 weeks of recovery.	Report summary of NN204268, NN204315, NN204338 and NN204413 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Liraglutide (NNC90-1170): 13 Week toxicity study in mice with subcutaneous administration. Calcitonin determinations	Subcutaneous	13 weeks	0, 0.2, 1.0, 5.0	Samples collected from satellite animals: 28 males and 28 females	Plasma calcitonin levels were higher in all liraglutide treated groups, when compared to the vehicle group, in both female and male mice, after one day of dosing as well as after 13 weeks of dosing	NN204289 (4.2.3.7.3 Mechanistic Studies)
Rat Studies						
Liraglutide (NNC 90-1170): Effects on calcium homeostasis after a single subcutaneous administration to male rats in a fasted condition – Combined evaluation of the in life phase and hormone analysis	Subcutaneous	Single dose	0, 0.75mg/kg	30 males	A transient increase in plasma calcitonin was observed after a single dose of liraglutide (0.75 mg/kg) to fasted rats. In parallel, low plasma calcium and increased PTH were observed. It is considered likely that an initial stimulatory effect of liraglutide on CT secretion was followed by loss of calcium in the urine which caused the hypocalcaemia and thereby a compensatory increase in PTH to mobilize calcium. The decreased plasma calcium possibly later counteracted any stimulatory effect of liraglutide on the calcitonin secretion. The observed reduction in thyroid calcitonin contents was compatible with secretion of stored protein and a parallel decrease in mRNA levels was interpreted as the result of increased translation to compensate for the increased calcitonin secretion.	Report summary of NN203281, NN203296 and NN204018 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Liraglutide (NNC 90-1170): The effects on calcium homeostasis after a single subcutaneous administration to male rats in a non-fasted condition - Combined evaluation of the in life phase and hormone analysis	Subcutaneous	Single dose	0, 0.75mg/kg	30 males	A marked increase in diuresis was a prominent finding after a single dose of liraglutide (0.75 mg/kg) in rats. The diuresis was paralleled by increased plasma albumin and decreased phosphate, low plasma calcium and increased PTH whereas no consistent pattern in the CT levels was observed. It is considered likely that the diuretic effect was the main mechanism behind the observed changes in the examined calcium homeostasis related parameters. An initial loss of calcium in the urine caused hypocalcaemia and thereby a compensatory increase in PTH to mobilize calcium. The decreased plasma calcium possibly masked any stimulatory effect of liraglutide – as seen in other studies - on the calcitonin secretion.	Report summary of NN203258 and NN203295 (4.2.3.7.3 Mechanistic Studies)
Liraglutide (NNC 90-1170): Study on acute effects on calcium homeostasis related hormones after single dose subcutaneous administration in fasted and calcium treated rats- Combined evaluation of the in life phase and hormone analysis	Subcutaneous	Single dose	0, 0.75mg/kg	30 males	A single acute subcutaneous injection with liraglutide to fasted calcium treated rats did not affect PTH and CT levels significantly. A rapid fall in PTH and rise in calcitonin during the first 15 minutes after vehicle or liraglutide administration and plasma calcium levels adjusted for pH were lower in liraglutide treated animals. However, the observed 2.4% decrease in calcium was judged to be of questionable biological significance	Report summary of NN203282 and NN203325 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Liraglutide (NNC 90-1170): Effects on calcium homeostasis related parameters and thyroid volume fractions after up to six weeks daily subcutaneous administration followed by a two week reversibility period in male rats - Combined evaluation of the in life phase, hormone analysis, vitamin D analysis, C-cell pathology of the thyroid gland and molecular analysis	Subcutaneous	6 weeks	0, 0.75mg/kg	64 males main study 15 males satellite	An increase in plasma calcitonin concentrations occurred consistently in both fed and fasted rats after 4-5 weeks of repeated dosing with liraglutide. In line with this, there was a trend towards increased transcriptional activity of calcitonin. The increase in plasma calcitonin was reversible after a two week treatment-free period with recovery levels being decreased. A moderate increase in urinary volume was seen after repeated dosing with liraglutide in fed rats and a corresponding but supra-proportional increase in the amount of calcium excreted in the urine was seen. Calcium homeostasis disturbances were further seen as decreases in plasma PTH, calcium and Vitamin D during the first weeks of treatment. No signs of C-cell proliferation or hyperplasia were observed after 4 weeks of dosing. In conclusion, a significant and sustained increase in plasma calcitonin was observed at a time point where no signs of C-cell hyperplasia or proliferation were present. Therefore, calcitonin release was an early event detecting C-cell stimulation by liraglutide in the absence of hyperplasia or proliferation in rats.	Report summary of NN200317, NN204031, NN204097, NN204020 and NN204018 (4.2.3.7.3 Mechanistic Studies)
Tissue/Species/ Strain	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)**	**Gender and No. per Group**	**Noteworthy Findings**	**Study Number (NN ref No.)**
Liraglutide (NNC 90-1170): Long term exploratory study in young and aged rats - Combined evaluation of the in life phase, calcitonin determination and C-cell pathology of the thyroid gland	Subcutaneous administration	Aged rats: 4, 17, 30 and 43 weeks; Young rats: 30, 43, 56, 69 weeks	0, 0.75	45 males/dose level/age	An increase in plasma calcitonin was seen after 4 weeks of dosing in young and aged rats. This confirmed plasma calcitonin as a biomarker of early C-cell stimulation in the rat. The incidence of focal C-cell hyperplasia and adenoma was increased in both young and aged rats when compared to control. In both aged and young rats, dosing with liraglutide accelerated the progression from hyperplasia to neoplasia also seen spontaneously in control rats. A positive correlation between plasma calcitonin and severity of diffuse and focal C-cell hyperplasia was observed. This confirmed plasma calcitonin as a biomarker of early C-cell proliferative lesions in the rat. No rats developed antibodies after 52 weeks of treatment with liraglutide.	Report summary of below NN204163, NN205119 and NN204310 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Cynomolgus monkey studies						
Liraglutide (NNC 90-1170): Preserved thyroid specimens from Sprague-Dawley rats (Crl:CD®(SD) IGS Br) rats from NN study ref No. 200239 and	Subcutaneous administration	26 weeks	0, 1.0	15 males and 15 females	Treatment of Cynomolgus monkeys for 12 months and rats for 26 weeks with liraglutide was not associated with any differences in thyroid C-cell mass or proliferation measured as the ratio between C-cells and follicular cells and PCNA labelling index, respectively.	NN204021 (4.2.3.7.5 Mechanistic Studies)
Preserved thyroid specimens from Cynomolgus monkeys (*Macaca fascicularis*) from NN study ref No. 200241	Subcutaneous administration	52 weeks	0, 5.0	4 males and 4 females		
Liraglutide (NNC 90-1170): 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	Subcutaneous administration	87 weeks	0, 0.25, 5.0	5 female + 5 male	Daily administration of NNC 90-1170 to cynomolgus monkeys at dose levels up to 5 mg/kg/day for 87 weeks was well tolerated and not associated with any toxicologically significant changes. Specifically, no treatment-related differences in thyroid C-cells were found by extended histopathological evaluation including immunohistochemistry. In accordance, no qualitatively discernable patterns of change in PTH levels and calcitonin levels were observed during 85 weeks of treatment.	Report summary of NN203262, NN204055 and NN204098 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Studies with Exendin-4 /exenatide (NNC 0113-0000-0000)						
Mouse studies						
Exendin-4 /exenatide (NNC 0113-0000-0000) : Study on Acute Effects on Calcitonin and Toxicokinetics after Single Dose Subcutaneous Administration in fasted Mice	Subcutaneous	Single dose	Exendin-4: 0, 0.25, 1 and 5	35 males and 35 females (vehicle) 30 males and 30 females (treated groups)	A treatment related increase in plasma calcitonin was seen following a single subcutaneous dose exendin-4 in CD-1 mice. This observation is compatible with the proposed GLP-1r mediated mechanism behind the similar observations for liraglutide. The toxicokinetic profile of exendin-4 in mice following a single subcutaneous dose was determined. A dose proportional exposure was observed in the dose range 0.25-5.0 mg/kg. The terminal plasma half-life after subcutaneous administration was found to be approximately 0.5 hour.	NN204402 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Exendin-4 /exenatide (NNC 0113-0000-0000): In vivo study with administration of NNC 0113-0000-0000 by subcutaneous administration as bolus injections (once, twice or three times daily) or continuous infusion in female mice	Subcutaneous injection or infusion via micro-osmotic pumps	1 day	Exendin-4: 0 or 0.25 mg/kg/day as: once daily (25 mg/kg/dosing) twice daily (0.125 mg/kg/dosing) tree times daily (0.083 mg/kg/dosing) or continuously via micro-osmotic pumps (0.25 mg/kg/24 hours)	30 females	With a single dose of 0.25 mg/kg, the compound was cleared from plasma after 12 h. When divided as bolus injections three times daily (0.083 mg/kg), the plasma levels were close to LOD of 45 pM at 6 and 8 h after injection. When a daily dose of 0.25 mg/kg/day was administered as a continuous infusion, a high steady state plasma level was observed over the 24 h. The mean CT levels were elevated in all groups treated with exendin-4. However, higher and more sustained CT levels were seen after continuous infusion than after injection once, twice or three times daily.	NN205074 (4.2.3.7.3 Mechanistic Studies)
Exendin-4 /exenatide (NNC 0113-0000-0000): Study on Calcitonin and Toxicokinetics after 3-days of Subcutaneous Administration in fasted Male Mice	Subcutaneous	3 days	Vehicle, Exendin-4: 0.06, 0.03, 0.25, 0.125 Liraglutide: 0.06, 0.25	36 males	Pre-dose and post-dose (1, 3, 6 and 12 hour) calcitonin values in treated animals were statistically significantly higher when compared to controls. Liraglutide induced significantly higher post-dose calcitonin values than exendin-4 administered once daily or as two separate doses. High doses of exendin-4 induced significantly higher calcitonin plasma levels 12 hours after dosing than low dose. No significant effect of dose regimen exendin-4 on post-dose values was found.	NN205050 (4.2.3.7.3 Mechanistic Studies)
Tissue/Species/ Strain	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)**	**Gender and No. per Group**	**Noteworthy Findings**	**Study Number (NN ref No.)**
Exendin-4 /exenatide (NNC 0113-0000-0000): Preliminary Investigative Study by Subcutaneous Administration (three 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	2 weeks	Exendin-4: 0, 0.25, 1.0 or 5.0 mg	6 males and 6 females	A dose-related increase in plasma calcitonin concentrations was seen. At 3.0 mg/kg/day or more in females this was associated with an adaptive increase of thyroid C-cell numbers when evaluated quantitatively. There were no qualitative microscopic findings in the thyroid glands after two or 13 weeks of treatment. Two weeks of treatment significantly increased calcitonin mRNA levels in the thyroid glands of mice in a dose-dependent fashion. In contrast, no significant effect on the GLP-1R mRNA levels was observed.	Report summary of NN205025 and NN205247 (4.2.3.7.3 Mechanistic Studies)
Exendin-4 /exenatide (NNC 0113-0000-0000): Investigatory toxicity study by osmotic mini-pump/subcutaneous administration to CD-1 mice for 12 or 16 weeks	Subcutaneous injection or infusion via micro-osmotic pumps	12 or 16 weeks	Exendin-4: Minipumps 0, 0.25 and 1 Single daily 0, 0.25	Group 1-2-3 36 males and females Group 4+5 24 males and females	Exendin-4 administered by continuous infusion via osmotic mini-pumps or by a single daily injection for 12 or 16 weeks caused a significant increase in plasma calcitonin in all treated groups compared to their respective control group. Levels of plasma calcitonin were higher in animals treated by continuous infusion via osmotic mini-pumps than by a daily subcutaneous injection of the same total daily dose (0.25 mg/kg/day). Statistically significantly increased incidence of C-cell hyperplasia after 12 and 16 weeks exposure was seen in groups administered exendin-4 by continuous infusion via osmotic mini-pumps but not in animals dosed the same total daily dose by a single daily injection for 12 or 16 weeks.	NN205205 (4.2.3.7.3 Mechanistic Studies)

Tissue/Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Study Number (NN ref No.)
Additional studies						
Modelling of Exendin-4 Concentration and Effect on Plasma Calcitonin in Mice from studies NN2044802, NN2050S0, and NN205074.	n.a.	n.a.	n.a.	n.a.	A model was established to describe the calcitonin (CT) response to exendin-4 in terms of ratio to CT in vehicle control groups. The model predicts that (near) maximal CT response is obtained when the exendin-4 concentration is constantly above a threshold which is achieved by a continuous infusion of 0.25 mg/kg/day. On the basis of the available data a similar calcitonin release was seen after 0.25 mg/kg/day of exendin-4 and liraglutide when dosing was done by continuous infusion with exendin-4 and once daily with liraglutide.	PAL2005.001 (4.2.3.7.3 Mechanistic Studies)
Modelling of Pharmacokinetics and Effect on Plasma Calcitonin after Once Daily Dose Administration of liraglutide From studies NN205106 and NN205050	n.a.	n.a.	n.a.	n.a.	A model was established in order to use PK predictions to explain calcitonin response in terms of calcitonin ratio to control after administration of liraglutide to mice. Due to prolonged level of drug concentration, liraglutide maintains a longer calcitonin response than exendin-4 after once daily dose administration of the same dose. With continuous infusion of exendin-4 the stimulation of calcitonin levels becomes similar to that of liraglutide after once daily dosing using the same total daily dose.	PAL2005.005 (4.2.3.7.3 Mechanistic Studies)
Tissue/Species/ Strain	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg/day)**	**Gender and No. per Group**	**Noteworthy Findings**	**Study Number (NN ref No.)**
Characterisation of the distribution of C-cells in thyroids from cynomolgus monkeys	n.a.	n.a.	n.a.	5 males and 5 females	Immunohistochemically staining for presence of calcitonin of thyroid tissue sections generates a higher sensitivity for identification of C-cells compared to HE stained sections. C-cells were typically present in the middle third region of the thyroid gland. It is therefore recommended to use this region of the gland for sampling and staining for presence of calcitonin to achieve a high quality microscopic evaluation of C-cells in the cynomolgus monkey thyroid gland	NN205121 (4.2.3.7.3 Mechanistic Studies)

[N000 2.6.7 P79 - 93]

OVERALL CONCLUSIONS AND RECOMMENDATIONS

NDA 22,341 is an electronic submission from Novo Nordisk to support approval of Victoza® (liraglutide injection), a lipidated human GLP-1(7-37) analog subcutaneously injected once a day indicated as an adjunct to diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus. Liraglutide is a long-acting GLP-1 analog intended for chronic use as monotherapy or in combination with an oral antidiabetic drugs (metformin, sulfonylureas, or thiazolidinediones). Liraglutide should be initiated at 0.6 mg/day for 1 week with dose escalation to 1.2 mg/day for at least week with possible further increase to the maximum recommended dose of 1.8 mg/day, depending on adequate efficacy. Because circulating liraglutide is cleared by nonspecific mechanisms, no dose adjustments are necessary based on age, body weight or BMI, race, or renal or hepatic impairment. Circulating liraglutide has a much longer elimination half-life compared to endogenous GLP-1 because it's resistant to GLP-1 metabolizing peptidases, including DPP-4 and NEP, it's highly bound to plasma proteins which further increases peptidase resistance and decreases renal excretion, and because liraglutide self-association at subcutaneous injection sites slows its absorption. In clinical studies, liraglutide significantly lowered HbA_{1C}. In humans, liraglutide improves glycemic control by increasing glucose-dependent insulin secretion, restoring first phase insulin secretion and improving second phase insulin secretion and increasing maximal insulin secretory capacity, decreasing glucagon secretion only at elevated blood

glucose levels, delaying gastric emptying, decreased food consumption, and decreasing body weight with weight loss attributed to reduced fat. Because incretin effects on blood glucose are glucose-concentration dependent, theoretically liraglutide should have a lower risk of causing hypoglycemia. Unlike other treatments for type 2 diabetes, liraglutide has neutral or beneficial effects of body weight and body composition. For a detailed review of clinical data, the reader is referred to the clinical reviews for this NDA by Dr. Mahoney and Dr. Yanoff.

[redacted] The final drug product is a clear, colorless solution of 6 mg/mL liraglutide in a multiple-dose, disposable, pre-filled cartridges for subcutaneous injection using a pen injector. In addition to liraglutide, each mL of aqueous drug solution contains disodium phosphate dihydrate (1.42 mg), phenol (5.5 mg), propylene glycol (14 mg), and [redacted] pH to 8.15. Liraglutide drug product is stable at 2 – 8C for up to 24 months and at temperatures up to 30C for up to [redacted] days, but liraglutide in solution is not photostable and should be protected from light. No unique photo-degradation products were identified. Liraglutide drug substance - related impurities [redacted] group A, group B, group C, and [redacted]. Pharmacologically active impurities occurred [redacted]. There were no new excipients. Toxicity of drug substance and drug product impurities was adequately assessed in repeat dose toxicity studies, but not in genetic toxicity studies. The reader is referred to the Chemistry review for a detailed review of the drug substance and drug product manuafacturing, charcacterization, and quality control.

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In vitro, liraglutide is a potent, selective GLP-1R agonist pharmacologically active at GLP-1Rs from mice, rats, rabbits, pigs, and monkeys. In vivo, liraglutide was pharmacologically active in all species used in nonclinical testing including mice, rats, rabbits, pigs, and monkeys lowering blood glucose in animal models of type 2 diabetes (mice, rats, pigs) or decreasing food consumption and body weight gain in normal animals or animal models of obesity (rats, rabbits, pigs, monkeys).

After subcutaneous injection, plasma levels of liraglutide increased with dose in all species tested. Liraglutide was highly plasma protein bound, but the extent of protein binding in rats was slightly lower than in humans, and in mice, it was slightly higher. Plasma half life was shorter in rats than in mice, monkeys, or humans. No major circulating metabolites of liraglutide were identified in humans after subcutaneous injection.

Local and systemic toxicity of liraglutide was determined after subcutaneous injection with repeat dose toxicity studies performed in mice (up to 13 weeks), rats (up to 26 weeks) and monkeys (up to 52 weeks) and local toxicity studies in pigs (single dose). In acute or subacute toxicity studies, rats were more sensitive to liraglutide toxicity than mice or monkeys resulting in doses yielding considerably lower multiples of human exposure in repeat dose toxicity studies. Lower levels of plasma protein binding may account for higher sensitivity of rats to liraglutide toxicity, at least in part. At the highest doses used in repeat-dose toxicity studies, multiples of human exposure were 8X in rats, 85X in mice, and 73X in monkeys based on plasma liraglutide AUC comparison at the maximum recommended human dose of 1.8 mg/day. Pregnancy or lactation did not affect the tolerability of liraglutide in rats, but non-pregnant and pregnant female rabbits were very sensitive to liraglutide toxicity with maximum tolerated doses yielding multiples of human exposure < 1X. Target tissues of liraglutide toxicity were thyroid (mice, rats) and injection sites (mice, pigs, monkeys), both occurring at clinically relevant exposures. Liraglutide is a multi-species, multi-sex carcinogen causing thyroid C-cell tumors at low multiples of human exposure in male and female rats and mice. Liraglutide formulated at a concentration 10-fold lower than the clinical formulation caused fibrosarcomas in the skin and subcutis near the injection site in male mice in a 2 year carcinogen bioassay. Drug distribution and excretion studies showed fetuses in liraglutide treated pregnant rats and rabbits were exposed to liraglutide and the intact drug was excreted in milk from lactating rats. Liraglutide increased the incidence of fetal abnormalities in rats, decreased fetal weight and increased fetal abnormalities in rabbits, and in a prenatal and postnatal toxicity study in rats, it decreased

body weight of F_0 and F_1 generation rats and increased agitated behavior in high dose F_1 generation males descended from 1 mg/kg liraglutide treated F_0 females. The following table summarizes NOAELs and the toxicity defining the NOAEL in pivotal toxicity studies.

Species	Study Duration / Doses (mg/kg/day)	NOAEL (Dose, mg/kg)	Toxicity Defining the NOAEL	Animal Exposure @ NOAEL		Human Safety Margin[G]	
				Cmax (nM)	AUC (nM.hr)	Cmax	AUC
CD-1 mice	13 weeks / 0.2, 1, 5	< 0.2	thyroid C-cell focal hyperplasia and ultimobranchial cysts	< 196	< 1959	< 4	< 2
	104 week carcinogenicity / 0.03, 0.2, 1, 3	Non-neoplastic: < 0.03	thyroid inflammatory cell infiltrate, femorotibial joint degradation, seminal vesicle lymphocyte infiltration, thymus tubular cystic hyperplasia, pigment accumulation from mild hemolysis	< 20	< 185	< 0.4	< 0.2
		Neoplastic: 0.2	C-cell tumors @ 1 mg/kg (preneoplastic focal hyperplasia @ ≥ 0.2 mg/kg)	141	1501	3	2
		Neoplastic, local: 1	dorsal skin and subcutis (surface for injections) fibrosarcomas @ 3 mg/kg	NA[D]	NA[D]	0.03[E]	
SD rat	26 weeks / 0.1, 0.25, 1	0.25	clinical signs, exocrine pancreas acinar cell hypertrophy with focal inflammation in females @ 1 mg/kg	107	1585	2	2
	104 week carcinogenicity / 0.075, 0.25, 0.75	Non-neoplastic: < 0.075	clinical signs (F) @ ≥ 0.075, thyroid C-cell focal hyperplasia @ ≥ 0.075 M, ≥ 0.25 F	< 31	< 423	< 0.7	< 0.5
		Neoplastic: < 0.075	thyroid C-cell tumors	< 31	< 423	< 0.7	< 0.5
cyno monkey	52 weeks / 0.05, 0.5, 5	< 0.05	injection site reaction (irreversible), increased rel wt of heart (M), increased rel wt of exocrine pancreas (M & F)	< 46	< 817	< 1	< 1
SD rat	fertility / 0.1, 0.25, 1	Males : 1	No effect on reproductive parameters or abnormalities in sperm, but rel wt of some reproductive organs altered by treatment	577[A]	9074[A]	13	11
		Females: 0.25	early embryonic deaths @ 1 mg/kg	214	2693	5	3
	embryofetal development / 0.1, 0.25, 1	Maternal: 0.25	clinical signs @ 1 mg/kg	214	2693	5	3
		Fetal: < 0.1	fetal abnormalities	< 75[B]	< 691[B]	< 2	< 0.9
	pre- & post-natal / 0.1, 0.25, 1	F_0 reproductive: < 0.1	gestation delayed to day 22	< 75	< 691	< 2	< 0.9
		F_1 development: < 0.1	lower body weight compared to controls starting lactation day 7, agitated behavior in males descended from 1 mg/kg treated F_0 females.	< 75[F]	< 691[F]	< 2[F]	< 0.9[F]
		F_1 reproductive: 1	none	1241[F]	9211[F]	28[F]	11[F]
		F_2 development: 1	none	1241[F]	9211[F]	28[F]	11[F]
NZW rabbit	embryofetal development / 0.01, 0.025, 0.05	Maternal: 0.05	none	22[C]	334[C]	0.5	0.4
		Fetal: < 0.01	fetal abnormalities	< 10[B]	< 137[B]	< 0.2	< 0.2

[A]Exposure estimated from day 28 TK parameters (males and females combined) from a 4-week repeat dose toxicity study in rats.

[B]Maternal plasma exposure on the last TK sample day.

[C]Estimated using gestation day 6 and 16 exposures from mated female NZW rabbits administered doses of 0.02 and 0.1 mg/kg.

[D]Not applicable.

[E]Exposure multiple based on comparison of liraglutide concentration in the 1 mg/kg dose formulation in the mouse carcinogenicity study (0.2 mg/mL) and the liraglutide concentration in the clinical formulation (6 mg/mL).

[F]Estimated ancestral F_0 maternal plasma exposures. F_1 generation exposed *in utero* and from milk during nursing period, F_2 generation never exposed.

[G]Human safety margins calculated by dividing liraglutide plasma exposure in animals (Cmax or AUC) by the plasma exposure in humans at the MRHD of 1.8 mg/day liraglutide (Cmax 45 nM, $AUC_{0\text{-}24h}$ 809 nM.hr).

General Toxicity

Immunogenicity

Anti-liraglutide antibodies were not detected in rats treated with up to 1 mg/kg liraglutide for up to 26 weeks or 0.75 mg/kg/day liraglutide for up to 104 weeks or in mice treated with up to 3 mg/kg/day liraglutide for up to 104-weeks. Anti-liraglutide antibodies were detected in 5 mg/kg/day liraglutide high dose cynomolgus monkeys treated for 52 weeks (one at the end of the study and 2 during the 4 week recovery) or monkeys treated with 0.25 or 5 mg/kg/day for 87 weeks. Anti-liraglutide antibodies in monkeys cross-reacted with endogenous GLP-1. Neutralizing effects of monkey anti-liraglutide antibodies were not determined. The data presented indicate anti-drug antibodies did not interfer with assessment of liraglutide toxicity in repeat dose studies in mice, rats, or monkeys. During clinical studies, anti-liraglutide antibodies occurred in 8.6% of patients, but antibodies were not associated with reduced efficacy.

Mortality

In a 7-day repeat dose toxicity study, rats administered 2 or 10 mg/kg liraglutide were sacrificed moribund within 2 days of dosing with clinical signs of toxicity including piloerection, rolling or high stepping gait, hunched posture, dark extremities, and decreased appetite with related symptoms of severe body weight loss, thin appearance, and decreased fecal output. The NOAEL for mortality in rats was 1 mg/kg in repeat dose studies, 8 to 11 times the human exposure (HEM 8 – 11X) based on AUC at the MRHD of 1.8 mg/day. It's notable that in a 4-day repeat dose *in vivo* micronucleus assay in male rats, doses of 7.5, 15, or 30 mg/kg/day liraglutide were tolerated with no clinical signs of toxicity. There were no mortalities considered treatment-related in toxicity studies in mice, rabbits, pigs, or monkeys. Liraglutide did not affect survival in repeat dose toxicity studies including a 26-week rat study (HEM $\leq$ 8X), a 52-week monkey study (HEM $\leq$ 73X), an 87-week mechanistic study in monkeys (HEM $\leq$ 64X) and 104-week carcinogenicity studies in mice (HEM $\leq$ 45X) and rats (HEM $\leq$ 8X).

Clinical Signs

Clinical signs of toxicity were confined to rats and consisted of one or more of the following: hunched posture, gait changes (rolling or high stepping), piloerection, red discharge from the eyes or nose, stained perigenital area, and dark extremities. Except in a 7-day repeat dose toxicity study, clinical signs generally didn't occur at doses < 1 mg/kg liraglutide (HEM $\leq$ 8X). In a single sc dose safety pharmacology study in rats, body temperature was decreased compared to controls at 0.2 and 2 mg/kg liraglutide. The NOEL for decreased body temperature in rats was 0.02 mg/kg liraglutide (estimated HEM < 1X). Clinical signs of toxicity in rats were not always dose-related and often occurred with specific batches of liraglutide suggesting they may be due to an impurity. In the-26 week chronic rat study, rolling gait, piloerection, and high stepping gait occurred in 1 mg/kg/day high dose groups (HEM $\leq$ 8X) during treatment with clinical signs of toxicity persisting in high dose groups after a 4-week recovery period.

Effects on Food Consumption and Body Weight Gain

Liraglutide dose-dependently decreased food consumption and body weight gain, and at higher exposures in some species, body weight. Decreased appetite with ensuing decreased fecal output, decreased body weight gain, and decreased body weight was dose-limiting in rats and unmated female rabbits. Decreased food consumption by liraglutide was often transient occurring during the first few days or weeks after initiating treatment, but decreased body weight gain was often more durable, except in mice. In mice, liraglutide did not have a durable effect on body weight gain or body weight in repeat dose toxicity studies up to 13 weeks or in the 2 year carcinogenicity study. Decreased food consumption and body weight gain are considered pharmacologic effects in nonclinical species including rats, rabbits, pigs, and monkeys. Body weight gain can increase to levels above normal after treatment is stopped. In clinical studies, liraglutide had neutral or beneficial effects on body weight.

Gastrointestinal Effects

GLP-1R agonists are expected to slow gastric emptying, and this effect contributes to lowering blood glucose by slowing absorption of ingested carbohydrates. In a single sc dose GI safety pharmacology study using longitudinal ileum strips from guinea pigs, 1.43 μM liraglutide, the highest concentration tested (32 fold higher than the Cmax of 45 nM at the MRHD) weakly and reversibly inhibited acetylcholine-induced smooth muscle contraction. In repeat dose studies, liraglutide decreased fecal output in rats and rabbits, and although the effect may be due to decreased GI motility, decreased food intake probably contributed. In the 52-week toxicity monkey study, inflammatory cell infiltrate in the lamina propria occurred at all doses, 0.05, 0.5, and 5 mg/kg/day liraglutide.

In clinical studies, the incidence of gastrointestinal adverse events was much higher in liraglutide treated groups (42.7%) compared to placebo (17.9%) or active comparator (18.5%). Gastrointestinal adverse events in decreasing order of incidence were nausea, diarrhea, vomiting, constipation, and dyspepsia. The incidence of serious adverse events was low and it was similar in liraglutide and comparator groups (placebo or active comparator). The most common adverse events leading to withdrawal for liraglutide-treated patients were nausea (2.8 % of patients) and vomiting (1.5 %). Withdrawal due to gastrointestinal adverse events mainly occurred within the first 2-3 months of starting treatment.

Thyroid Effects

Thyroid is a target of liraglutide toxicity in mice and rats. In 2-year carcinogenicity studies in CD-1 mice or Sprague Dawley rats, liraglutide caused C-cell focal hyperplasia, a precursor to tumors, and C-cell tumors at clinically relevant exposure in both mice and rats. Carcinogenicity study results from exenatide and liraglutide along with toxicity study results from other GLP-1R agonists indicate rodent C-cell focal hyperplasia and tumors are a pharmacologic class effect associated with prolonged GLP-1R activation. Novo Nordisk performed mechanistic studies aimed at determining a mode of action for liraglutide-induced thyroid tumors in rodents, and based on this mode of action, their relevance to humans. This mode of action was based on liraglutide activating GLP-1Rs on thyroid C-cells leading to persistent calcitonin secretion and calcitonin synthesis, and increased calcitonin secretion and synthesis driving C-cell hyperplasia with progression of hyperplasia to tumors. Mechanistic studies did not support the proposed mode of action. Results from repeat-dose, mechanistic, and carcinogenicity studies suggest liraglutide transforms normal thyroid C-cells into focal hyperplastic C-cells and tumors without causing proliferation of normal C-cells (diffuse hyperplasia). In a December 2008 meeting of CDER's ECAC, the committee concurred the weight of evidence from rodent carcinogenicity studies, mechanistic studies, and clinical data was not sufficient to conclude liraglutide-induced thyroid-cell tumors are rodent-specific. A large majority of an Advisory Committee convened on 2 April 2009 to address the clinical safety of liraglutide concluded the applicant did not provide adequate data on animal thyroid C-cell tumor findings to demonstrate these findings are not relevant to humans (The committee voted 1-yes and 12-no on the question "Has the applicant provided adequate data on the animal thyroid C-cell tumor findings to demonstrate that these findings are not relevant to humans?"). In a June 2009 meeting, the applicant agreed the proposed mode of action is probably not correct.

C-cell focal hyperplasia and adenomas are common in rats and their incidence increases with age, along with plasma calcitonin levels, but C-cell carcinomas are rare. Seven approved drugs cause C-cell tumors in rats; usually only in one sex and/or at high multiples of human exposure. The No Observed Adverse Effect Level (NOAEL) for thyroid C-cell tumors in rats was < 0.075 mg/kg/day liraglutide (HEM < 1X), below the lowest dose tested. BIn Sprague Dawley rats, benign C-cell adenomas dose-dependently increased at ≥ 0.25 mg/kg in males (HEM ≥ 2X) and at ≥ 0.075 mg/kg in females (HEM ≥ 0.5X). Malignant C-cell carcinomas increased with dose in males at 0.75 mg/kg (HEM 8X) and the incidence was above the historical control group range at ≥ 0.075 mg/kg in males (HEM ≥ 0.5X) and at ≥ 0.25 mg/kg in females (HEM 2X). Combined C-cell carcinomas and adenomas increased dose-dependently at ≥ 0.25 mg/kg in males (HEM 2X) and at ≥ 0.075 mg/kg in females (HEM ≥ 0.5X). Focal C-cell hyperplasia, a preneoplastic lesion, increased above concurrent control groups and above the

historical control group range at ≥ 0.075 mg/kg liraglutide in males (HEM ≥ 0.5X) and at ≥ 0.25 mg/kg in females (HEM ≥ 2X). Rats < 8 months old were insensitive to liraglutide's effects on C-cells, so focal C-cell hyperplasia and tumors didn't occur in the chronic 6-month repeat-dose toxicity study using rats ≤ 2 months old at the start of treatment. Plasma calcitonin, a biomarker for C-cell activation and increased C-cell mass (proliferation of normal cells or tumors) was not a biomarker for either liraglutide-induced focal C-cell hyperplasia or tumors in rats. Liraglutide increased the incidence of age-related focal C-cell hyperplasia in rats, but without accelerating its onset. Liraglutide increased the incidence of C-cell adenomas and carcinomas, and accelerate red the onset of adenomas.

In mice, spontaneous or drug-induced C-cell focal hyperplasia, adenomas, or carcinomas are rare and plasma calcitonin does not increase with age. There are no approved drugs that cause C-cell tumors in mice. The NOAEL for C-cell tumors in mice was 0.2 mg/kg/day liraglutide (safety margin 2X). The incidence of benign C-cell adenomas dose-dependently increased compared to concurrent controls and above the historical controls at ≥ 1 mg/kg liraglutide in males and females (HEM ≥ 10X). Malignant C-cell carcinoma occurred in 2 high dose females (HEM 45X), and the incidence of combined C-cell carcinoma and adenomas dose-dependently increased at ≥ 1 mg/kg in females (HEM ≥ 10X). C-cell hyperplasia dose-dependently increased above concurrent and historical control groups at ≥ 0.2 mg/kg liraglutide in males and females (HEM ≥ 2X). High dose liraglutide induced focal C-cell hyperplasia within 4 – 9 weeks (5 mg/kg/day) and C-cell tumors within 64 – 78 weeks (3 mg/kg/day) in mice. In the 2-year mouse carcinogenicity study, plasma calcitonin increased with liraglutide dose and treatment-duration at ≥ 0.2 mg/kg (HEM ≥ 2X) and thyroid inflammatory cell infiltrate occurred at ≥ 0.03 mg/kg liraglutide (HEM ≥ 0.2X). In addition to liraglutide inducing focal C-cell hyperplasia in thyroid in the 13 week toxicity study, it also increased incidence of ultimobranchial cysts at ≥ 0.2 mg/kg (HEM ≥ 2X).

Liraglutide had no effect on plasma calcitonin or thyroid C-cell histopathology in monkeys treated with high dose liraglutide for up to 52 weeks (HEM 73X) or up to 87 weeks (HEM 64X).

Plasma calcitonin and thyroid morphology were monitored in phase 3 clinical studies. Liraglutide increased plasma calcitonin and there was a numerical imbalance in the number of thyroid adverse events in liraglutide treated groups compared to placebo or active comparator, including a higher incidence of papillary carcinomas. At the 2 April 2009 Advisory Committee meeting convened to address the safety of liraglutide, liraglutide associated increase in plasma calcitonin were not considered clinically relevant while the imbalance in thyroid papillary tumors seen in liraglutide treated groups was not attributed to treatment. There is a numerical imbalance in the number of thyroid C-cell proliferative findings with more cases occurring in liraglutide-treated groups, but the clinical significance is unknown because of the small total number of cases in clinical studies.

Injection Site Reactions and Local Toxicity

Injection site reactions or pathology findings at or near the injection site occurred in single dose local tolerance studies in pigs and repeat dose studies in mice (104-week) and monkeys (3-day dose-escalation and 14-day, 28-day, 13-week, and 52-week studies). In repeat-dose toxicity and carcinogenicity studies, the concentration of liraglutide in dosing formulations was typically much lower than the 6 mg/mL liraglutide concentration used in phase 3 clinical studies, even for high dose groups. Local toxicity from repeat dosing with high concentration liraglutide (~6 mg/mL) was not adequately assessed in repeat-dose toxicity studies.

High dose male mice treated with 3 mg/kg/day liraglutide in the 2-year carcinogenicity study developed fibrosarcomas on the dorsal skin and subcutis, the body surface used for drug injection. The incidence of fibrosarcomas at the injection site, which were considered separately from dorsal skin and subcutis fibrosarcomas, exceeded concurrent and historical control group levels at 0.03, 0.2 and 3 mg/kg liraglutide. In male mice, rhabdomyosarcomas on the dorsal skin and subcutis at ≥ 0.2 mg/kg and injection site fibrosarcomas at 3 mg/kg exceeded concurrent and historical controls. Liraglutide solution was injected at a constant volume of 5 mL/kg, so at the highest dose, the concentration of liraglutide in the dosing solution was 0.6 mg/mL (diluted from a 6 mg/mL stock solution), 10-fold lower than the concentration of liraglutide used clinically (6 mg/mL).

Injection site reactions were noted in repeat dose toxicity studies in rats, but with no substantive differences between vehicle or liraglutide treated rats. Liraglutide concentrations in dosing formulations for rat studies were typically < 1 mg/mL.

Local tolerance studies in pigs showed subcutaneous injection of 200 µL 0 (vehicle) or 5 mg/mL liraglutide in a phase 2 formulation (containing mannitol as an osmotic agent) caused injection site reactions characterized by subacute inflammation occurring 2 days after dosing, but there were no substantive differences between vehicle control and liraglutide sites. Macrophages and epitheloid / giant cells with collagen formation and fat necrosis occurred 5 days after dosing. Injection site toxicity of propylene glycol containing phase 3 formulations with different pHs ranging from 7.7 to 8.15 showed no substantive differences between formulations with different pHs or between vehicle and liraglutide injection sites. Up to slight edema, cellular infiltrate, and necrosis with or without hemorrhage occurred 2 days after dosing with moderate cellular infiltrate, collagen formation, and fat necrosis with or without hemorrhage occurring 5 days after injection. The 200 µL dose volume used in these pig studies was 100 µL less than the 300 µL volume for the highest clinical of 1.8 mg/day.

In cynomolgus monkeys, injection site reactions occurred in all toxicity studies. In 13-week and 52-week repeat dose monkey studies, NOAELs were not established due to injection site reactions at the lowest dose of 0.05 mg/kg liraglutide in both studies. Repeat dose studies ≥ 28 days had clinical signs of thickening at the injection sites. In the 28-day study, injection site reactions consisted of clinical signs of thickening and reddening and microscopic pathology findings of subacute or chronic fasciitis, hemorrhage, and pigmented macrophages, but these findings occurred in all dose groups (0, 0.05, 0.5, 5 mg/kg liraglutide) and attributed to the vehicle. In a 13-week study, injection site reactions occurring at all doses (0.05, 0.5, and 5 mg/kg liraglutide) were characterized by clinical signs of subcutaneous thickening with correlative active fasciitis and increased blood eosinophils. Injection site reactions were not reversed in high dose recovery group females after a 2 week treatment-free period. In a 52-week study, the concentration of liraglutide in the dosing solution for the 5 mg/kg high dose formulation was 2 mg/mL, a 3-fold lower drug concentration compared to the clinical formulation. Injection site findings were thickening with correlative subcutaneous inflammatory cell infiltrate increased with liraglutide dose at all doses (0.05, 0.5, 5 mg/kg). In the 5 mg/kg liraglutide high dose group, subcutaneous sclerosis, foreign material, and foreign body giant cells occurred at the injection site, and thickening with inflammation persisted in 4-week high dose recovery groups, albeit with diminished severity.

Mild injection site reactions occurred in approximately 2% of subjects receiving liraglutide in clinical trials 26 weeks or longer. These reactions did not lead to discontinuation of liraglutide.

Mild Anemia

Mild anemia, characterized by decreased RBC, hematocrit and hemoglobin occurred in repeat dose studies in mice, rats, and monkeys.

In mice, liraglutide caused mild anemia at 0.2, 1, and 3 mg/kg in the 104-week carcinogenicity study (HEM ≥ 2X), at ≥ 0.1 mg/kg in a 4-week study (HEM ≥ 1X), and at ≥ 0.2 mg/kg in a 13-week study (HEM > 2X). Hemosiderin accumulation in spleen and liver Kupffer cells suggests anemia in mice may be hemolytic. In the 13-week study, RBC count was 4.9 – 12.4% lower than controls in males and 3.1 – 5.9% lower than controls in females in liraglutide-treated groups, but without correlative decreases in hematocrit or hemoglobin.

In rats, mild anemia occurred at all doses (0.1, 0.25, 1 mg/kg liraglutide) in a 4-week toxicity study (HEM > 0.6X), but it didn't occur in 13- or 26-week studies using doses up to 1 mg/kg (HEM ≤ 14X). RBC parameters were not measured in the 104-week rat carcinogenicity study using doses up to 0.75 mg/kg. In an *in vivo* micronucleus assay dose range-finding study in male rats, bone marrow toxicity characterized by a decreased ratio of bone marrow polychromatic erythrocytes to normochromatic erythrocytes occurred 5 days after a single 40 mg/kg liraglutide dose. In the definitive 4-day repeat dose micronucleus assay in male rats, all doses of liraglutide (7.5, 15, or 30 mg/kg/day) caused bone marrow toxicity and decreased the relative level of reticulocytes in peripheral blood from 41.9 – 65.1% of controls, but without significantly affecting RBC parameters.

In monkeys, mild anemia occurred in 2-, 13-, and 52-week toxicity studies, but not in the 28-day study. Fourteen days of treatment with 4 mg/kg/day liraglutide (HEM 73X) resulted in mild anemia with decreased RBC parameters (RBC count, hemoglobin, and hematocrit) and increased reticulocytes. In a 13-week study, mild anemia occurred in males treated with 5 mg/kg/day liraglutide (HEM ~74X). In the 52 week monkey study, anemia occurred at 0.5 and 5 mg/kg/day liraglutide (HEM ≥ 9X) in both male and female monkeys. Anemia was reversed after a 4 week recovery period.

No clinically relevant changes in hematology parameters were observed in clinical studies.

Cardiovascular Effects

Liraglutide had cardiovascular effects in isolated rabbit hearts and *in vivo* in repeat dose studies in rats and monkeys.

Liraglutide (1.43 μM) slightly increased heart rate in isolate rabbit hearts. In a single dose cardiovascular safety study in rats, ≥ 0.2 mg/kg liraglutide (estimatedHEM ≥ 2X) increased heart rate. Liraglutide increased heart rate and cardiac output in a pig model of myocardial infarction, but liraglutide had no effect on heart rate in single or repeat dose studies in monkeys.

In a single-dose safety pharmacology study in rats, liraglutide increased systolic, diastolic, and mean arterial blood pressure at doses ≥ 0.2 mg/kg liraglutide (estimated HEM ≥ 2X). The NOEL was 0.02 mg/kg.

Pigs were treated with 0.01 mg/kg/day liraglutide once a day for 3 days prior to infarction induced by balloon angioplasty in the left descending coronary artery. Liraglutide treated pigs had higher heart rates (~10 - 20 bpm higher) at baseline prior to infarction, during ischemia, and during reperfusion. Liraglutide increased cardiac output (23% higher 60 min after reperfusion, 14% higher 120 min post infarction).

Liraglutide decreased heart weight in rats and increased heart weight in cynomolgus monkeys. In male rats, liraglutide decreased absolute heart weight 11 - 18% in males at ≥ 0.25 mg/kg in 1- and 4-week studies (HEM ≥ 2X), and 11 – 17% at all doses (0.1, 0.25, 1 mg/kg liraglutide) in 13- and 26-week studies (HEM ≥ 0.5X). In females, decreased heart weight was more sporadic with 11 - 17% decreases occurring in 0.125 and 0.25 mg/kg liraglutide in a 1-week study (but not in the 1 mg/kg high dose group) and at 0.25 and 1 mg/kg groups in the 26-week study (HEM ≥ 2X). Because dose-related decreased body weight in liraglutide treated rats, heart weight normalized to body weight was generally decreased < 10% in studies ≥ 4 weeks long. In the 13 week rat study, CPK increased 25% compared to controls in males (0.1 and 1 mg/kg) and females (0.25 and 1 mg/kg), but since the CPK isozyme was not determined, the relation to decreased heart weight was uncertain.

Rats

	Sex	Males			Females		
	LGT Dose (mg/kg/day)	0.1 - 0.125	0.25	1	0.1 - 0.125	0.25	1
	Human EM (AUC_{0-24} ratio)	0.6 - 0.9	2 - 4	8 - 13	0.6 - 0.9	2 - 4	8 - 13
Study Duration	Parameter	% Change From Control					
1 week	heart wt, absolute	-4	-15*	-18*	-11	-17	-7
	heart wt, relative to bw	-5	-11	-6	-11	-16	2.2
4 week	heart wt, absolute	-6	-11*	-16*	-2	-9*	-8*
	heart wt, relative to bw	-5	-9	-10	-6	-11	-6
13 week	heart wt, absolute	-11	-17	-15	0	-4	-3
	heart wt, relative to bw	-3	-7	-2	-2	-6	-5
	CPK	162	21	29*	24	54*	26
26 week	heart wt, absolute	-15*	-20*	-17*	-9*	-14*	-15*
	heart wt, relative to bw	-3*	-7*	-1*	-4*	-5*	-8*

In cynomolgus monkeys, absolute heart weight increased 11 - 15% in males at all doses in a 52 week study, and because liraglutide decreased body weight, heart weight normalized to body weight increased from 23 to 49%. In a 3-day ascending dose monkey study, CPK increased up to 4.4 fold at 5 mg/kg in

both males and females, but since the CPK isozyme was not determined, the relation to liraglutide's effects on the heart was uncertain

Cynomolgus Monkeys

	Sex	Male			Female		
	LGT Dose (mg/kg/day)	0.05	0.5	5	0.05	0.5	5
	Human EM (AUC_{0-24h} ratio)	0.2 - 1.0	2.3 - 9.0	31 - 85	0.2 - 1.0	2.3 - 9.0	31 - 85
Study Duration	Parameter	% Change From Control					
4 week	heart wt, absolute	3	-13	-11	-1	0	-8
	heart wt, relative to bw	-2	-3	-6	-7	-5	-3
13 week	heart wt, absolute	-6.8	-10.5	-8.9	0.9	-8.1	-9.5
	heart wt, relative to bw	-2.4	-6.3	0.2	10.5	5.7	-0.9
52 week	heart wt, absolute	15	13	11	-5	2	-2
	heart wt, relative to bw	23	49	45	-11	-1	-4
		Fold Increase Over Controls					
3 Day (ascending dose)	CPK	-	-	0.4 - 4.4X	-	-	1.1 - 4.0X

In clinical studies, liraglutide causes a small increase in heart rate and a small decrease in systolic blood pressure. Cardiovascular safety of liraglutide was an issue addressed at the 2 April 2009 Advisory Committee Meeting and analysis of data from phase 2/3 clinical studies indicate liraglutide was not associated with excessive cardiovascular risk.

Kidney Effects

Liraglutide had effects on kidneys in mice, rats, and monkeys.

In a single dose renal safety pharmacology study in male rats, liraglutide caused diuresis with increased urine volume and increased excretion of sodium, potassium, and chloride within 6 hours of dosing at 0.2 and 2 mg/kg, decreased urine specific gravity and osmolarity at all doses (0.02, 0.2, and 2 mg/kg), and increased protein in urine from 6 – 24 hours after dosing at 2 mg/kg.

Transient diuresis (increased urine volume and excretion of sodium, chloride, and phosphate and decreased specific gravity and decreased excretion of magnesium, potassium, and calcium) occurred in the 5 mg/kg liraglutide high dose group in the first day of dosing in a 13 weeks mouse toxicity (HEM 91X).

There were no substantive effects on urine composition in cynomolgus monkeys treated for up to 52 weeks with up to 5 mg/kg/day liraglutide, but in that study week study, focal interstitial inflammatory cell infiltrate in kidneys occurred in males at 5 mg/kg (HEM 73X) and in females at 0.05 mg/kg (HEM 1X)

There were no clinically relevant effects on urinalysis parameters observed in clinical studies of liraglutide.

Pancreas Effects

Treatment with liraglutide for 15 days increased beta cell proliferation in obese diabetic db/db mice. Pancreatic beta cell and islet volume increased in diabetic Zucker diabetic fatty rats treated with 30 or 0.15 mg/kg/injection liraglutide administered twice a day for 6 weeks, but liraglutide had no effect on beta cell mass in pre-diabetic ZDF rats treated with 0.2 mg/kg/injection twice a day for 8 weeks. In non-diabetic Sprague Dawley rats, 0.2 mg/kg/injection liraglutide administered twice a day transiently increased beta cell mass after 1 week, but had no effect at the end of 6 weeks. In rats, liraglutide-induced increased beta cell volume or mass depends on the presence of diabetes.

Liraglutide affected the exocrine pancreas in rats and monkeys. In a 26-week repeat dose toxicity study in rats, the incidence of up to mild pancreatic acinar cell atrophy was increased in 1 mg/kg male and female high dose groups and the incidence of minimal focal inflammation in the exocrine pancreas increased in high dose females (HEM 8X).In repeat dose toxicity studies in cynomolgus monkeys,