CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209637Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date: December 1, 2017
From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA: 209637
Agency receipt date: December 5, 2016
Drug: Ozempic (Semaglutide)
Sponsor: Novo Nordisk

Indication: Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of semaglutide for the indication listed above.

The established pharmacologic class for semaglutide is glucagon-like peptide-1 (GLP-1) receptor agonist. Exenatide (Byetta and Bydureon), dulaglutide (Trulicity), albiglutide (Tanzeum), lixisenatide (Adlyxin) and liraglutide (Victoza) are previously approved GLP-1 agonists in the US.

Semaglutide is a novel GLP-1 analogue that is engineered to have a low clearance and long elimination half-life, achieved by albumin binding that is facilitated by a fatty di-acid attached to the peptide backbone. The peptide backbone has also been modified to reduce degradation by the DPP-4 enzyme. The maximum recommended clinical dose is a 1 mg once weekly subcutaneous injection.

An appropriate nonclinical program was conducted by the sponsor to support approval of semaglutide. Semaglutide elicited expected pharmacological responses in rats, diabetic mice, and minipigs. The primary nonclinical toxicity studies were conducted in mice, rats and monkeys; dosing was limited by pharmacologically mediated reductions in food intake and body weight. Primary findings included liver necrosis, focal C-cell hyperplasia, C-cell nests, and dilated ultimobranchial ducts in mice and ECG abnormalities and myocardial vacuolation and degeneration. These findings were observed at doses associated with exposures that were 17- to 27-times the anticipated clinical exposure.

A standard battery of genetic toxicity studies was conducted and produced no evidence of genotoxic potential. Carcinogenicity studies in mice and rats demonstrated a risk for tumorigenesis, similar to other GLP-1 agonists, at exposures that were 2-fold (mice) and 0.4-fold (rats) or greater than the anticipated clinical AUC. The findings included thyroid C-cell adenomas and carcinomas. The totality of the available data indicates that rodents are more sensitive to the C-cell proliferative effects than nonrodents and presumably humans. Although the human relevance of GLP-1 receptor agonist-induced C-cell

tumorigenesis in rodents is unknown, human relevance to drug-induced C-cell tumors cannot be discounted and, therefore, a boxed warning and appropriate epidemiological monitoring continue to be warranted for GLP-1 agonists.

Reproductive and developmental toxicity studies were conducted in rats, rabbits and monkeys. Key findings included reduced growth in rats and monkeys, fetuses with visceral and/or skeletal abnormalities at clinically relevant exposures in all species, and early pregnancy losses in rabbits and monkeys. The identified developmental findings generally occurred in the presence of significant maternal toxicity so the direct relationship to drug administration is not clear. Given that they occurred at clinically relevant exposures, I agree that they merit discussion in the product label.

Conclusion:
I agree with the division pharmacology/toxicology conclusion that semaglutide can be approved from the pharmacology/toxicology perspective. I have reviewed the proposed labeling and agree with the recommendations made by the division regarding the relevant nonclinical sections.

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/s/

TIMOTHY J MCGOVERN
12/01/2017

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 209637
Supporting document/s:	SDN 1
Applicant's letter date:	12/05/2016
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Product:	Semaglutide
Indication:	Type 2 Diabetes Mellitus
Applicant:	Novo Nordisk
Review Division:	DMEP
Reviewer:	Federica Basso, Ph.D
Supervisor/Team Leader:	Ron Wange, Ph.D
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Project Manager:	Peter Franks

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

Semaglutide is a novel glucagon-like peptide-1 (GLP-1) analogue for once-weekly subcutaneous administration in patients with type 2 diabetes. Semaglutide has been engineered to have a low clearance and thereby a long elimination half-life, which makes the compound suitable for once-weekly administration. The extended half-life is achieved by albumin binding facilitated by a fatty di-acid attached to the peptide backbone through a hydrophilic linker at lysine in position 26. In addition, the peptide backbone has been modified in position 8 (alanine to 2-aminoisobutyric acid) in order to reduce degradation by the DPP-4 enzyme.

1.2 Brief Discussion of Nonclinical Findings

In vitro and in vivo pharmacology studies have demonstrated that semaglutide potently activates the human GLP-1 receptor. Dose-related increase in glucose-dependent insulin secretion, and decrease in glucose levels were observed in rats, diabetic mice, and minipigs.

The toxicity profile of semaglutide was evaluated in mice, rats, and monkeys for up to 3, 6 and 12 month duration, respectively. In all species dose levels were limited by pharmacologically mediated reductions in food intake and body weight. A dose-escalation approach was utilized in the pivotal toxicology studies to minimize the initial treatment-related effects on body weight.
Mild focal C-cell hyperplasia, C-cell nests, and dilated ultimobranchial ducts were observed after 3-month of dosing in mice starting at 17X the clinical exposure. Liver necrosis and centrilobular hypertrophy were observed at higher doses, mostly in males (175X MRHD). Minimal to moderate Brunner's gland hypertrophy was noted in nearly all treated rats at the clinical exposure. This finding was reversible and was not considered adverse, given the absence of associated inflammatory or degenerative changes. In monkey, there were no definitive signs of toxicity other than the expected effects on body weight and food consumption. ECG abnormalities (a bigeminal rhythm with two episodes of sinus tachycardia in Week 13 and a continuous left bundle branch block-like recording that persisted from Week 26 to Week 52) and slight multifocal myocardial vacuolation and degeneration, with karyomegaly, in the left ventricle were observed in one high-dose female and male, respectively (27X MRHD). A relationship to treatment could not be excluded; NOAEL for cardiac effects was established at 5-fold the clinical exposure. No adverse microscopic lesions were observed in the monkey thyroid at doses up to 27X MRHD.

In two-year carcinogenicity studies in mice and rats, a statistically significant increase in the incidence of C-cell adenoma and combined C-cell adenoma and carcinomas was observed in both species. These tumors occurred at the clinical exposure in rats and at 2X and 5X the clinical exposure in female and male mice, respectively. C-cell carcinomas were statistically significantly increased in male rats at ≥0.025 mg/kg/day

(0.7X the clinical exposure). A numerical increase in C-cell carcinoma was noted in mice (n=2, 2, 2 in LD, MD and HD male mice; n=1, 2, 2 in LD, MD and HD female mice). C-cell tumors are known class effects of GLP-1R agonists and have been reported in two-year rodent studies with other long acting GLP-1R agonists. The human relevance of these tumors is unknown.

A standard development and reproductive toxicology program was conducted in rats, rabbits, and monkeys. In combined fertility and embryonic development studies in rats, no effects were observed on male fertility. In females, an increase in estrus cycle length was observed at all doses, together with a small reduction in the number of corpora lutea. Both findings occurred at the clinical exposure, but were likely an adaptive response secondary to the pharmacological effect of semaglutide on food consumption and body weight. Decrease in maternal body weight gain, embryofetal mortality, growth retardation, skeletal (scapula, long bones, ribs, digits, vertebrae and cranial bones) and visceral (cardiac blood vessels) malformations were observed in rats at approximately the clinical exposure. Mechanistic studies showed that semaglutide caused embryotoxicity in rats through a GLP-1 receptor-mediated impaired function of the inverted yolk sac. However, involvement of additional mechanisms leading to embryotoxicity in rats cannot be completely excluded.

In embryofetal development studies, marked maternal body weight loss and/or decrease in body weight gain were observed in rabbits and monkeys at the clinical exposure. Increased post-implantation loss, skeletal malformations in the sternebra and digits, and visceral malformations in the kidney and liver were observed in rabbits at the clinical exposure. A direct drug-related effect on fetal development cannot be ruled out. Sporadic malformations were noted in monkeys at $\geq$5X clinical exposure (shifts in the alignment of the vertebrae, ribs and sternebra at the cervico-thoracic border and blood accumulation under the skull causing misshapen right brain hemisphere), but were considered secondary to the effect on maternal body weight. No treatment related embryotoxic effects were noted in monkeys at the clinical exposure.

In a pre- and post-natal development study in monkeys, early pregnancy losses observed at 3X the clinical exposure were most likely related to maternal weight loss during the first trimester. There were no treatment related external abnormalities or histopathological findings in the offspring at doses up to 7X the clinical exposure.

Administration of semaglutide to juvenile SD rats for 11 weeks, from postnatal day 21 to 97, caused reduction in food consumption, body weight gain, and delayed sexual maturation at the clinical exposure. There were no consequential effects upon fertility or reproductive performance at doses up to 2X the clinical exposure.

1.3 Recommendations

1.3.1 Approvability

On the basis of the nonclinical data reviewed in this marketing application, semaglutide is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

8.1 Pregnancy

Risk Summary

[redacted] limited [redacted] data with semaglutide in pregnant women [redacted] to inform [redacted] a drug-associated ris [redacted]

In pregnant rats administered semaglutide during organogenesis, embryofetal mortality, [redacted] structural abnormalities occurred at maternal [redacted] exposures. In [redacted] rabbits and cynomolgus monkeys administered semaglutide during organogenesis, early pregnancy losses and structural abnormalities were observed at [redacted] ≥5-fold [redacted] These findings coincided with a marked maternal body weight loss in both animal species *[see Animal Data]*.

The estimated background risk of major birth defects for women [redacted] is 6 to 10%. [redacted] has been reported to be as high as 20 to 25% in women with a Hemoglobin A1C >10. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Clinical Considerations

Disease associated maternal and fetal risk

Poorly controlled diabetes during pregnancy increases the maternal risk for diabetic ketoacidosis, pre- eclampsia, and delivery complications [redacted] Poorly controlled diabetes increases the fetal risk for [redacted]

Animal Data

In a combined fertility and embryofetal development study in rats, subcutaneous doses of 0.01, 0.03 and 0.09 mg/kg/day (0.1-, 0.4-, and 1.1-fold the MRHD) were administered to males for 4 weeks prior to and throughout mating and to females for 2 weeks prior to mating, and throughout organogenesis to Gestation Day 17. In parental animals, pharmacologically mediated reductions in body weight gain and food consumption were observed at all dose levels. In the offspring, reduced growth and fetuses with visceral (heart blood vessels) and skeletal (cranial bones, vertebra, ribs) abnormalities were observed at the human exposure.

In an embryofetal development study in rabbits, subcutaneous doses of 0.0010, 0.0025 or 0.0075 mg/kg/day (0.03-, 0.3-, and 2.3-fold the MRHD) were administered throughout organogenesis, from Gestation Day 6 to 19. Pharmacologically mediated reductions in maternal body weight gain and food consumption were observed at all dose levels. Early pregnancy losses and increased incidences of minor visceral (kidney, liver) and skeletal fetal abnormalities (sternebra) were observed at ≥0.0025 mg/kg/day (at clinically relevant exposures).

In an embryofetal development study in cynomolgus monkeys, subcutaneous doses of 0.015, 0.075, and 0.15 mg/kg twice weekly (1.0-, 5.2-, and 14.9-fold the MRHD) were administered throughout organogenesis, from Gestation Day 16 to 50. Pharmacologically mediated, marked initial maternal body weight loss and reductions in body weight gain and food consumption coincided with the occurrence of sporadic skeletal abnormalities (vertebra, sternebra, ribs) at ≥0.075 mg/kg twice weekly (≥5X human exposure).

In a pre- and postnatal development study in cynomolgus monkeys, subcutaneous doses of 0.015, 0.075, and 0.15 mg/kg twice weekly (0.7-, 3.3-, and 7.2-fold the MRHD) were administered from Gestation Day 16 to 140. Pharmacologically mediated marked initial maternal body weight loss and reductions in body weight gain and food consumption coincided with an increase in early pregnancy losses and led to delivery of slightly smaller offspring at ≥0.075 mg/kg twice weekly (≥3X human exposure).

8.2 Lactation

Risk summary

There are no data on the presence of semaglutide in human milk, the effects on the breastfed infant, or the effects on milk production. Semaglutide was present in the milk of lactating rats *[see Animal Data]*. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for semaglutide and any potential adverse effects on the breastfed infant from semaglutide or from the underlying maternal condition.

(b) (4) Data

In lactating rats, semaglutide was detected in milk at levels 3-12 fold lower than in maternal plasma.

8.3 Females and Males of Reproductive Potential

(b) (4)

Women (b) (4) discontinue OZEMPIC at least 2 months before a planned pregnancy due to the long washout period for semaglutide

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 2-year carcinogenicity study in CD-1 mice, subcutaneous doses of 0.3, 1 and 3 mg/kg/day (5-, 17-, and 59-fold the maximum recommended human dose (MRHD) of 1

mg/week, based on AUC) was administered to the males, and 0.1, 0.3 and 1 mg/kg/day (2-, 5-, and 17-fold MRHD) was administered to the females. A statistically significant increase in thyroid C-cell adenomas and a numerical increase in C-cell carcinomas were observed in males and females at all dose levels (>2X human exposure).

In a 2-year carcinogenicity study in Sprague Dawley rats, subcutaneous doses of 0.0025, 0.01, 0.025 and 0.1 mg/kg/day were administered (below quantification, 0.4-, 1-, and 6-fold the exposure at the MRHD). A statistically significant increase in thyroid C-cell adenomas was observed in males and females at all dose levels, and a statistically significant increase in thyroid C-cell carcinomas was observed in males at ≥0.01 mg/kg/day.

Human relevance of thyroid C-cell tumors in rats is unknown and could not be determined by clinical studies or nonclinical studies *[see Boxed Warning and Warnings and Precautions (5.1)]*.

Semaglutide was not mutagenic or clastogenic in a standard battery of genotoxicity tests (bacterial mutagenicity (Ames), human lymphocyte chromosome aberration, rat bone marrow micronucleus).

In a combined fertility and embryo-fetal development study in rats, subcutaneous doses of 0.01, 0.03 and 0.09 mg/kg/day (0.1-, 0.4-, and 1.1-fold the MRHD) were administered to male and female rats. Males were dosed for 4 weeks prior to mating, and females were dosed for 2 weeks prior to mating and throughout organogenesis until Gestation Day 17. No effects were observed on male fertility. In females, an increase in estrus cycle length was observed at all dose levels, together with a small reduction in numbers of corpora lutea at ≥0.03 mg/kg/day (at clinically relevant exposures). These effects were likely an adaptive response secondary to the pharmacological effect of semaglutide on food consumption and body weight.

2 Drug Information

2.1 Drug

CAS Registry Number: RN910463-68-2

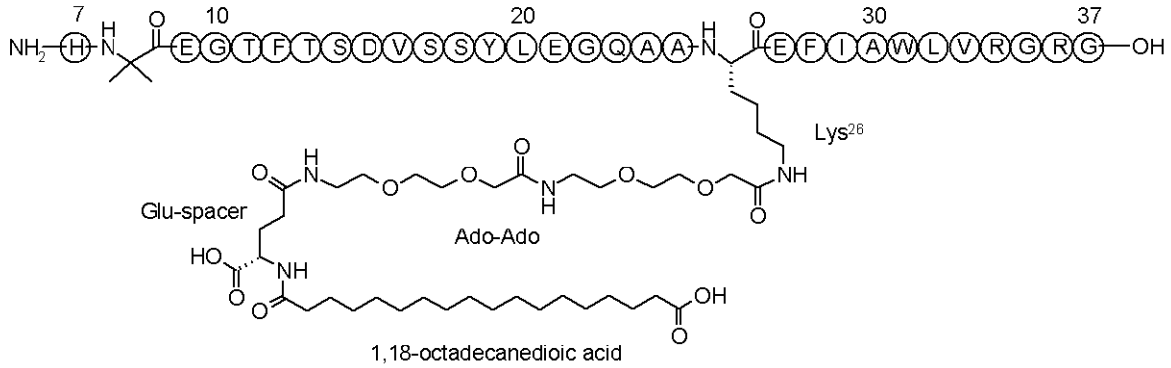
Generic Name: Ozempic

Code Name: Semaglutide, NNC0113-0217

Chemical Name: Nε26 [(S)-(22,40-dicarboxy-10,19,24-trioxo-3,6,12,15-tetraoxa-9,18,23-triazatetracontan-1-oyl)] [Aib8, Arg34]GLP-1-(7-37) peptide.

Molecular Formula/Molecular Weight: C_{187} H_{291} N_{45} O_{59} / 4113.6 Da.

Structure or Biochemical Description



Pharmacologic Class: Long acting glucagon-like peptide-1 (GLP-1) receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 022200 - Exenatide, Astra Zeneca, once weekly formulation
BLA 125469 - Dulaglutide, Eli Lilly, once weekly formulation
BLA 125431 - Albiglutide, GlaxoSmithKline, once weekly formulation
NDA 208471 - Lixisenatide, Sanofi Aventis, once daily formulation
NDA 22341 - Liraglutide, Novo Nordisk, once daily formulation

2.3 Drug Formulation

Semaglutide 1.34 mg/ml solution for injection is a clear and colorless solution filled in a 1.5 ml cartridge, assembled in a PDS290 pen-injector. The drug product contains disodium phosphate dihydrate, propylene glycol, phenol and water. HCl and NaOH are used as needed to adjust pH to7.4

Table 1. Composition of semaglutide 1.34 mg/ml solution for injection (Applicant's table)

Name of ingredients	Quantity per ml	Function	Reference to standards
Active substance			
Semaglutide	1.34 mg	Active drug substance	Novo Nordisk A/S
Excipients			
Disodium phosphate, dihydrate	1.42 mg	(b) (4)	USP/Ph. Eur.
Propylene glycol	14.0 mg		USP/JP/Ph. Eur.
Phenol	5.50 mg[a]		USP/JP/Ph. Eur.
Hydrochloric acid	q.s[b]	pH adjustment	USP/JP/Ph. Eur.
Sodium hydroxide	q.s[b]	pH adjustment	USP/JP/Ph. Eur.
Water for injections	(b) (4)	(b) (4)	USP/JP/Ph. Eur.

[a] (b) (4)

[b]To reach pH 7.4

2.4 Comments on Novel Excipients

There are no novel excipients. Semaglutide is (b) (4) composed of a (b) (4) (1.42 mg/mL disodium (b) (4) phosphate dihydrate) (b) (4), phenol (5.50 mg/mL) (b) (4), propylene glycol (14 mg/mL) (b) (4) and water for injection. These excipients are well known from other pharmaceuticals approved for chronic subcutaneous administration and are included in major pharmacopoeias (Ph. Eur., USP, JP). All excipients are further listed in the FDA's database for "Inactive Ingredient Search for Approved Drug Products" for subcutaneous use.

2.5 Comments on Impurities/Degradants of Concern

No impurities or degradants of concern have been identified.

2.6 Proposed Clinical Population and Dosing Regimen

Semaglutide is proposed to be used as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus (T2DM). The recommended clinical maintenance doses are 0.5 mg or 1 mg once-weekly. To minimize gastrointestinal side effects, dosing is initiated by titration. The starting dose is 0.25 mg once-weekly. After 4 weeks, the dose should be increased to 0.5 mg once-weekly. After at least 4 weeks with a dose of 0.5 mg once-weekly, the dose can be increased to 1 mg once-weekly.

2.7 Regulatory Background

The following is limited primarily to the nonclinical development.

The semaglutide pre-IND was opened in October 2007 with the submission of mouse and rat carcinogenicity study protocols for special protocol assessment by the ECAC (Executive Carcinogenicity Assessment Committee). The IND was opened in September 2008. In February 2012 the applicant requested a Type C meeting to discuss the adequacy of the carcinogenicity data and to seek the Division's concurrence on the no need of further mechanistic studies to assess risk of human medullary thyroid cancer. The Division responded that there was insufficient data to conclude that humans are not at risk for GLP-1 receptor agonist-induced thyroid C-cell tumors.
In March 2013, the applicant also sought FDA concurrence about the human relevance of the rat embryotoxicity findings. The Division acknowledged that the mechanistic data suggest a role for an effect on the visceral yolk sac; however, the data did not eliminate the possible involvement of other mechanisms and did not necessarily imply species (rat) specificity or a lack of human relevance. The Division suggested that evaluation of GLP-1 receptor expression in the monkey yolk sac/ chorioallantoic placenta might be of value in further assessing a rodent-primate difference in histiotrophic modulation mode of action for this drug.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacodynamics	Route	Study #
Cloned human receptor activation	In vitro	Alal15468-086
Rat pancreas/insulin secretion	In vitro	JStu050701
Rat/potency	SC	JStu051101
Diabetic mouse/potency	SC	DXG050301-0113
Diabetic mouse/glucose	SC	MmLa070620
Obese rats/body weight	SC	KiRa080803
Mouse/rat/brain access	SC	JHES151201
Obese mouse/appetite	SC	JCFJ151203
Pig/insulin	SC	BidR050301
Pig/food intake	SC	MmLa050901

Secondary Pharmacodynamics	Route	Study #
Glucagon receptor selectivity	In vitro	LEri16090-035
Broad Receptor Profile	In vitro	211228

Safety Pharmacology	Route	Study #
Irwin's test in the rat (CNS)	SC	206443
Pulmonary function in the rat	SC	206518
hERG ion channel patch clamp	Perfusion	206446
Rabbit Purkinje fibre*	Perfusion	206445
Cardiovascular function in primates	SC	206442
Renal function in the rat*	SC	206444

Distribution	Route	Study #
In vitro protein binding	In vitro	208380/213228
QWBA in the Wistar rat	SC and IV	206132
Tissue distribution and placenta transfer in the Wistar rat	SC	207267
QWBA in Lister Hooded pigmented rats	SC	210172
Excretion into milk in SD rat	SC	213315
Distribution in rat embryo		210018

Metabolism	Species	Route	Study #
[3H]Tyr-semaglutide: Metabolism in hepatocytes	Rat, monkey, human	In vitro	206642
[3H]Oct-semaglutide: Metabolism in hepatocytes	Rat, monkey, human	In vitro	214064
[3H]Oct-semaglutide: Metabolite profiling of plasma	Mouse CD1	SC	210299/210171
3H-Tyr-semaglutide: Metabolite profiling of plasma	Wistar rat	SC	207044
[3H]Oct-semaglutide: Metabolite profiling of plasma	Wistar rat	SC	207347
[3H]Oct-semaglutide: Metabolite profiling of urine, feces and bile	Wistar rat	SC	208008
[3H]Oct-semaglutide: Metabolite profiling of plasma after repeated dosing	SD rat	SC	213526
[3H]Oct-semaglutide: Metabolite profiling of plasma, urine and feces	Cyno monkey	SC	209041

Excretion	Species	Route	Study #
Normal and bile cannulated rat	Wistar	SC	207265
Monkey	Cyno	SC	208349

General toxicity	Route	Study #
CD1 Mouse, 13 weeks	SC	20663
SD rat, 4 wk comparative study (aged vs. fresh DP)	SC	209159
SD rat, 13 wk comparative study ((b) (4) DP)	SC	210195
SD rat, 26 wk + recovery	SC	207377
Cyno monkey, 52-wk + recovery	SC	207288
SD rat, 2-year carcinogenicity study	SC	207363
CD1 mouse, 2-year carcinogenicity study	SC	207362

Carcinogenicity	Route	Study #
SD rat, 2-year carcinogenicity study	SC	207363
CD1 mouse, 2-year carcinogenicity study	SC	207362
Activation of GLP-1R in rat thyroid C-cell line	In vitro	LBKN150301
CD1 mouse, calcitonin release after one dose	SC	208422/213448
SD rat, calcitonin release after 6 weeks dosing	SC	208456

Genotoxicity	Route	Study #
Ames with (b) (4) semaglutide	In vitro	210193
Chromosome aberrations in cultured HPBL with (b) (4) semaglutide	In vitro	210194
Micronucleus assay in SD rat with (b) (4) semaglutide	SC	206409

DART	Route	Study #
SD rat, preliminary combined fertility and EFD	SC	206616
SD rat, preliminary combined fertility and EFD	SC	207359
SD rat, combined fertility and EFD	SC	207361
NZW rabbit, EFD	SC	207358
Cyno monkey, EFD s	SC	208486
Cyno monkey, PPND	SC	210061
SD rat, juvenile toxicity study	SC	214479

Mechanistic studies on semaglutide-induced embryotoxicity

Study description	Element of hypothesis tested	Study ID
WEC study with semaglutide including comparison with *in vivo* development evaluated on GD11, 13 or 15.	Time dependency	4.2.3.7.3, 209213
WEC range finding study testing high concentrations (uM) of semaglutide	Time dependency	4.2.3.7.3, 210019
WEC main study with semaglutide + GLP-1R antagonist	Time dependency Presence of functional GLP-1R	4.2.3.7.3, 211213
WEC main study with native GLP-1 + GLP-1R antagonist	Time dependency Presence of functional GLP-1R	4.2.3.7.3, 211342
WEC main study with NNC 0113-0236 + GLP-1R antagonist	Time dependency Presence of functional GLP-1R	4.2.3.7.3, 211341
Prelim EFD study of NNC 0113-0236 by continuous infusion	Time dependency	4.2.3.7.3, 211411
Main EFD study of NNC 0113-0236 by continuous infusion (during specific periods of organogenesis)	Time dependency	4.2.3.7.3, 211412
In vivo study exploring: • cAMP levels in yolk sac from GD12 embryos • Pinocytotic activity in yolk sac membranes determined by Ussing chamber. • The developing bone structures on GD14 whole embryos by 3D digital imaging	Presence of functional GLP-1R Functional effect on yolk sac Time dependency	4.2.3.7.3, 212080
WEC study with tissue sampling for assessment of a) pinocytotic activity by TEM b) cAMP levels in *in vitro* semaglutide exposed GD13 yolk sac membranes.	Functional effect on yolk sac Presence of functional GLP-1R	4.2.3.7.3, 211489
Pinocytotic activity in *in vitro* semaglutide exposed GD13 yolk sac membranes determined by Ussing chamber.	Functional effect on yolk sac	4.2.3.7.3, 212241
In vivo distribution study (MARG)	Distribution of semaglutide to yolk sac membrane	4.2.3.7.3, 2100 18
GLP-1R localisation in yolk sac from untreated rats (pilot study)	Presence of functional GLP-1R	4.2.3.7.3, 211238
GLP-1R localisation in yolk sac from untreated rats (main study)	Presence of functional GLP-1R	4.2.3.7.3, 212301
Evaluation of presence of GLP-R in cynomolgus monkey yolk sac	Presence of functional GLP-1R in primates	4.2.3.7.3, JHES150306

WEC: Whole embryo culture

Reviewed previously under the IND

ADME	Species	Route	Study #
Absorption - rats	Wistar Han	SC	207264
Absorption - rabbits	NZW rabbit	SC	207005
Absorption - monkey	Cyno monkey	IV/SC	207205
Effect on hepatic levels of CYP450	SD rat	IV/SC	208501

General toxicity	Route	Study #
CD1 Mouse, single dose	SC	296524
CD1 Mouse, single dose	IV	207117
SD rat, single dose	SC	206523
SD rat, single dose	IV	207114
CD1 Mouse, 8-18 days DRF with dose escalation	SC	207029
CD1 Mouse, 2 week (+ 0-3 week dose escalation)	SC	206447
SD rat, 14-25 days DRF with dose escalation	SC	206062
SD rat, 2 week (+ 0-3 week dose escalation)	SC	206448
SD rat, 13 week (+ 0-2 week dose escalation)	SC	206662
Cyno monkey, 2 week	SC	206449
Cyno monkey, 13 week	SC	206450

Genotoxicity	Route	Study #
Ames with (b) (4) semaglutide	In vitro	206415
Chromosome aberrations in cultured HPBL with (b) (4) semaglutide	In vitro	206417

DART	Route	Study #
NZW rabbit, preliminary EFD	SC	206536
NZW rabbit, preliminary EFD	SC	207358
Cyno monkey, preliminary EFD	SC	208477
Juvenile toxicity DRF study	SC	214276

Local tolerance studies	Route	Study #
NZW rabbits	IV/IM	212073
Pig	SC	206664

3.2 Studies Not Reviewed

	Route	Study #
ApoE-KO mouse/aorta	SC	GURA150803
LDLr-KO mouse/aorta	SC	BiDR150901
Assay validation studies	In vitro	Several

3.3 Previous Reviews Referenced

Pharm/Tox reviews under IND 79754 in DARRTS.

4 Pharmacology

4.1 Primary Pharmacology

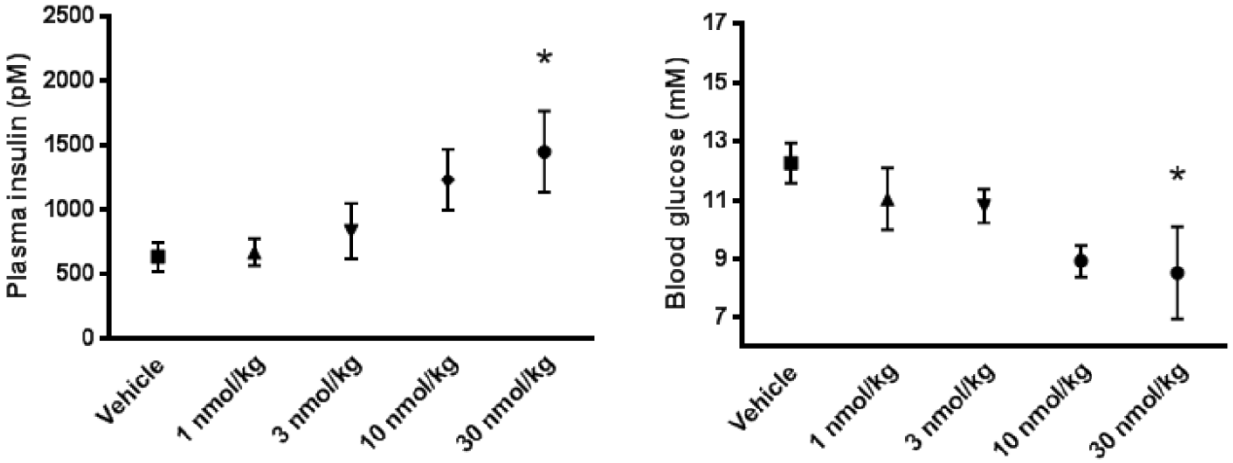
Pharmacology studies have been conducted *in vitro* and *in vivo* in diabetic mice, normal and obese rats and normal pigs and minipigs.

Semaglutide activated the human GLP-1 receptor measured as cAMP release from baby hamster kidney (BHK) cells expressing the cloned human GLP-1 receptor with a potency comparable to liraglutide and approximately 8-fold lower than native GLP-1. The receptor binding affinity of semaglutide (NNC 011300217) is comparable to liraglutide and GLP-1 in the presence of 0.005% albumin, but it decreased more than for liraglutide in the presence of 2% albumin compared with GLP-1. The lower affinity at the higher albumin concentration is explained by a stronger albumin binding of semaglutide than liraglutide. In isolated perfused rat pancreas semaglutide stimulated insulin secretion with an EC_{50} of approximately 13 nM (data not shown).

Analogue	Potency (nM)	Binding affinity (nM) with albumin 0.005%	2%
GLP-1(7-37)OH	0.019 ± 0.013 (n=7)	0.44 ± 0.059 (n=11)	0.19 ± 0.015 (n=11)
liraglutide	0.15 ± 0.037 (n=3)	0.25 ± 0.26 (n=4)	5.2 ± 0.60 (n=4)
NNC 0113-0217	0.16 ± 0.069 (n=7)	0.78 ± 0.12 (n=12)	91 ± 88 (n=12)

Increase in glucose-stimulated plasma insulin concentration, decrease in blood glucose, and decrease in body weight gain were observed in Wistar rats and diabetic db/db mice after a single subcutaneous administration. Semaglutide (NNC 0113-0217) was more potent than liraglutide (NNC 0090-1170) in lowering blood glucose level (ED_{50}: 0.30 and 6.9 nmol/kg, respectively, at 6h post-dose); however, reduction in blood glucose was similar between semaglutide and liraglutide. In C57BL mice, food intake was lower with semaglutide than with liraglutide.

Figure 1. Plasma insulin and blood glucose in Wistar rats (Applicant's figure)



Plasma insulin 10 min after glucose bolus injection and 190 min after compound administration.
Blood glucose 60 min after glucose bolus injection and 240 min after compound administration.

Figure 2. Non-linear curve fits for estimating ED_{50} of 6h blood glucose lowering (Applicant's figure)

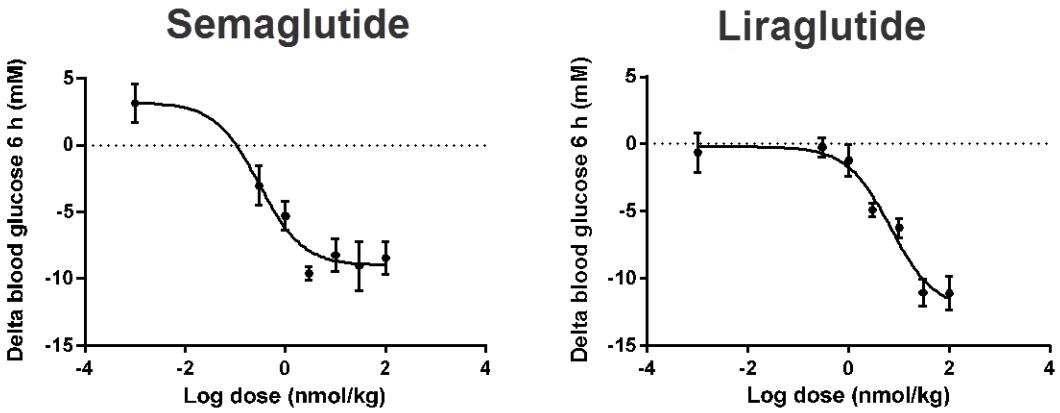


Figure 3. Delta blood glucose in diabetic db/db mice (Applicant's figure)

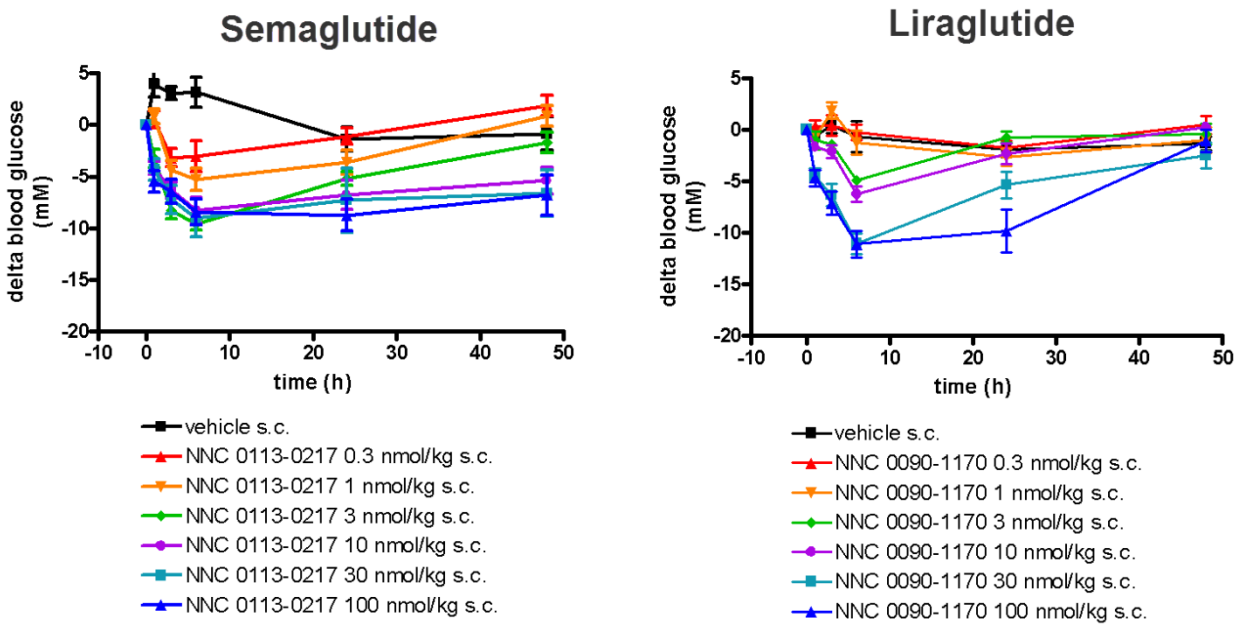


Figure 4. Body weight change at 48h post-dose in diabetic db/db mice (Applicant's figure)

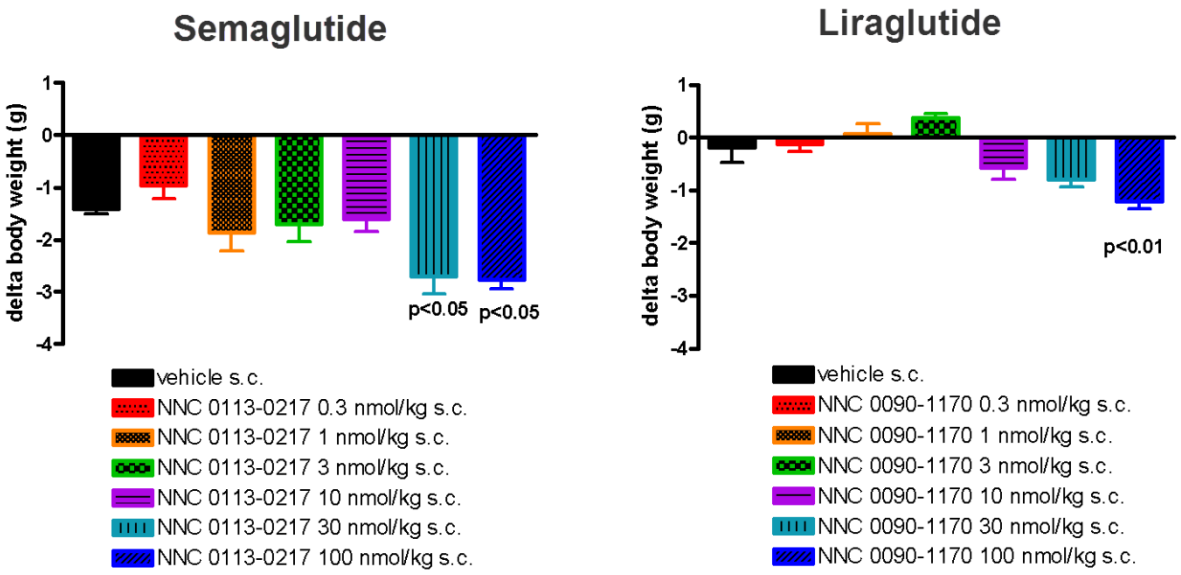
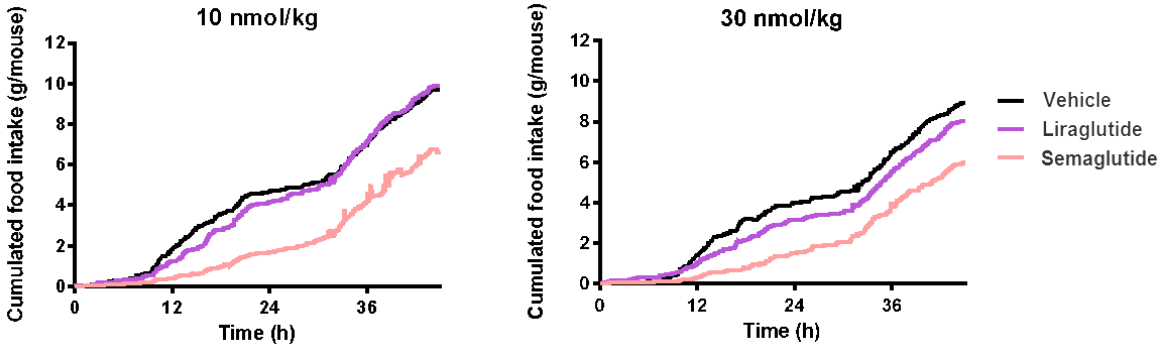


Figure 5. Cumulative food intake in C57Bl/6 mice (Applicant's figure)



Following repeated doses of semaglutide, the following effects were observed:

- Lower blood glucose and HbA1c, increased insulin secretion and beta-cell mass in diabetic db/db mice treated for 4 weeks.
- Decreased body weight and selectively reduced chocolate intake in diet-induced obese (DIO) rats fed with chow and chocolate ad libitum for approximately 9 months prior to initiation of treatment (77 day).
- Sustained decrease in food intake and increased insulin secretion for up to 7 days after the last dose in minipigs.
- Increased mRNA levels of the satiety peptide cocaine- and amphetamine-regulated transcript (CART) and decrease in the hunger signals neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the hypothalamic arcuate nucleus (ARC) of diet induced obese mice administered semaglutide for 18 days. No effects were observed in the paraventricular hypothalamic nucleus (PVN; data not shown).
- Semaglutide activated hypothalamic POMC/CART neurons, and inhibited NPY/AGRP neurons in mice. Semaglutide was found in select circumventricular organs {(the area postrema (AP), the median eminence (ME), the subfornical organ (SFO), the organum vasculosum of lamina terminalis (OVLT), and the choroid plexus (ChP)}, in the arcuate nucleus (ARC), the lateral septal nucleus (LS), the septofimbral nucleus (SFi), the nucleus tractus solitarus (NTS) and the dorsal motor nucleus of vagus (10N). The detection of the semaglutide in these brain regions, except from the ChP and the fenestrated capillaries in ME, was dependent on GLP-1R as the signal was absent in GLP-1R-/- mice.

Figure 6. HbA1c in db/db mice during once-daily SC dosing of semaglutide (Applicant's figure)

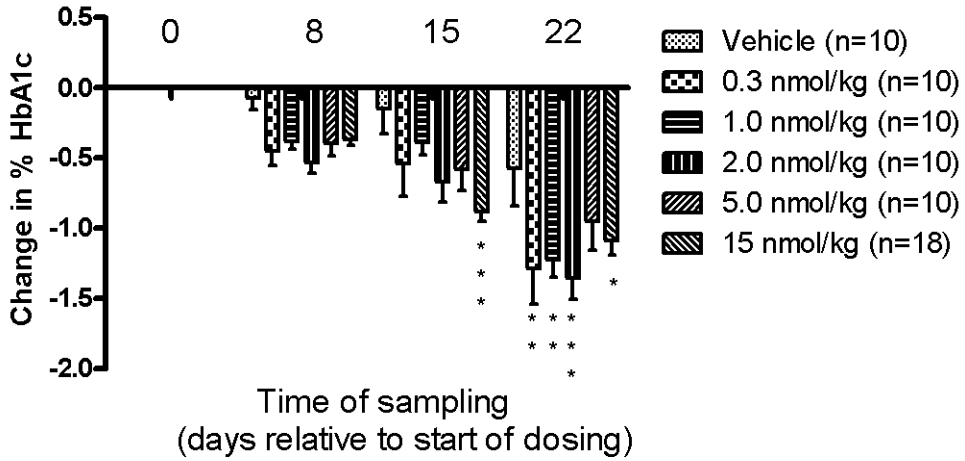


Figure 7. Beta-cell islet number and glucose-induced insulin secretion in diabetic db/db mice after four weeks semaglutide treatment (Applicant's figure)

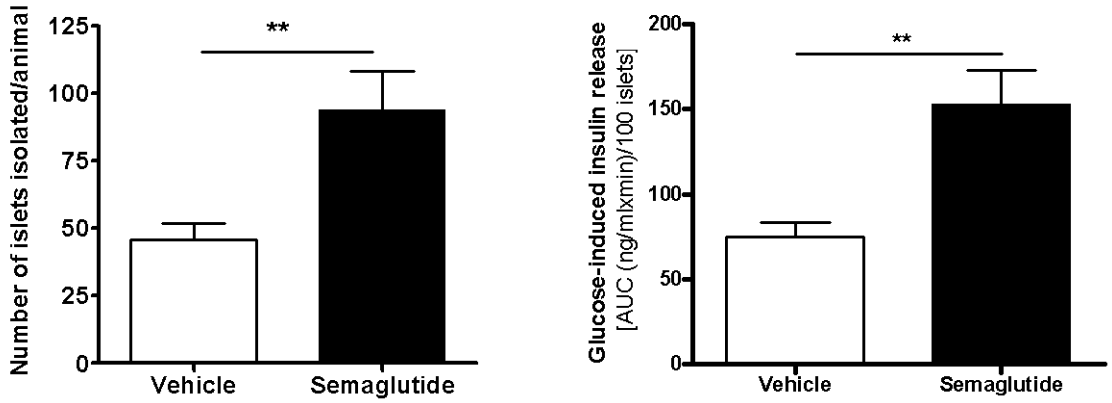


Figure 8. Body weight in DIO rats after 77 days semaglutide treatment (Applicant's figure)

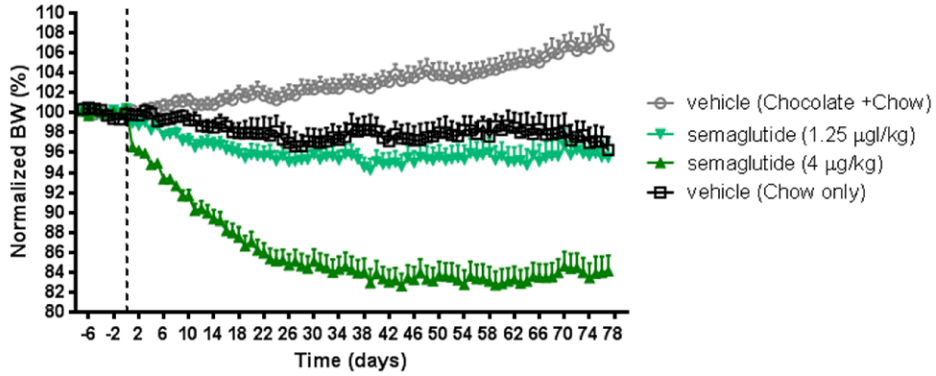


Figure 9. Chow (C) and chocolate (CH) intake in rats after 77 day of dosing (Applicant's figure)

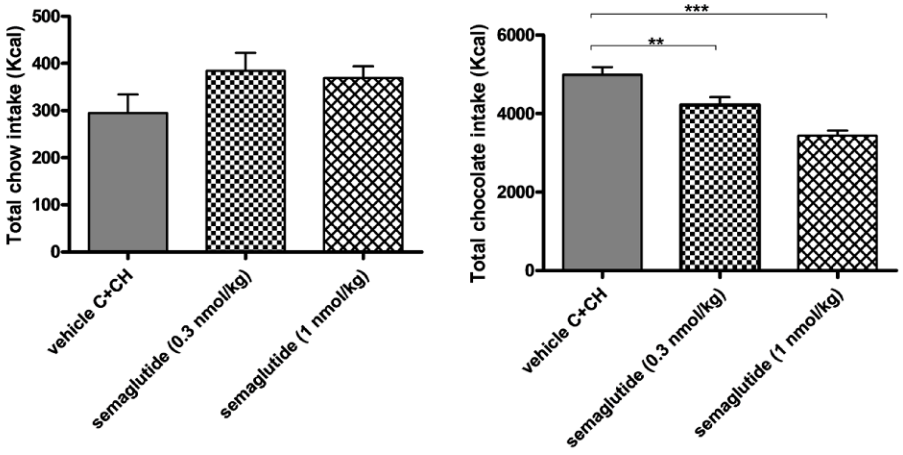


Figure 10. Insulin secretion in clamped minipigs (Applicant's figure)

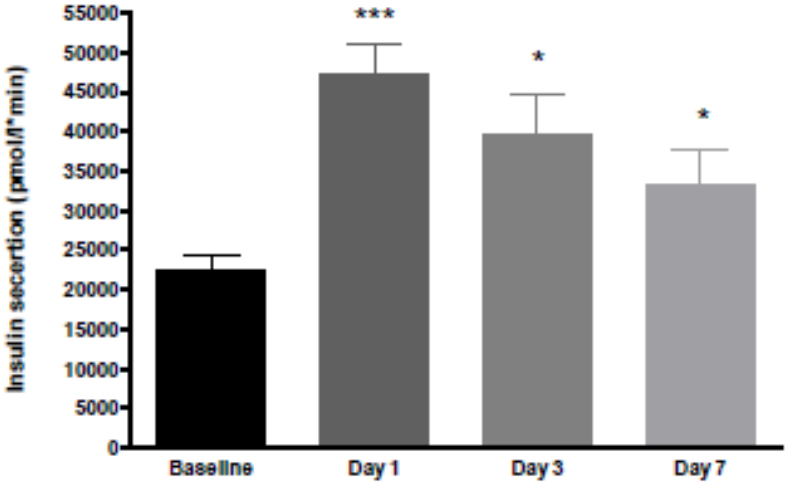


Figure 11. Food intake in minipigs after subcutaneous dosing of semaglutide (Applicant's figure)

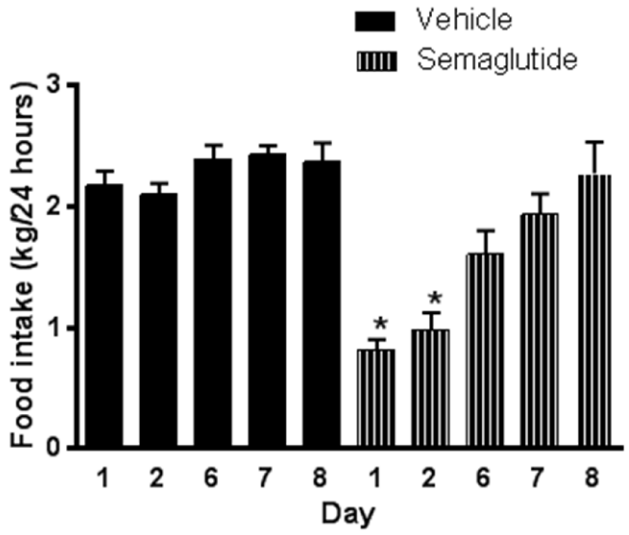


Table 2. Effect of semaglutide on AgRP, NPY, CART, and POMC mRNA in the arcuate nucleus (Applicant's figure)

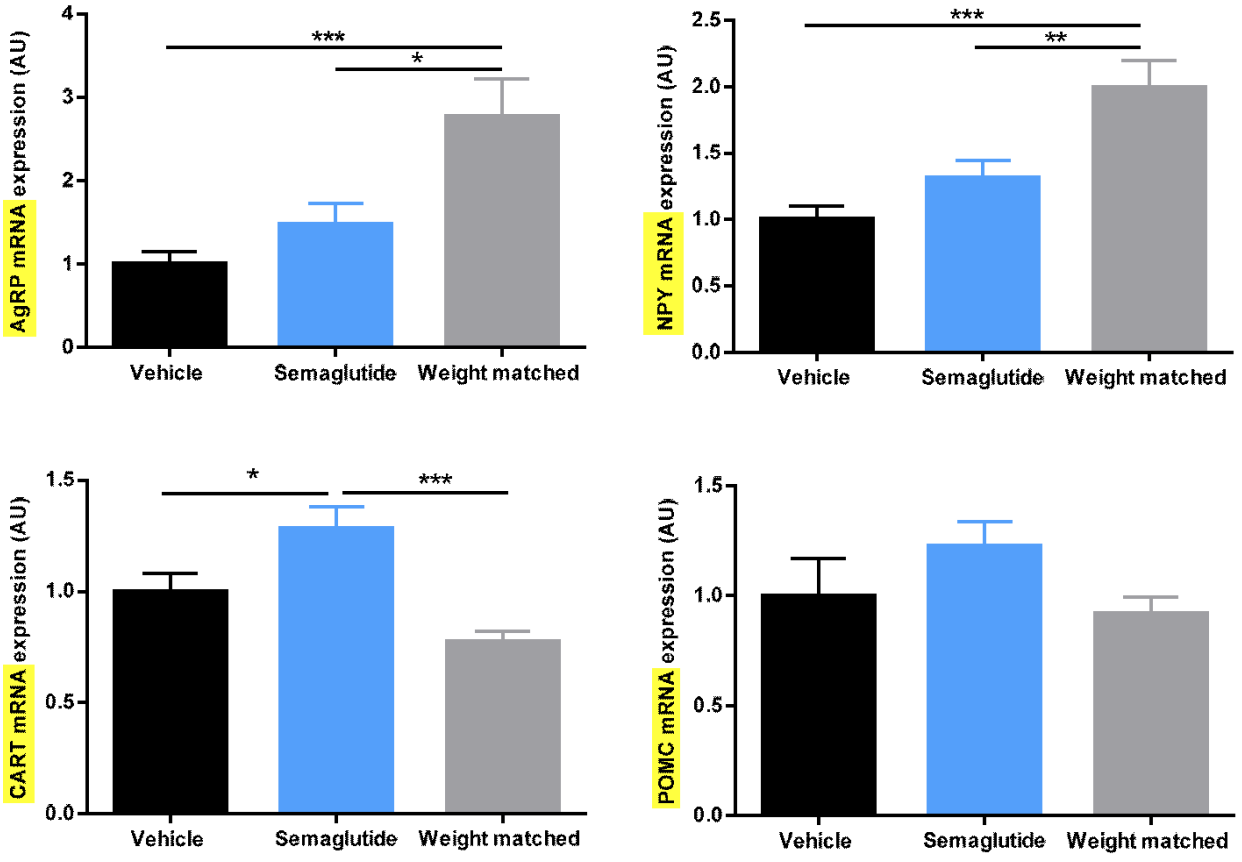
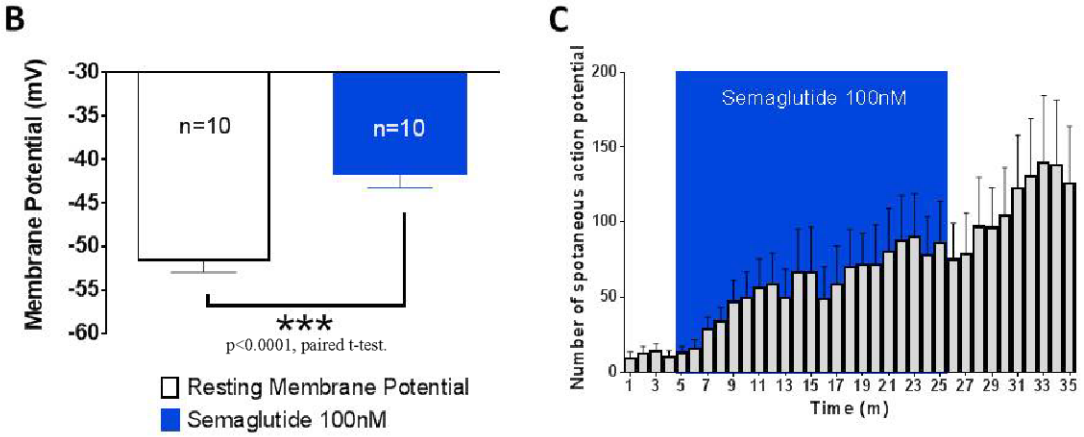
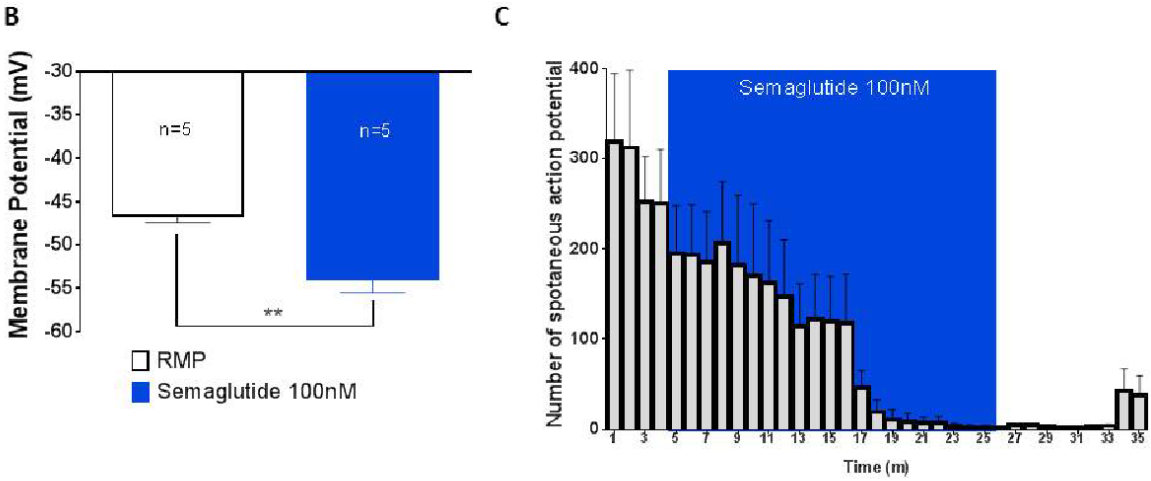


Figure 12. Effect of semaglutide on the neuronal activity of POMC neurons (Applicant's figure)



(B) Bar graph showing changes in the membrane potential following application of semaglutide.
(C) Histogram showing the effects of 100 nM semaglutide on the number of spontaneous action potentials in POMC neurons.

Figure 13. Effect of semaglutide on the neuronal activity of NPY/AgRP neurons (Applicant's figure)



(B) Summary graph showing changes in membrane potential in NPY-GFP neurons after semaglutide application.
(C) Histograms showing the effects of semaglutide in the firing rate of NPY-GFP neurons.
Results are shown as mean ± SEM, 5 cells out of 12 neurons from 4 animals.
RMP= Resting Membrane Potential p=0.0068, paired t-test.

Table 3. VivoTag750-semaglutide signal in the brain (Applicant's table)

Region	Brain area		Signal in C57/Bl6, 6 hours	Signal in GLP-1R-/-, 6 hours	Signal in C57/Bl6, 4 days
Forebrain	PVN		-	-	+
	Arc		+	-	+
	ME	ZE	+	+	+
		ZI	+	-	+
	SFO		+	-	+
	ChP		+	+	+
	OVLT		+	-	+
	SO		-	-	+
	SOD		+	-	+
	LS		+	-	+
	SFi		+	-	+
	PO		+	-	+
Brain stem	AP		+	-	+
	NTS		+	-	+
	10N		+	-	+

Presence (+) or absence (-) of fluorescent signal in the mouse brain following acute or repeated peripheral injections with VivoTag750-semaglutide. Black indicates if the signal is only seen in mice with functional GLP-1R expression.

4.2 Secondary Pharmacology

Semaglutide does not bind the human glucagon receptor. In a Broad Receptor Profile assay, semaglutide showed an approximately 72% negative inhibition of binding/activity at the thyroid hormone receptor. Negative inhibition (augmentation of ligand binding) is generally considered non-specific, and is rarely of pharmacological consequence. The repeat dose toxicity studies showed no indication of thyroidtoxicosis. A result of greater than 50% inhibition or stimulation was not observed for any other receptor, channel, or transporter.

4.3 Safety Pharmacology

Safety pharmacology studies were conducted *in vitro* and *in vivo* to investigate the effect of semaglutide on central nervous system, cardiovascular and respiratory system, and renal function.

CNS

Abnormal gait (walking on toes), decreased touch response, passivity, increased urination, dirty muzzle, lethargy and piloerection were observed in rats administered 0.095 mg/kg at 0-8 hours after administration (~6X the estimated clinical starting dose, based on Cmax). The increased urination and passivity were still present 24 hours post-

dose. The NOAEL was 0.022 mg/kg (~2X the estimated clinical starting dose, based on Cmax).

Table 4. Irwin test in the rat (Applicant's table)

Observation	DC = Dispersion in cage ↓LA = Decreased locomotor activity A = Apathy T = Tremor ↓AL = Decreased alertness ↓SR = Decreased startle response AG(T) = Abnormal gait (walking on toes) DM = Dirty muzzle	AG(Sp) = Abnormal gait (spread) AC(Hu) = Abnormal carriage (Hunched) ↓TR = Decreased touch response ↓F = Decreased fearfulness ↓PR = Decreased pinna reflex ↓CR = Decreased corneal reflex L = Lethargy	C = Catalepsy PA = Passivity ↓BT = Decreased body tone PT = Ptosis CHR = Chromodacryorrhoea P = Piloerection L = Lacrimation ↑U = Increased urination ↓G = Decreased grooming NAD = No Abnormalities Detected

Treatment	**Observation Time points post-dose**			
	2 h	**4 h**	**8 h**	**24 h**
Vehicle 1 mL/kg, s.c.	AG(T)-2, PA-3	PA-3	PA-5	PA-6
NNC 0113-0217 0.001 mg/kg, s.c.	PA-4	NAD	PA-4	PA-3
NNC 0113-0217 0.022 mg/kg, s.c.	PA-3	PA-4	PA-2	PA-3
NNC 0113-0217 0.095 mg/kg, s.c.	AG(T)-3, PA-6	↓LA, AG(T)-2, ↓TR-6, PA-5, ↑U-5	↓LA, AG(T)-2, ↓TR-5, PA-6, DM-2, L-6	P-2, PA-6
Chlorpromazine 2 mg/kg, i.v.	DC, ↓LA, A-6, T-3, ↓AL-6, ↓SR, AC(Hu)-5, AG(Sp)-6, P-6, ↓TR-6, ↓F-6, ↓PR-6, ↓CR-6, C-6, PA-6, ↓BT-6, L-6, CHR-3, PT-6, ↑U-6, ↓G-6, DM-6	DC, A-2, T-4, ↓AL-2, AC(Hu)-3, AG(T)-3, PA-2	PA-3	NAD

All values represent the number of rats showing the observation at a respective time point.
The vehicle for NNC 0113-0217 was disodium phosphate dihydrate (1.42 mg/mL), Propylene glycol (14 mg/mL), Phenol (5.5 mg/mL), WFI (b) (4), pH 7.40.
n = 6 animals per group.

Table 5. Pharmacokinetic parameters for male rats following single SC dose (Applicant's table)

Dose (mg/kg)	C_{max} (nmol/L)	t_{max} (h)	$AUC_{(0-10h)}$ (h*nmol/L)	AUC_{last} (h*nmol/L)	AUC (h*nmol/L)	$AUC_{\%extrap}$	λ_z (1/h)	$t_{1/2}$ (h)
0.001	0.726	10	5.94	5.94	NC	NC	NC	NC
0.022	14.0	6	101	195	234	17	0.0805	8.6
0.095	47.2	6	347	757	NC	28	0.0549	13

Renal function

Increased urine volume and sodium, potassium, and chloride concentrations were observed at ≥0.023 mg/kg between 0 and 8 hours post dose (<1X MRHD, estimated on AUC from the CNS study). Diuresis is a known pharmacological effect of GLP-1R agonists in the rat (Moreno C 2002; Larsen PJ 2001).

Table 6. Effect on renal function in rats at 0-8h (Applicant's table)

Treatment	Sodium (mmol/kg)	Potassium (mmol/kg)	Chloride (mmol/kg)	Urine Volume (mL)	pH
Vehicle (1 mL/kg s.c.)	1.45 ± 0.10	1.33 ± 0.07	1.05 ± 0.09	2.33 ± 0.25	7.26 ± 0.16
NNC 0113-0217 (0.005 mg/kg s.c.)	2.61 ± 0.22	1.77 ± 0.16 *	2.07 ± 0.21	2.85 ± 0.35	7.40 ± 0.14
NNC 0113-0217 (0.023 mg/kg s.c.)	5.11 ± 0.32 ††	2.12 ± 0.10 **	3.41 ± 0.18 †††	6.26 ± 0.31 **	8.27 ± 0.06 †††
NNC 0113-0217 (0.089 mg/kg s.c.)	6.56 ± 0.24 †††	2.29 ± 0.06 **	3.94 ± 0.10 †††	8.40 ± 0.31 **	8.21 ± 0.04 ††
Furosemide (5 mg/kg i.v.)	4.26 ± 0.17 +	1.69 ± 0.12 +	4.00 ± 0.17 +	5.51 ± 0.27 +	6.84 ± 0.17

n = 8 rats per group.
Data are expressed as mean ± s.e. mean.
* P < 0.05, ** P < 0.01 compared to the vehicle (ANOVA and Dunnett's test).
†† P < 0.01, ††† P < 0.001 compared to the vehicle (Kruskal-Wallis and Dunn's test).
+ P < 0.05 compared to the vehicle (unpaired, Student's t-test).

Table 7. Effect on renal function in male SD rats at 8-24h (Applicant's table)

Treatment	Sodium (mmol/kg)	Potassium (mmol/kg)	Chloride (mmol/kg)	Urine Volume (mL)	pH
Vehicle (1 mL/kg s.c.)	3.08± 0.39	3.12 ± 0.28	2.84 ± 0.28	2.64 ± 0.47	7.43 ± 0.16
NNC 0113-0217 (0.005 mg/kg s.c.)	2.69 ± 0.39	3.00 ± 0.44	2.66 ± 0.40	2.05 ± 0.34	7.09 ± 0.18
NNC 0113-0217 (0.023 mg/kg s.c.)	1.45 ± 0.25 **	1.54 ± 0.27 †	1.28 ± 0.25 †	1.28 ± 0.22 *	6.93 ± 0.19
NNC 0113-0217 (0.089 mg/kg s.c.)	1.19 ± 0.08 **	1.52 ± 0.11 †	0.62 ± 0.06 †††	1.43 ± 0.12 *	6.51 ± 0.14 **
Furosemide (5 mg/kg i.v.)	1.77 ± 0.51	2.32 ± 0.70 #	1.43 ± 0.38 +	1.48 ± 0.46	7.13 ± 0.22 (7)

n = 8 rats per group unless otherwise indicated in parentheses.
Data are expressed as mean ± s.e. mean.
* P < 0.05, ** P < 0.01 compared to the vehicle (ANOVA and Dunnett's test).
# P < 0.05, compared to the vehicle (Mann-Whitney U-test).
† P < 0.05, ††† P < 0.001 compared to the vehicle (Kruskal-Wallis and Dunn's test).
+ P < 0.05 compared to the vehicle (unpaired, Student's t-test).

Cardiovascular system

Semaglutide did not inhibit hERG tail current and had no effect on cardiac action potential parameters in cardiac Purkinje fibers isolated from New Zealand white rabbits at concentration up to 7.8 uM and 8.2 uM (corresponding to a 242- and 255-fold above the maximal plasma concentration of 32.2nM, respectively).

There were no treatment related ECG changes in monkeys following single doses up to 0.5 mg/kg or 440 nM (14X MRHD). In the 52-week study with twice-weekly administration, a chronic left bundle-branch-block occurred in one high-dose female at an exposure 27-fold above the exposure at the maximum recommended human dose, based on AUC. Cardiac bundle-branch-blocks are occasional findings in monkeys and in humans and are, in most cases, a consequence of other underlying cardiac diseases (Bristow JD,1965; Francia P, 2007). However, a relation to treatment could not be excluded. NOAEL was set at the mid dose, 5-fold above the clinical exposure.

Respiratory system

No treatment related effect on the respiratory system was noted in male SD rats up to 0.084 mg/kg (1X MRHD, estimated on AUC from the CNS study).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The pharmacokinetics of semaglutide was evaluated in CD-1 mouse, Sprague Dawley rat, New Zealand White rabbit and Cynomolgus monkey. The distribution, metabolism and excretion of semaglutide was evaluated *in vitro* and *in vivo*, with two different radiolabeled tracers; one with tritium positioned in tyrosine in the peptide backbone ([^{3}H]Tyr-semaglutide) and another with tritium positioned in the octadecanedioic acid side chain ([^{3}H]Oct-semaglutide).

Absorption

Following subcutaneous administration of semaglutide, C_{max} was observed after 3-4 hours in mice and rats, and between 12 and 24 hours in monkeys and minipigs. Semaglutide was well absorbed from the subcutaneous injection site, with a bioavailability of 86% in monkeys. Mean terminal half-life was between 7 and 54 hours, in the order mouse < rat < monkey. The binding to plasma proteins was high in all species (>99%) and albumin was shown to be the primary binding site. In the monkey, the volume of distribution was 0.2 L/kg which corresponds to the volume of extracellular water, indicating that semaglutide distributes to plasma and peripheral tissues to the same extent as albumin.

Table 8. Interspecies comparison of dose-normalized (1 mg/kg) pharmacokinetics for repeated subcutaneous administration of semaglutide (Applicant's table)

Species	s.c. administration (steady-state)					
	C_{max} (nmol/L)	t_{max} (h)	$AUC_{(tau)}$ (h×nmol/L)	Tau (hours)	C_{avg} (nmol/L)	$t_{½}$ (h)
Mouse	1040	4	12500	24	522	7.5
Rat	1340	3	23700	24	927	12
Minipig	1950	24	42300	72	1760	-
Monkey	2860	12	150000	72	2080	54
Human	3000	36	437000	168	2600	149

Data in mouse, rat, minipig and monkey are presented as arithmetic means, except t_{max} and $t_{½}$ which are presented as median and harmonic mean, respectively. Human data are presented as geometric mean except t_{max} and $t_{½}$ which are median and geometric mean. Data from studies in mouse: 206447, 206663, 207362; rat: 206448, 206662, 207377, 207363; monkey: 206449, 206450, 207288; minipig: 208404; human: trial 3635.

Tissue distribution

Protein binding

Determination of semaglutide plasma protein binding was attempted using equilibrium dialysis and ultracentrifugation. However, the protein binding could not be assessed using these techniques, likely due to non-specific binding of semaglutide to the test system surfaces.

In vitro protein binding was subsequently investigated in humans and animals using validated assays based on surface plasmon resonance technology. Two studies were conducted using different assay conditions. The binding assays were performed at 37°C with diluted plasma samples from mice, rats, rabbits, monkeys and humans which were passed over immobilized semaglutide. A kinetic analysis based on a 1:1 binding model was performed to determine the dissociation constant, K_D, and percentage fraction unbound (f_u) of semaglutide in each pool of plasma.

The variation of the f_u between species in the two assays was approximately 2-3 fold, however in both assays, the fu, was < 1%, corresponding to a plasma protein binding of more than 99% for all species investigated.

Binding studies with rat and human serum albumin indicated that albumin was the primary protein for binding of semaglutide in plasma.

Table 9. In vitro plasma protein binding (Applicant's table)

	Study ID: 208380							Study ID: 213228						
Species	**Sex**	**% f_u Mean ±SD**			**Plasma albumin K_D (µM)**			**Sex**	**% f_u Mean ±SD**			**Plasma albumin K_D (µM)**		
Mouse (CD-1)	M	0.56	±	0.23	2.21	±	0.93	M/F	0.28	±	0.05	1.07	±	0.19
	F	0.36	±	0.16	1.36	±	0.60	-	-	-	-	-	-	-
Rat (Wistar)	M	0.65	±	0.23	2.86	±	1.03	-	-	-	-	-	-	-
	F	0.60	±	0.16	2.77	±	0.77	-	-	-	-	-	-	-
Rat (Sprague Dawley)	M	0.59	±	0.25	2.42	±	1.05	M/F	0.19	±	0.04	0.80	±	0.14
	F	0.67	±	0.12	3.14	±	0.55	-		-	-		-	-
Rabbit (NZ White)	F	0.036	±	0.011	0.14	±	0.05	F	0.07	±	0.01	0.27	±	0.05
Minipig (Göttingen)	F	0.22	±	0.19	1.37	±	1.19	-	-	-	-	-	-	-
Monkey (cynomolgus)	M	0.19	±	0.05	0.94	±	0.26	M/F	0.46	±	0.09	2.19	±	0.45
	F	0.10	±	0.02	0.49	±	0.08	-	-	-	-	-	-	-
Human	M	0.18	±	0.05	1.06	±	0.30	M/F	0.36	±	0.05	2.10	±	0.31
	F	0.19	±	0.04	1.08	±	0.21		-	-		-	-	-
HSA	-	-	-	-	-	-	-	-	0.17	±	0.02	1.09	±	0.10
HSA (fatty acid free)	-	-	-	-	-	-	-	-	0.04	±	0.002	0.27	±	0.02

Additional Information: In general, the plasma protein binding was comparable between species and the observed difference between the assays was 2-3 fold. The f_u was <1% for all species investigated

Distribution in the rat by quantitative whole body autoradiography

- In partially pigmented Lister-Hooded rats, distribution of [^{3}H]Oct-semaglutide was detected in all tissues with the exception of the lens of the eye. Concentrations of radioactivity in nearly all tissues were less than those in plasma and blood, except in the injection site (5 to115 fold above the plasma level) and bile ducts (1-2 fold above the plasma levels) during the first 24 hours post-dose. Highest levels of radioactivity were noted in the tooth pulp, renal cortex (inner and outer), adrenal medulla, and preputial gland. On day 28 post-dose, radioactivity was 2 to 20 fold above the plasma levels in the injection site, intestinal mucosa and in the fat (peri-renal >> brown and white fat). The amount of semaglutide related material in the melanin-containing tissues in the pigmented rat was similar to the levels measured in the albino rats (uveal tract/retina and skin), suggesting that semaglutide does not bind to melanin.

- In the Wistar rat, distribution of [^{3}H]Oct-semaglutide was similar between male and pregnant female rats following a single subcutaneous administration (administered on gestation day 18 in females). Semaglutide was slowly absorbed from the subcutaneous dose site, with peak levels occurring between 6 and 24 hours after dosing. Plasma contained the most abundant level of radioactivity at all time points followed by lung, tooth pulp, kidney (cortex and medulla), adrenal medulla, and the uterus of pregnant animals. Although the placenta contained relatively high levels of radioactivity, concentrations in the fetal tissues were generally very low, <4% of the dam's plasma radioactivity at 24 hours post dose. In the fetal tissues, radioactivity was found in the brain, heart, liver, lung and skin.

- In both rat strains, distribution to the brain and spinal cord was low at all sampling times.

Table 10. Tissue:plasma radioactivity concentration ratio in pigmented rats (Applicant's table)

	Tissue : plasma radioactivity concentration ratios								
Animal number and sex	363M	365M	361M	362M	366M	367M	368M	369M	370M
Sample / Sampling time	1 hour	4 hours	8 hours	12 hours	1 day	3 days	7 days	14 days	28 days
Plasma*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Adrenal cortex	0.228	0.150	0.126	0.024	0.061	0.105	0.515	2.63	NC
Adrenal medulla	0.637	0.314	0.543	0.389	0.375	0.184	0.212	0.403	NC
Aortic wall	0.356	0.321	0.263	0.228	0.261	0.282	NC	NC	NC
Bile ducts	1.01	2.13[2]	1.44[2]	0.824	1.42	1.92	NC	NC	NC
Blood	0.829	1.07	1.07	0.907	0.853	0.543	0.102	NC	NC
Bone marrow	0.211	0.146	0.179	0.150	0.121	0.165	0.125	NC	NC
Brain	0.014	0.013	0.011	0.009	0.011	0.025	0.068	NC	NC
Brown fat	0.555[1]	0.146[1]	0.041[1]	0.080	0.301	0.080	0.210	0.309	2.19
Bulbo-urethral gland	0.843	0.405	0.617	0.625	0.511	0.298	0.139	NC	NC
Choroid plexus	0.189	0.065	0.058	0.065	0.066	0.114	NC	NC	NC
Dose site	115[2]	81.6[2]	38.5[2]	20.2[2]	5.75[2]	0.572	1.40	9.74	23.1
Epididymis	0.043	0.010	0.026	0.270	0.087	0.335	0.147	0.477	NC
Exorbital lachrymal gland	0.131	0.108	0.115	0.117	0.135	0.155	0.101	NC	NC
Harderian gland	0.033	0.054	0.042	0.044	0.090	0.201	0.178	NC	NC
Intra-orbital lachrymal gland	0.162	0.147	0.124	0.107	0.127	0.147	0.093	NC	NC
Lens	NC	NC	NC	NC	NC	NC	NC	NC	NC
Liver	0.181	0.266	0.283	0.261	0.349	0.411	0.159	NC	NC
Lung	0.606	0.648	0.737	0.529	0.660	0.376	0.108	NC	NC
Mandibular lymph nodes	0.069	0.103	0.084	0.294	0.233	0.219	0.069	NC	NC
Meninges	0.139	0.108	0.083	0.065	0.084	0.104	NC	NC	NC
Muscle	0.025	0.027	0.037	0.053	0.058	0.054	0.067	NC	NC
Myocardium	0.127	0.205	0.275	0.256	0.180	0.158	0.084	NC	NC
Nasal mucosa	0.313	0.092	0.124	0.108	0.081	0.111	0.068	NC	NC
Pancreas	0.185	0.218	0.176	0.184	0.145	0.124	0.100	NC	NC
Periodontal membrane	0.086	0.286	0.330	0.247	0.414	0.211	NC	NC	NC
Peri-renal fat	NC	0.005	0.003	0.004	0.009	NC	0.080	0.538	21.0
Pineal body	0.279	0.128	0.195	0.185	0.129	0.111	0.113	NC	NC
Pituitary	0.152	0.153	0.130	0.193	0.147	0.174	0.103	NC	NC
Preputial gland	0.822	0.523	0.162	0.527	0.482	0.368	0.101	2.29	NC
Prostate	0.052	0.023	0.098	0.054	0.048	0.112	0.095	NC	NC
Renal cortex inner	0.269	0.362	0.319	0.269	0.916	3.81	0.264	NC	NC
Renal cortex outer	0.313	0.570	0.458	0.495	0.764	1.09	0.166	NC	NC
Renal medulla	0.385	0.581	0.362	0.281	0.301	0.238	0.094	NC	NC
Salivary glands	0.174	0.132	0.083	0.128	0.114	0.162	0.101	NC	NC
Seminal vesicles	NC	0.034	0.021	0.039	0.007	0.016	0.306	NC	NC
Skin (Non-pigmented)	0.028	0.056	0.062	0.047	0.086	0.127	0.078	NC	NC
Skin (Pigmented)	0.125	0.074	0.124	0.180	0.223	0.329	0.088	NC	NC
Spinal cord	0.008	0.011	0.011	0.006	0.007	0.021	0.067	NC	NC
Spleen	0.106	0.119	0.124	0.105	0.116	0.082	0.089	NC	NC
Testis	0.027	0.031	0.098	0.093	0.085	0.126	0.107	NC	NC
Thymus	0.012	0.049	0.044	0.046	0.057	0.111	0.095	NC	NC
Thyroid	0.279	0.351	0.195	0.118	0.167	0.122	0.113	NC	NC
Tongue	0.102	0.123	0.095	0.162	0.261	0.278	0.077	NC	NC
Tooth pulp	NS	0.953	0.803	0.484	0.924	0.991	NC	NC	NC
Urinary bladder wall	0.045	0.152	0.110	0.458	0.639	0.535	0.270	NC	NC
Uveal tract/retina	0.093	0.132	0.189	0.213	0.245	0.261	NC	NC	NC
White fat	NC	0.004	NC	0.005	0.004	NC	NC	NC	2.47
Oesophageal wall	0.046	0.090	0.140	0.122	0.169	0.389	NC	NC	NC
Stomach mucosa (fundus)	0.221	0.147	0.194	0.280	0.128	0.085	0.086	NC	NC
Stomach mucosa (non-fundic)	0.062	0.079	0.040	0.228	0.485	0.111	NC	NC	NC
Small intestine mucosa	0.074	0.113	0.110	0.098	0.214	0.126	0.141	0.548	4.96
Caecum mucosa	0.054	0.070	0.126	0.015	0.163	0.318[3]	0.181	0.612	11.3
Large intestine mucosa	0.077	0.151	0.150	0.200	0.106	0.227	1.39	0.507	12.6
Rectum mucosa	0.096	0.116	0.138	0.199	0.151	0.263	0.100	NC	NC

* – Radioactivity determined by liquid scintillation counting (LOD Plasma = 0.020 ng eq./g),
NC – Not calculable (tissue concentration below lower limit of quantification)
NS – Not sectioned
[1] - Measurement affected by high level of radioactivity in the dose site
[2] - Measurement above the upper limit of quantification

Table 11. Tissue:plasma radioactivity concentration ratio in male Wistar rats (Applicant's table)

		Tissue : plasma concentration ratios			
Tissue type	Tissue Sampling time	3 hours	6 hours	24 hours	72 hours
Vascular/ lymphatic	Plasma[1]	1.00	1.00	1.00	1.00
	Blood	0.575	0.663	0.455	0.149
	Mandibular lymph nodes	0.036	0.037	0.063	0.099
Metabolic/ excretory	Kidney cortex	0.311	0.387	0.683	0.395
	Kidney medulla	0.196	0.219	0.333	0.062
	Liver	0.095	0.117	0.174	0.126
CNS	Brain	0.006	0.004	0.005	NC
	Choroid plexus	0.167	0.340	0.168	0.080
	Meninges	0.067	0.068	0.097	NC
	Pineal body	0.115	0.090	0.063	0.050
	Spinal cord	NC	0.003	0.002	NC
Endocrine	Adrenal cortex	0.093	0.085	0.057	0.107
	Adrenal medulla	0.241	0.259	0.192	0.108
	Pituitary	0.083	0.085	0.110	0.031
	Thymus	0.014	0.022	0.031	0.038
	Thyroid	0.129	0.114	0.189	0.066
Secretory	Exorbital lachrymal gland	0.055	0.042	0.083	0.054
	Harderian gland	0.031	0.026	0.063	0.063
	Intra-orbital lachrymal gland	0.043	0.046	0.112	0.047
	Salivary glands	0.072	0.060	0.055	0.047
Fatty	Brown fat	0.056	0.044	0.043	0.043
	White fat	NC	NC	0.002	NC
Reproductive	Bulbo-urethral gland	0.144	0.152	0.113	0.088
	Epididymis	0.013	0.033	0.135	0.037
	Prostate	0.040	0.062	0.079	0.053
	Seminal vesicles	0.008	NC	0.012	0.012
	Testis	0.034	0.048	0.077	0.040
Muscular	Muscle	0.014	0.027	0.048	0.019
	Myocardium	0.128	0.078	0.097	0.045
	Tongue	0.036	0.046	0.153	0.062
Other	Bone marrow	0.073	0.093	0.092	0.076
	Lung	0.394	0.255	0.302	0.102
	Nasal mucosa	0.042	0.075	0.046	0.042
	Pancreas	0.070	0.085	0.072	0.051
	Skin	0.036	0.024	0.076	0.067
	Spleen	0.064	0.077	0.075	0.059
	Tooth pulp	0.386	0.454	0.649	0.305
	Trachea	0.047	0.054	0.060	0.020
	Uveal tract	0.078	0.166	0.163	0.101
Gastrointestinal	Stomach mucosa (fundus)	0.082	0.067	0.140	0.036
	Stomach mucosa (non-fundic)	0.086	0.073	0.086	0.047
	Small intestine mucosa	0.071	0.109	0.083	0.075
	Caecum mucosa	0.060	0.049	0.139	0.057
	Large intestine mucosa	0.056	0.054	0.204	0.120
	Rectum mucosa	0.044	0.062	0.118	0.066

[1] - Plasma concentrations determined by liquid scintillation counting

NC - Not calculable (tissue below limit of quantification)

Table 12. Tissue:plasma radioactivity concentration ratio in pregnant albino rats (Applicant's table)

		Tissue : plasma concentration ratios		
	Animal number and sex	486F	485F	489F
Tissue type	Tissue Sampling time	3 hours	6 hours	24 hours
Vascular/ lymphatic	Plasma[1]	1.00	1.00	1.00
	Blood	0.717	0.471	0.403
	Mandibular lymph nodes	0.027	0.028	0.164
Metabolic/ excretory	Kidney cortex	0.261	0.330	0.481
	Kidney medulla	0.107	0.154	0.148
	Liver	0.086	0.094	0.142
CNS	Brain	NC	0.004	NC
	Choroid plexus	0.312	0.101	0.050
	Meninges	0.192	0.031	0.050
	Pineal body	0.114	0.081	0.056
	Spinal cord	NC	NC	NC
Endocrine	Adrenal cortex	0.030	0.047	0.046
	Adrenal medulla	0.095	0.131	0.097
	Pituitary	0.100	0.058	0.072
	Thymus	0.016	0.015	0.043
	Thyroid	0.100	0.076	0.095
Secretory	Harderian gland	0.031	0.012	0.065
	Intra-orbital lachrymal gland	0.015	0.046	0.145
	Salivary glands	0.084	0.047	0.073
Fatty	Brown fat	NC	0.008	0.008
	White fat	NC	NC	NC
Reproductive	Clitoris	0.117	NC	0.448
	Ovary	0.026	0.058	0.050
	Uterus	0.161	0.333	0.335
Muscular	Muscle	0.011	0.008	0.030
	Myocardium	NC	NC	0.125
	Tongue	0.031	0.038	0.102
Other	Bone marrow	0.036	0.041	0.058
	Lung	0.230	0.213	0.234
	Nasal mucosa	0.031	0.010	0.068
	Pancreas	0.054	0.080	0.123
	Skin	0.010	0.010	0.023
	Spleen	0.059	0.048	0.071
	Tooth pulp	0.611	0.439	0.376
	Trachea	NC	0.044	0.107
	Uveal tract	0.045	0.120	0.164
Gastrointestinal	Stomach mucosa (fundus)	0.014	0.051	0.079
	Stomach mucosa (non-fundic)	NC	0.047	0.075
	Small intestine mucosa	0.142	0.096	0.229
	Caecum mucosa	0.030	NC	0.065
	Large intestine mucosa	0.034	0.027	0.010
	Rectum mucosa	0.042	NC	0.165

[1] - Plasma concentrations determined by liquid scintillation counting.
NC - Not calculable (tissue below limit of quantification)

Table 13. Tissue : plasma radioactivity concentration ratio in fetal tissues from pregnant female albino rats (Applicant's table)

	Tissue : plasma concentration ratios		
Animal number and sex	486F	485F	489F
Tissue Sampling time	3 hours	6 hours	24 hours
Placenta	0.153	0.190	0.207
Foetal brain	NC	NC	0.018
Foetal heart	NC	NC	0.011
Foetal liver	NC	0.008	0.034
Foetal lung	NC	NC	0.014
Foetal skin	NC	NC	0.026

NC - Not calculable (tissue below limit of quantification)

Metabolism

Metabolism of semaglutide has been evaluated in vitro and in vivo using [^{3}H]Tyr-semaglutide and [^{3}H]Oct-semaglutide.

In vitro

In vitro metabolism of [^{3}H]Tyr-semaglutide and [^{3}H]Oct-semaglutide was investigated following incubations in hepatocytes from rat, monkey and human. Limited metabolism was observed, with the amounts of unchanged semaglutide being greater than 99% of total peak area in all species. No unique human metabolites were observed (Study # 206642, 214064).

Plasma metabolite profile

Metabolite profile in plasma was evaluated following a single subcutaneous administration of [^{3}H]semaglutide in mouse, rat, and monkey. Semaglutide was the primary component circulating in plasma in all species tested (between 69 and 93%). Twelve metabolites were detected; each metabolite was found to be in the range of 0.3-9% of the total amount of semaglutide, based on AUC (Study # 210299, 207044, 207347, 209041). The metabolite profile was similar following single and repeated administration in the rat, with 9 to12 metabolites detected in the plasma. The exposure ratios between semaglutide and the metabolites were comparable after 1 and 5 daily administrations indicating no accumulation (Study # 213526). Overall, the metabolite profile of semaglutide in the nonclinical species was similar to that of humans.

Table 14. Overview of plasma metabolite profiling data (Applicant's table)

Species	Sex	Label position	No. of doses	Dose	Samples profiled	Exposure levels*			Study ID
						Semaglutide (% of total AUC)	No. of metabolites	Individual metabolites (% of total AUC)	
Mouse	M+F	[^{3}H]Oct	1	0.4 mg/kg 41 MBq/kg	1-36 h	83	11	< 5	210299
Rat	M	[^{3}H]Tyr	1	0.3 mg/kg 16 MB/kg	2-72 h	93	1	6.9	207044
Rat	M+F	[^{3}H]Oct	1	0.3 mg/kg 10 MBq/kg	2-72 h	69	10	< 7	207347
Rat	M+F	[^{3}H]Oct	1	0.04 mg/kg 16 MBq/kg	6-24 h	84	9-12	< 3	213526
Rat	M+F	[^{3}H]Oct	5	0.04mg/kg/day 16 MBq/kg/day	6-96 h	76	12	< 4	213526
Monkey	M	[^{3}H]Oct	1	0.3 mg/kg 15 MBq/kg	0.5-168 h	71	4	0.3- 9**	209041
Human	M	[^{3}H]Oct	1	0.5 mg 16.7 MBq (450µCi)	24-840 h	83	6	< 7.7	213363***

* Tritiated water was excluded from the exposure calculations.

** One component represented an exposure of 17%, but was shown to represent a range of unresolved metabolites.

*** Part of clinical trial report 3789.

Table 15. [^{3}H]Oct-semaglutide: Metabolite profiling of rat plasma after single and repeated doses (Applicant's table)

Days of Adm.	PK	Sex	Sema-glutide	P1 Water	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	Total (water incl)	Total (no water)
1	C_{max} (nmol/L)	M	61	5	2	3	-	<1	1	2	-	<1	2	-	<1	-	<1	72	68
		F	66	5	2	1	<1	<1	3	2	<1	<1	2	<1	<1	-	<1	77	74
	T_{max} (h)	M	6	24	12	12	-	24	12	6	-	12	6	-	6	-	6	12	6
		F	6	24	24	12	24	24	6	6	24	24	6	24	24	-	12	6	6
	T_{last} (h)	M	24	24	24	24	-	24	12	24	-	12	24	-	12	-	12	24	24
		F	24	24	24	24	24	24	24	24	24	24	24	24	24	-	12	24	24
	AUC_{last} (nmol*h/L	M	1090	59	33	39	-	4	4	24	-	8	16	-	4	-	3	1350	1240
		F	1050	64	10	20	6	7	36	25	2	15	28	2	4	-	<1	1330	1210
5	C_{max} (nmol/L)	M	71	23	5	4	2	1	3	2	<1	2	2	1	-	2	<1	114	89
		F	92	23	5	5	1	1	3	2	<1	2	2	-	<1	2	<1	129	107
	T_{max} (h)	M	12	12	6	6	12	-	6	6	12	6	12	24	-	6	6	12	6
		F	6	24	12	12	24	12	12	12	12	24	6	-	12	24	6	6	6
	T_{last} (h)	M	96	96	24	24	12	24	24	24	12	24	24	24	-	48	6	96	96
		F	96	96	24	48	24	24	24	48	12	48	24	-	24	24	6	96	96
	AUC_{last} (nmol*h/L)	M	1970	1840	95	73	6	22	51	40	2	32	25	7	-	25	3	4450	2530
		F	2410	1930	72	113	7	25	47	51	4	57	20	-	10	9	1	4950	2930
	T½ (h)	M	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33	11
		F	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	9

Additional Information: P1 is considered to be tritiated water

Metabolite profile in urine and feces

Metabolite profile in urine and feces was evaluated in rats and monkeys following a single dose of [^{3}H]Oct-semaglutide (Study # 208008 and 209041).

- In rats, low amounts of intact semaglutide were observed in feces and urine (~1%). Up to 8 and 14 metabolites were detected in urine and feces, respectively. Each metabolite accounted for less than 4% of the administered dose.

- In the bile cannulated rat, approximately 14% of unchanged semaglutide was detected in bile. Up to 13 metabolites were detected as excretion products in bile, each accounting for less than 5% of the administered dose.

- In monkeys, 9 and 15 metabolites were detected in urine and feces, each accounting for less than 4.9% and 0.6% of the administered dose, respectively. Intact semaglutide was not detected in urine.

Table 16. [^{3}H]Oct-semaglutide: Metabolite profile of urine, feces and bile in the rat (Applicant's table)

Species	Sample	Profiling of samples	Amount of drug related material in % of dose (%)	Compound in % of dose (%)				
				Number of components	Semaglutide*	Most abundant metabolite	Second most abundant metabolite	Front peak
Rat, male	Urine	0-120 hours	31	6	ND	2.4 (U7)	1.7 (U2)	25.5 (U1)
	Faeces	0-120 hours	26	14	1.4 (F9)	3.2 (F14)	3.0 (F3)	4.0 (F1)
	Bile	0-72 hours	43	13	12.8 (B13)	4.1 (B10)	4.0 (B11)	4.2 (B1)
Rat, female	Urine	0-120 hours	34	8	0.6% (U8)	3.9 (U7)	1.4 (U6)	24.2 (U1)
	Faeces	0-120 hours	24	13	0.8 (F9)	3.2 (F6)	2.6 (F7)	4.3 (F1)
	Bile	0-72 hours	48	12	14.6 (B13)	4.5 (B11)	4.3 (B9)	3.1 (B1)

Additional Information:

Peaks in the metabolite profiles were named by a letter code and a number. The prefix U, F and B represents the matrix (urine, faeces and bile). The front peak was tritiated water as it was shown to be volatile and disappeared from plasma after freeze drying of the sample and is thus not regarded being a true primary metabolite.

Bile was obtained from a separate group of bile-cannulated rats.

ND= not detected.

*Expected to be semaglutide based on similar retention times.

Table 17. [^{3}H]Oct-semaglutide: Metabolite profile of urine, feces and bile in the monkey (Applicant's table)

Species	Sample	Sampling Time or Period	% of Dose in sample	Compound in % of total AUC (water excl.)			
				Parent		Most abundant metabolite	2nd most abundant metabolite
Cynomolgus monkey (M)	Plasma	0.5-168 h	NA	71% (P3)		17% (P2)	9% (P4)
				Fraction of dose in sample (%)			
				Parent	Front peak*	Most abundant metabolite	2nd. Most abundant metabolite
	Urine	0-216 h	22%	NA.	13% (U1)	4.9 (U4)	2.6 (U3)
	Faeces	0-216 h	10%	0.6% (F9)	7% (F1)	0.5 (F6)	0.4 (F5)

NA= not applicable

Additional Information:

The metabolite P2 is expected to contain more than one component

6 components were detected in plasma, 9 components were detected in urine and 15 in faeces

* The front peak is tritiated water as it was volatile and disappeared after freeze drying of the sample and is thus not regarded being a metabolite.

Excretion

The excretion of semaglutide related radioactivity was evaluated following subcutaneous administration of [^{3}H]Oct-semaglutide in intact and bile duct cannulated rats, in lactating rats, and in cynomolgus monkeys.

- Urine and feces were the main excretion routes for semaglutide related material.
- Semaglutide was metabolized prior to excretion and only limited amounts of intact semaglutide were observed in urine.
- In lactating rats administered a single subcutaneous dose of [^{3}H]Oct-semaglutide at day 10 post-partum, maximal concentrations of radioactivity in blood, plasma, reconstituted plasma and milk was observed at 6, 12, 6 and 12 hours post-dose, respectively. Excretion of semaglutide-related material into milk was observed in low amounts (3 to 12-fold lower than in dam plasma) with semaglutide being the most abundant component.

Table 18. Excretion of [^{3}H]Oct-semaglutide in intact rat following a single SC dose (Applicant's table)

Excretion route (%), male	Urine	Faeces	Expired air	Other	Total
0-24 h	3.71	0.03	0.15	0.48[b]	**4.4**
0-168 h	35.63	32.63	0.15[a]	26.10[c]	**94.5**
Excretion route (%), female					
0-24 h	3.79	0.05	0.16	0.42[b]	**4.4**
0-168 h	39.01	36.32	0.16[a]	26.1[c]	**101.6**

[a]Expired air was only collected 0-24 h
[b]Cage wash
[c]Including cage wash, cage debris and carcass

Table 19. Excretion of [^{3}H]Oct-semaglutide following a single SC dose to the bile cannulated rat (Applicant's table)

Excretion route (%), Male	Urine	Faeces	Bile	Other	Total
0-24 h	2.66	0.04	18.39	1.04[a]	22.13
0-96 h	29.91	2.77	45.65	24.77[b]	103.1
Excretion route (%), Female					
0-24 h	3.56	0.02	17.02	0.72[a]	21.32
0-96 h	36.65	2.06	50.98	13.73[b]	104.0

[a]Cage wash
[b]Final cage wash, cage debris and carcass.

Table 20. Excretion of [^{3}H]Oct-semaglutide in male cynomolgus monkey (Applicant's table)

Excretion route (% of administered dose, mean)	Total Radioactivity (Wet samples)				Total Radioactivity (Freeze dried samples)		
	Urine	Faeces	Cage Wash	Total	Urine	Faeces	Total
Time							
0-24 h	0.598	0.089	0.195	0.88	0.417	0.084	0.501
0-168 h	16.81	12.04	2.74	31.59	8.769	11.03	19.80
0-336 h	30.33	20.67	7.19[a]	58.20	13.14	17.75	30.89

Table 21. Secretion of [^{3}H]Oct-semaglutide in lactating rats (Applicant's table)

Sampling time	Mean nmol of [^{3}H]Oct-NNC0113-0217/g of tissue				
Hours	Blood	Plasma	Reconstituted plasma	Milk	Reconstituted milk
Pre-dose	0.000	0.000	0.000	0.000	0.000
6	0.207	0.407	0.411	0.034	0.049
12	0.190	0.419	0.402	0.091	0.116
24	0.155	0.290	0.293	0.090	0.209

6 General Toxicology

The toxicity profile of semaglutide was evaluated in mice, rats, and monkeys up to 13, 26, and 52 weeks, respectively. These studies were reviewed under the IND by Dr. Dalin Yao (13-week mice study) and Dr. Tim Hummer.

6.2 Repeat-Dose Toxicity

Study title: 13 Week toxicity Study in Mice with Subcutaneous Administration

Study no.:	200663
Study report location:	Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 2, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, Batch #ow GLP-1-06-101C, 91.9% pure

Key Study Findings

- Piloerection in all treated male groups, and transient hunched posture (week 1-3) in males at ≥3 mg/kg (59X MRHD) and in HD females (175X MRHD).
- Body weight loss occurred at all doses during the first week, followed by lower body weight gain thereafter (up to 21 and 29% in males and females, respectively; 17X MRHD).
- The primary microscopic finding was focal C-cell hyperplasia, C-cell nests, and dilated ultimobranchial ducts in all treated groups (≥17X MRHD). Lobar necrosis in one male and centrilobular hypertrophy of the liver were observed in some of the HD treated animals with a minimal severity (173X MRHD).
- NOAEL could not be established based on the thyroid findings.

Methods

Doses:	0, 1, 3, and 10 mg/kg/d
Frequency of dosing:	Once daily (no dose escalation phase)
Route of administration:	Subcutaneous
Dose volume:	1 mL/kg
Formulation/Vehicle:	Disodium (b) (4) phosphate dehydrate (1.42 mg/mL), propylene glycol (14.0 mg/mL), phenol (5.50 mg/mL), and water for injection
Species/Strain:	Mouse / CD-1
Number/Sex/Group:	10
Age:	5 weeks old on arrival
Weight:	25-30 g for males and 22-26 g for females
Satellite groups:	15/sex/group for TK analysis 5/sex/group for ADA analysis
Unique study design:	No
Deviation from study protocol:	None affecting the integrity of the data

Observations and Results

Mortality

None treatment related.

Clinical Signs

Piloerection in all treated male groups, and hunched posture in males at ≥3 mg/kg and in HD females.

Table 22. Clinical signs (Applicant's table)

	Males				Females			
Nominal Dose (mg/kg/day)	**0 (Control)**	**1**	**3**	**10**	**0 (Control)**	**1**	**3**	**10**
Clinical signs (main study animals)[b]								
Hunched posture	0	0	2	6	0	0	0	2
Piloerection	0	2	2	6	0	0	0	0

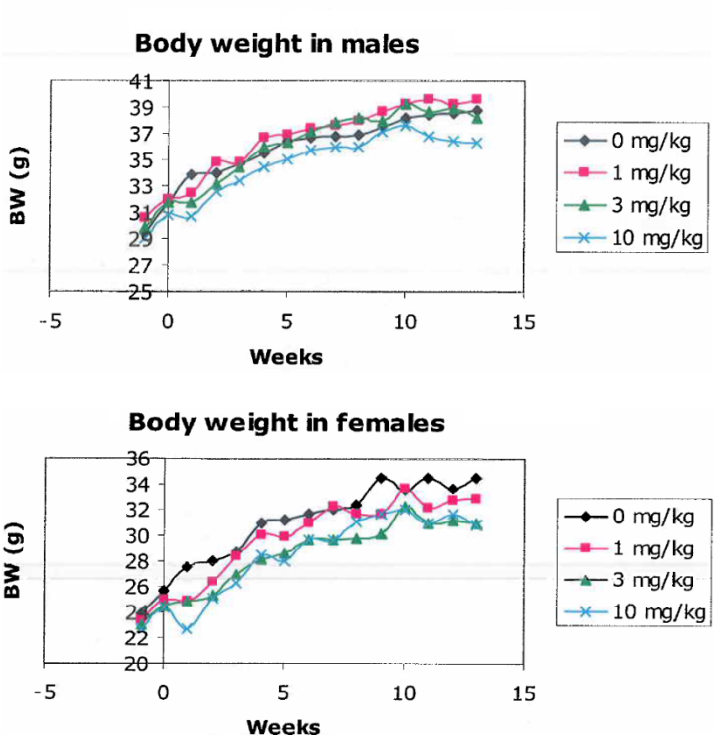
Body Weights

Initial weight loss occurred one week after the start of treatment, followed by a decrease in weight gain over week 2 to 13 when compared to controls (11% and 21% in males at MD and HD, and 12%, 27% and 29% in females at LD, MD and HD, respectively).

Table 23. Body weight gain (Applicant's table)

		Males				Females		
Nominal Dose (mg/kg/day)	**0 (Control)**	**1**	**3**	**10**	**0 (Control)**	**1**	**3**	**10**
Body weight gain (main study animals)								
Week 1-13 (g)	7.1	7.5	6.3	5.6	8.9	7.8	6.5	6.3
Week 1-13 (% difference from control)	-	+6	-11	-21	-	-12	-27	-29

Figure 14. Body weight, mice (Applicant's figure)



Feed Consumption

Food consumption was transiently decreased in all treated groups in the first few days of dosing compared to controls; it was comparable to controls thereafter, except in the HD females, in which the reduction clearly persisted.

Table 24. Food consumption (Applicant's table)

		Males				Females		
Nominal Dose (mg/kg/day)	**0 (Control)**	**1**	**3**	**10**	**0 (Control)**	**1**	**3**	**10**
Food consumption (main study animals)								
Day 1 (g/animal/day)	5.3	4.1***	3.6***	3.3***	4.2	2.9***	2.5***	2.2***
Mean Week 1-13 (g/animal/day)	5.5	5.5	5.1	5.2	5.0	4.8	4.3	4.0

***p≤0.001 vs. control (pairwise test)

Water Consumption

Visual inspection noted no intergroup differences in either sex.

Ophthalmoscopy

No treatment related changes were observed.

Hematology/Clinical Chemistry

There were no obvious effects on the clinical pathology parameters. Some slight differences that achieved statistical significance were considered to be incidental as there was no clear relationship to dose.

Calcitonin

Calcitonin levels were increased in all treated animals, without apparent dose relationship.

Subject	Treatment Description	Visit	Hour Nominal	Run ID	Dilution Factor	Concentration (pg/mL)	LLOQ
(b) (4)							

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Subject	Treatment Description	Visit	Hour Nominal	Run ID	Dilution Factor	Concentration (pg/mL)	LLOQ
(b) (4)							

Urinalysis

Not conducted

Gross Pathology

There were no treatment related findings.

Organ Weights

There was a slight statistical significant decrease in absolute and relative liver weight in HD males (-15% and -11%, respectively). This correlated with decreased liver glycogen content observed in 6/10 of the HD males. The reduced liver weights and the decreased glycogen content of the liver are considered to be secondary to the pharmacological effect of semaglutide on food consumption.

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

Thyroid gland

- Increased incidence of minimal to mild focal C-cell hyperplasia in all treated groups, without dose relationship.
- Increased incidence of C-cell nests in females at ≥3mg/kg and in HD males
- Increased incidence of minimal to moderate dilated ultimobranchial duct in all treated groups, not dose-related.

Liver

- Increased incidence of minimal centrilobular hypertrophy in HD males.
- Decreased glycogen in HD males and females.

Table 25. Histopathology findings, mice, 13-week (Applicant's table)

HISTOLOGICAL FINDINGS	GROUP TOTALS							
	Males				Females			
GROUP DOSE	Grp 1 0 mg/kg /day	Grp 2 1 mg/kg /day	Grp 3 3 mg/kg /day	Grp 4 10 mg/kg /day	Grp 1 0 mg/kg /day	Grp 2 1 mg/kg /day	Grp 3 3 mg/kg /day	Grp 4 10 mg/kg /day
THYROID GLAND	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(14)
No abnormality detected	8	5	5	2	9	0***	2*	1**
Focal C-cell hyperplasia								
minimal	0	2	2	2	0	3	1	5*
mild	0	0	0	0	0	0	0	1
Total Incidence	0	2	2	2	0	3	1	6**
C-cell nests	1	1	2	5	0	6*	3	0
Ultimobranchial duct, dilated								
minimal	4	8	5	6	5	6	10	8
mild	2	2	3	3	1	7*	3	4
moderate	0	0	0	1	0	0	0	0
Total Incidence	6	10	8	10	6	13*	13*	12*
Ectopic thymus	0	0	0	0	0	1	1	1
LIVER	(10)	(2)	(1)	(10)	(10)			(10)
No abnormality detected	7	0	1	2	6			5
Lobar necrosis	0	0	0	1	0			0
Focal necrosis	2	0	0	0	1			2
Microgranuloma(ta)	0	0	0	1	0			0
Centrilobular hypertrophy								
minimal	0	0	0	4	0			0
Total Incidence	0	0	0	4	0			0
Centrilobular hepatocyte vacuolation	0	2*	0	0	0			0
Glycogen, decreased	0	0	0	6*	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

Toxicokinetics

Following single SC doses of semaglutide, C_{max} and AUC_{0-24} increased in a broadly dose-proportional manner across the entire dose range, indicating linear kinetics with increasing dose. Total systemic exposure based on AUC_{0-24} during Week 13 was marginally greater than that observed on Day 1, suggesting a slight accumulation of semaglutide following repeated dosing. There were no appreciable gender related differences in any of the TK parameters.

Table 26. Toxicokinetic parameters, mice, Day 1 and week 13 (Applicant's table)

Sex	Group	Dose (mg/kg/day)	Day/Week	Cmax(obs) (nmol/L)	AUC(0-t) (nmol.h/L)	AUC(0-24) (nmol.h/L)	AUC(0-∞) (nmol.h/L)
Male	2	1	D1	837	11100	11100	13000
			W13	1030	12100	12100	15000
	3	3	D1	3160	26500	26500	NR
			W13	4060	39800	39800	46000
	4	10	D1	11700	112000	112000	127000
			W13	11600	116000	116000	126000
Female	2	1	D1	1210	11100	11100	12600
			W13	780	10700	10700	12500
	3	3	D1	2630	31600	31600	34000
			W13	3270	36900	36900	39800
	4	10	D1	13700	115000	115000	130000
			W13	9200	117000	117000	129000

NR = Not reported in accordance with Novo Nordisk SOP (040664)

Sex	Group	Dose (mg/kg/day)	Day/ Week	Tmax(obs) (h)	T½el (h)	CL/F (L/h/kg)	Vd/F (L/kg)	Rac (observed)	Rac (predicted)
Male	2	1	D1	4.00	8.17	0.0187	0.221	1.09	1.15
			W13	2.00	9.79	0.0201	0.284		
	3	3	D1	2.00	NR	NR	NR	1.50	NR
			W13	2.00	8.01	0.0183	0.212		
	4	10	D1	2.00	7.79	0.0192	0.215	1.04	1.13
			W13	1.00	5.96	0.0209	0.180		
Female	2	1	D1	4.00	7.74	0.0193	0.216	0.96	1.13
			W13	4.00	8.17	0.0228	0.269		
	3	3	D1	2.00	5.50	0.0214	0.170	1.17	1.05
			W13	4.00	6.10	0.0198	0.174		
	4	10	D1	2.00	8.16	0.0187	0.220	1.02	1.15
			W13	1.00	6.32	0.0207	0.189		

NR = Not reported in accordance with Novo Nordisk SOP (040664)

Anti-drug antibody

There were no antibodies detected in the serum samples.

Study title: Toxicity study by subcutaneous administration to CD rats for at least 26 weeks followed by a 4-week recovery period

Study no.:	JLY0162; applicant #NN207377
Study report location:	Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 16, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, Batch #LP0217G1T01, 93.64% pure

Key Study Findings

- There were no treatment-related mortalities or adverse clinical signs.
- Treatment resulted in a dose-related decrease in body weight gain, which was most noteworthy after dose escalations. Effects on body weight gain generally correlated with decreased food consumption.
- Minimal Brunner's gland hypertrophy was observed in nearly all treated animals but not in control animals. This finding was not observed after recovery.
- No treatment-related adverse microscopic lesions were observed in the pancreas or thyroid.
- The noteworthy findings observed in this study were pharmacologically mediated and/or were not considered to be adverse. The NOAEL for this study was considered to be the high dose level, 0.6 mg/kg/d, 27X MRHD based on AUC_{0-168}.

Methods

Doses:

Group	Dose Level (mg/kg/d)		
	Week 1	Week 2	Week 3-28
1	0	0	0
2	0.03	0.03	0.03
3	0.03	0.13	0.13
4	0.03	0.13	0.60

Frequency of dosing: Once daily
Route of administration: Subcutaneous
Dose volume: 0.25 mL/kg
Formulation/Vehicle: 1.42 mg/mL disodium (b) (4) phosphate, 14 mg/mL propylene glycol, and 5.5 mg/mL phenol in water; pH 7.4
Species/Strain: Rat/Sprague-Dawley
Number/Sex/Group: 20/sex/group
Age: 7-8 weeks at start of treatment
Weight: 201 to 257 g (males) and 156 to 211 g (females)
Satellite groups: Recovery animals: 5/sex/group
Unique study design: NA
Deviation from study protocol: No protocol deviations occurred that impacted the integrity or interpretation of the study.

Observations and Results

Mortality

There were no treatment related deaths. One male in the low dose group died during Week 15 due to a lymphoma in the brain. One control group male was euthanized during the second week of recovery because of a severe respiratory impairment resulting from a schwannoma within the thorax.

Clinical Signs

No treatment-related adverse effects on behavior were noted. Some treated animals from all dose groups were observed as being thin due to treatment-related effects on body weight.

Table 27. Clinical signs (Applicant's table)

Nominal Dose (mg/kg/day)	0 (Control)	0.03	0.13	0.6	0 (Control)	0.03	0.13	0.6
Initial Number of Animals Main	M: 20	M: 20	M: 20	M: 20	F: 20	F: 20	F: 20	F: 20
Clinical signs Thin build	0	1	1	1	0	1	5	3

Body Weights

Dose-related decrease in body weight was observed in all treated groups compared to controls.

Table 28. Body weight, rat (Applicant's table)

Dose (mg/kg/d)	0		0.03		0.13		0.60	
Sex	M	F	M	F	M	F	M	F
Weight (g) - Week 28	657	332	577	291	536	276	517	272
Diff from control (g)			-80	-41	-121	-56	-140	-60
% diff from control			↓12%	↓12%	↓18%	↓17%	↓21%	↓18%
Body Weight Gain during 4-Week Recovery Period								
Weight gain (g)	21	5	51*	27	87*	35	84*	44
Diff from control (g)			30	22	66	30	63	39
% diff from control			↑143%	↑440%	↑314%	↑600%	↑300%	↑780%

*p<0.05. F = female; M = male.

Figure 15. Body weight, male rats (Applicant's figure)

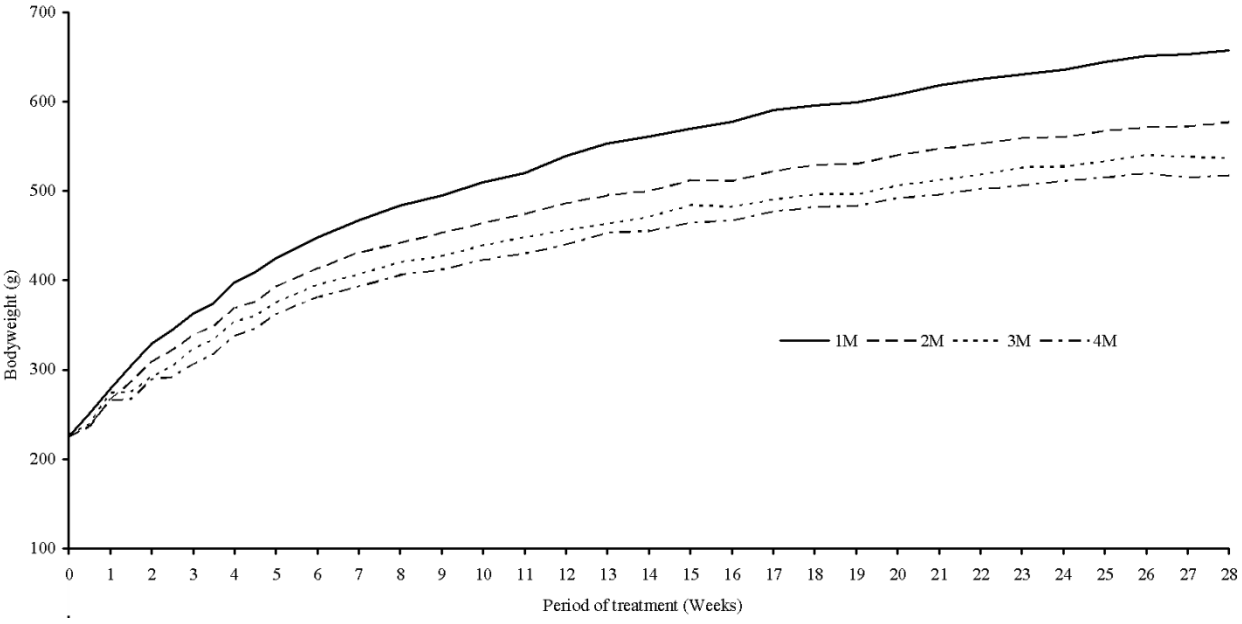
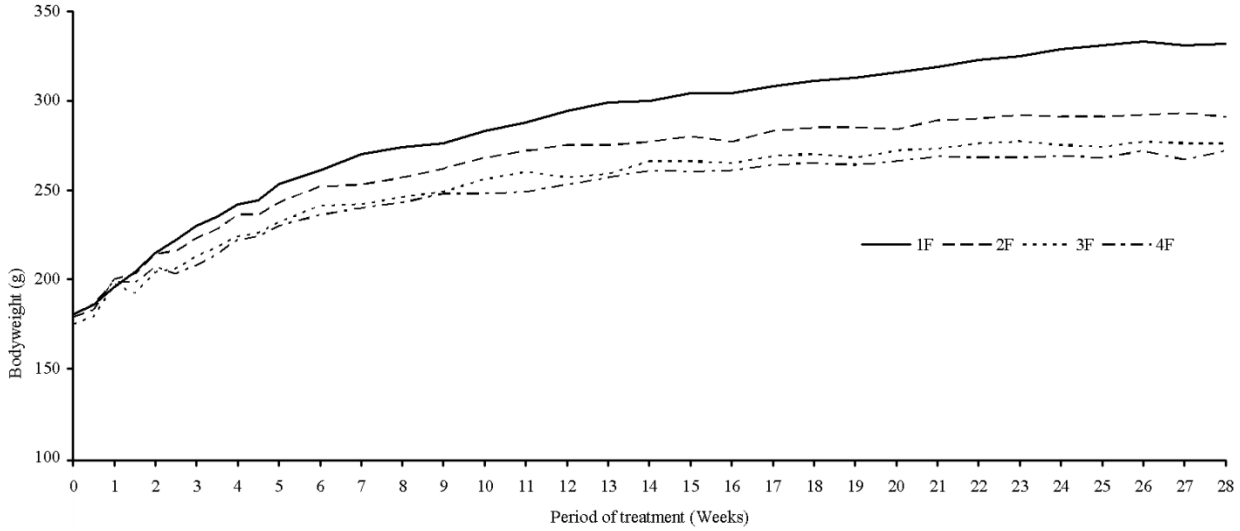


Figure 16. Body weight, female rats (Applicant's figure)



Feed Consumption

Dose-related decrease in food consumption was observed in males at ≥0.13 mg/kg/day and in all treated females.

Table 29. Feed consumption (Applicant's table)

Dose (mg/kg/d)	0		0.03		0.13		0.60	
Sex	M	F	M	F	M	F	M	F
Daily intake (g/rat/d)	200	136	198	131	190	129	185	125
% diff from control			↓1%	↓4%	↓5%	↓5%	↓8%	↓8%

F = female; M = male.

Ophthalmoscopy

There were no treatment-related ophthalmoscopy findings.

Hematology

There was a slight reduction in reticulocyte counts in males and females, and a minor increase in hematocrit, hemoglobin, and erythrocyte count in females. There were no clear dose responses for these findings and the difference from controls did not always attain statistical significance. Decreased food consumption has been shown to cause increases in erythroid parameters and decreases in reticulocyte counts (Moriyama T et al. 2008). Consequently, these findings were likely to be secondary to the pharmacological effect of semaglutide and were considered to be non-adverse.

Table 30. Hematology, percent difference from control (Applicant's table)

Group/Sex	1M	2M	3M	4M
Dose level (mg/kg/day)	**0**	**0.03**	**0.13**	**0.6**
Hct	0.438	-3*	-1	+1
Hb	15.2	-3	-1	+2
RBC	8.59	-3	-4*	0
Retic	2.15	-13	-17*	-17*
MCH	17.7	0	+2	+2
MCHC	34.7	+1	0	0
MCV	51.0	0	+3	+1
WBC	10.99	-3	-8	-6
N	1.70	+5	+18	+8
L	8.58	-3	-12	-7
E	0.180	-12	-26**	-21*
B	0.0490	0	-7	-6
M	0.394	-22	-19	-26
LUC	0.0835	-16	-22	-23
Plt	1186	-5	-13***	-9*
PT	15.4	+1	+1	+4
APTT	17.3	+6	+6	+11

Group/Sex	1F	2F	3F	4F
Dose level (mg/kg/day)	**0**	**0.03**	**0.13**	**0.6**
Hct	0.407	+4**	+4**	+2
Hb	14.3	+3	+4*	+3
RBC	7.61	+4	+3	+3
Retic	1.87	-11	-17*	-16*
MCH	18.8	0	0	-1
MCHC	35.1	-1	-1	0
MCV	53.4	+1	+1	-1
WBC	5.94	+26*	+32**	+15
N	0.74	+17	+28	+23
L	4.84	+30**	+34***	+16
E	0.106	-8	+15	-2
B	0.0115	+83*	+83**	+70
M	0.203	0	-1	-8
LUC	0.0460	+38	+42	+22
Plt	1129	-3	+2	+11*
PT	15.0	+3	+2	+3
APTT	14.2	+19**	+19**	+21**

Clinical chemistry

Slight reduction in plasma triglycerides, creatinine, total protein, albumin and α1 globulin concentrations and slightly increase in urea concentration were observed and were considered to be secondary to the pharmacological effect of semaglutide.

Table 31. Clinical chemistry, percent difference from control (Applicant's table)

Group/Sex	1M	2M	3M	4M
Dose level (mg/kg/day)	**0**	**0.03**	**0.13**	**0.6**
ALP	62.0	+2	+10	+12
ALT	44.6	-9	-7	-6
AST	79.8	-8	-10	-7
Bili	2.00	-3	-3	-5
Urea	4.87	+10	+17**	+18**
Creat	45.8	-9**	+4	+5
Gluc	7.85	-6	-7	-12**
Chol	1.86	-17	-12	-9
Trig	1.095	-46***	-51***	-57***
Na	140	+1	+1	+1
K	5.78	-11**	-8*	-7
Cl	102	0	0	0
Ca	2.64	+2	+1	+1
Phos	1.96	-1	-2	-6
Total Prot	68.7	-1	+1	+1
Alb	30.2	+1	+1	+1
a1	13.2	-3	-7	-3
a2	4.05	+20***	+22***	+16**
Beta	16.4	-6	-3	-2
Gamma	4.70	+3	+22*	+13
A/G Ratio	0.790	+2	0	-1

Group/Sex	1F	2F	3F	4F
Dose level (mg/kg/day)	**0**	**0.03**	**0.13**	**0.6**
ALP	26.8	+45***	+23	+39**
ALT	45.4	-22*	-23*	-26**
AST	103.2	-27	-30*	-32**
Bili	2.65	+4	-9	-13
Urea	5.89	-7	+2	-3
Creat	47.7	-3	+1	-7*
Gluc	7.30	-6	-5	-3
Chol	2.31	-6	-1	+2
Trig	0.905	-42***	-43***	-51***
Na	140	+1**	+1*	0
K	4.10	+5	+2	-3
Cl	101	0	0	-1
Ca	2.81	-2*	-2	-3**
Phos	1.46	+11**	+10*	0
Total Prot	76.6	-4*	-4*	-5**
Alb	40.1	-4	-7**	-9***
a1	11.8	-9*	-8*	-12***
a2	4.30	-3	+3	+3
Beta	14.6	-1	0	-2
Gamma	5.80	+3	+9	+10
A/G Ratio	1.100	-1	-7*	-6

Urinalysis

Urine volumes were increased together with decreased urinary protein concentrations, increased sodium and decreased potassium concentrations. The increased urinary volume and sodium concentrations are likely to be due to known inhibitory effects of GLP-1 on sodium reabsorption from the proximal tubules (Moreno C et al. 2002), whereas the decreased protein levels are considered secondary to the reduced food consumption.

Table 32. Urinalysis, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	4M
Dose level (mg/kg/day)	**0**	**0.03**	**0.13**	**0.6**
Vol	8.79	↑33**	↑7	↑24*
pH	7.44	-4	-7**	-7**
SG	1032	-1**	0	0
Prot	0.878	-23**	-14	-25***
U-Na	64.6	-1	-15	+12
U-K	156	-29***	-9	-21**
U-Cl	60.2	-19	-32**	-3

Group/Sex	1F	2F	3F	4F
Dose level (mg/kg/day)	**0**	**0.03**	**0.13**	**0.6**
Vol	4.22	+35*	+32	+47**
pH	6.42	0	-3	-4
Prot	0.279	-46*	-46*	-49**
U-Na	51.3	+58***	+46**	+54**
U-K	200	-23*	-30**	-30**
U-Cl	37.0	+27	+42	+41

Prot = protein; SG = specific gravity; U-Cl = urine chloride; U-K = urine potassium; U-Na = urine sodium; Vol = urine volume.

Gross Pathology

Increased incidence in uterine fluid distension was seen in treated females, most notably in the high dose group.

Table 33. Macroscopic findings (Applicant's table)

Tissue and finding	Group/sex: / Number Examined:	1M 20	2M 19	3M 20	4M 20	1F 20	2F 20	3F 20	4F 20
Uterus									
Cyst(s)		-	-	-	-	0	1	0	0
Elongated		-	-	-	-	0	0	1	0
Fluid distension		-	-	-	-	5	7	7	13
Thickened		-	-	-	-	0	1	2	0
Uterine cervix									
Thickened		-	-	-	-	0	0	0	2
General comments									
Animal thin		0	0	0	0	0	0	2	2

Organ Weights

Most mean absolute organ weights for treated groups were decreased compared with control, often being statistically significant. In contrast, when organ weights were evaluated relative to body weight, mean organ weights were generally higher than controls, again often being statistically significant. This suggests that the effect on organ weights was related to decreased body weights compared with control values. An exception to this effect was the uterus/cervix, which showed a dose-related increase in mean organ weight in spite of lower body weights. It was speculated that uterine weights were higher in the HD group because of increased incidence of fluid distension (13/20 females) compared with controls (5/20 females), which relates to the stage of the estrus cycle at the time of necropsy. There were no treatment-related changes in uterine weight/estrus cycle after a 4-week recovery period.

Table 34. Organ weight, females, percent change from control

Group/Sex		2F	3F	4F
Dose (mg/kg/d)		0.03	0.13	0.6
Body weight		-11***	-16***	-20***
Uterus + Cervix	Absolute	+2	+14	+28
	Relative to body weight	+16	+36	+60**

Histopathology

Adequate Battery: Yes

Peer Review: An internal and external (b) (4) peer review was conducted. Additionally, the Applicant's pathologist conducted a peer review of Brunner glands.

Histological Findings

Duodenum

Nearly all treated animals showed minimal Brunner's gland hypertrophy, with no control animals having the same finding. The Brunner's glands are involved in the secretion of alkaline mucus as a medium for enzymes in the upper intestinal tract and also for protection of the upper intestinal tract against the acidity of the stomach luminal content. The glands also show high GLP-1 receptor expression. The finding was therefore considered related to the pharmacological action of semaglutide.

Liver

Treated males had a decreased incidence in generalized hepatocyte rarefaction. It is uncertain whether this is a treatment-related effect or if the male control animals had an unusually high incidence of this finding.

Uterus
A dose-related increase in uterine luminal dilatation was noted, which may suggest an imbalance of estrus cycle stage for HD females. Similar findings were not observed after a 4-week recovery period.

According to the applicant, the findings of fluid distension (macroscopically) and luminal dilatation (microscopically) were due to the stage of the sexual cycle and variations between treated groups and controls were fortuitous.

No treatment-related lesions were observed for pancreas or thyroid.

Table 35. Histopathological findings, dosing period (Applicant's table)

Tissue and Finding	Group/Sex: / Number:	1M 20	2M 19	3M 20	4M 20	1F 20	2F 20	3F 20	4F 20
Duodenum	Number Examined:	19	19	20	20	20	20	20	20
Hypertrophy of Brunner's glands		0	19	20	18	0	20	20	18
Liver	Number Examined:	20	19	20	20	20	20	20	20
Hepatocyte Rarefaction (Generalised)		17	6	6	6	8	9	8	5
Uterus	Number Examined:	-	-	-	-	20	8	9	20
Luminal Dilatation		-	-	-	-	4	7	8	12

Note: all cases of Brunner's gland hypertrophy were minimal severity; uterine luminal dilatation was minimal to slight, except for a single moderate finding in a Group 2 female - severity did not increase with dose level.

Possible target tissues for drug class - no treatment-related findings observed

Tissue and Finding	Group/Sex: / Number:	1M 20	2M 19	3M 20	4M 20	1F 20	2F 20	3F 20	4F 20
Pancreas	Number Examined:	20	0	0	20	20	0	0	20
Acinar Atrophy		1	0	0	3	1	0	0	3
Inflammatory Cell Infiltration		2	0	0	1	3	0	0	2
Periductular Pigment		1	0	0	0	0	0	0	0
Note: all cases of acinar atrophy were minimal severity									
Thyroids	Number Examined:	20	0	1	20	20	1	2	20
Ectopic Thymic Tissue		0	0	0	0	1	0	0	1
Prominent Ultimobranchial Cyst(s)		2	0	1	3	2	0	0	4
Reduced Colloid		0	0	0	0	0	1	0	0

Table 36. Histopathological findings, recovery period (Applicant's table)

Tissue and Finding	Group/Sex: / Number:	1M 4	2M 5	3M 5	4M 5	1F 5	2F 5	3F 5	4F 5
Duodenum	Number Examined:	4	4	5	4	5	5	5	5
Brunner's Glands - Focal Inflammation		0	0	0	1	0	0	0	0

Toxicokinetics

Samples (3/sex/group/ time point) were collected on:
Day 1 - Groups 1 and 2 at 0, 1.5, 3, 6, 12, and 24 hours after dosing.
Groups 3 and 4 at 0 and 6 hours after dosing.
Week 28 - All Groups at 0, 1.5, 3, 6, 12, and 24 hours after dosing.

The C_{max} and AUC_{0-24h} increased with dose for both male and female animals. The increase in exposure was proportional to the dose, and some accumulation (range 1.9-2.5) was observed for both males and females. No clear sex differences were observed in the toxicokinetics.

Table 37. Toxicokinetic parameters (Applicant's table)

Phase	Group	Gender	Dose (mg/kg)	C_{max} (nM)	T_{max} (h)	T_{Last} (h)	AUC_{last} * ((h)*(nM))	$AUC_{\%extrap}$ (%)	Rac_{Pred}	Rac_{Obs}
Day 1	2	Female	0.03	17.9	3	12	*115**	NC	NC	-
		Male		29.5	3	24	325	20.2	1.2	-
	3	Female	0.13	159	6	24	1890	NC	NC	-
		Male		132	3	24	1680	31.1	1.4	-
	4	Female	0.6	*622#*	*1.5#*	*24#*	*11300#*	NC	NC	-
		Male		*423#*	*1.5#*	*24#*	*6480#*	NC	NC	-
Wk 28	2	Female	0.03	55.8	3	24	989	NC	NC	NC
		Male		41.1	3	24	815	NC	NC	2.5
	3	Female	0.13	208	6	24	3620	NC	NC	1.9
		Male		213	12	24	4090	NC	NC	2.4
	4	Female	0.6	1030	6	24	18500	NC	NC	*1.6#*
		Male		897	6	24	17700	NC	NC	*2.7#*

* AUC_{last}= AUC_{tau} = $AUC_{0-24\ hr}$ with the exception of the lowest dose level at SD, 0.03 mg/kg (females), where the last measured time-point was detected at 12 hr (AUC_{last}= $AUC_{0-12\ hr}$).
# Several animals not included in the plasma concentration time profiles, i.e. parameters only rough estimates

Anti-Drug Antibody Analysis

No samples were found to be positive for anti-semaglutide antibody formation.

Dosing Solution Analysis

Dosing formulations were between 91% and 101% of the nominal concentrations. Semaglutide was not detected in any vehicle control formulation.

Study title: Toxicity study by subcutaneous administration to Cynomolgus monkeys for 52 weeks followed by a 4 week recovery period

Study no.:	JLY0140; applicant #NN207288
Study report location:	Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 16, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, Batch #LP0217G1T02, 92.98% pure

Key Study Findings

- Transient dehydration, decreased activity, and hunched posture were observed in the HD groups (27X MRHD) during the first few weeks of treatment.
- Body weight loss occurred during the dose-escalation phase and first few weeks of the target dose phase, after which time decreased body weight gain occurred. After treatment ended there was a significant improvement in mean body weight gain. Effects on body weight correlated with decreased food consumption.
- One HD female showed a bigeminal rhythm, with two episodes of sinus tachycardia in Week 13 and a continuous left bundle branch block-like recording that persisted from Week 26 to Week 52. No cardiac lesions were identified in this animal. Because this is a rare background finding in Cynomolgus monkeys, a relationship to treatment could not be excluded.
- Microscopic evaluation of the heart showed one HD male with slight multifocal myocardial vacuolation and degeneration, with karyomegaly, in the left ventricle. There was no significant ECG abnormality in this animal. A relationship to treatment could not be excluded.
- Increased mean absolute and relative thyroid weights in treated females and increased mean weights for prostate and testes were observed, without correlative microscopic findings.
- No treatment-related microscopic findings were observed for the thyroid, pancreas, or Brunner's glands.
- Because of the possible treatment-related finding of myocardial vacuolation in a HD male and continuous left bundle branch block in a HD female as well as the requirement for a dosing holiday in 2 HD animals, the NOAEL was considered to be 0.06 mg/kg, 5X MRHD based on AUC_{last}.

Methods

Doses:

Group	Dose Level (mg/kg)		
	Week -2	Week -1	Week 1-52
1	0	0	0
2	0	0	0.01
3	0	0.01	0.06
4	0.01	0.06	0.36

Frequency of dosing: Twice weekly (1st and 4th day of the week)
Route of administration: Subcutaneous
Dose volume: 0.2 mL/kg
Formulation/Vehicle: 1.42 mg/mL disodium (b) (4) phosphate, 14 mg/mL propylene glycol, and 5.5 mg/mL phenol in water; pH 7.4
Species/Strain: Monkey/Cynomolgus
Number/Sex/Group: 4/sex/group
Age: 30 to 34 months
Weight: 2.15 to 3.59 kg (males); 2.15 to 3.01 kg (females)
Satellite groups: Recovery animals: 2/sex/treated groups
Unique study design: NA
Deviation from study protocol: Two HD animals (1M and 1F) received a brief dosing holiday during Weeks 3 and 4 and 1 MD male during Week 21 due to excessive effects on body weight. There were no protocol deviations that affected the integrity or validity of the study.

Observations and Results

Mortality

There were no unscheduled mortalities.

Clinical Signs

Adverse clinical signs were primarily limited to the HD group and generally occurred during the first few weeks of treatment. Two males were observed with skin tenting, suggestive of dehydration, in Week 2 of dose escalation. Decreased activity and hunched posture were observed in 2 males and 2 females during the first 2 weeks of receiving the target dose of 0.36 mg/kg. One HD female also showed slight transient body tremors during Week 5. Due to effects on food and water intake leading to decreased body weight and dehydration, 2 HD animals (1M and 1F) received a brief dosing holiday during Weeks 3 and 4 and 1 MD male received a dosing holiday during Week 21.

Table 38. Clinical signs (Applicant's table)

Nominal Dose (mg/kg twice-weekly)	0 (Control)	0.01	0.06	0.36	0 (Control)	0.01	0.06	0.36
Initial Number of Animals Main	M: 4	M: 4	M: 4	M: 4	F: 4	F: 4	F: 4	F: 4
Clinical signs[a]								
Thin	0	0	1	1	0	0	0	0
Underactive	0	0	0	2	0	0	0	2
Hunched posture	0	0	0	2	0	0	0	2
Skin tenting	0	0	0	2	0	0	0	0

Body Weights

Treatment resulted in mean body weight loss during the dose-escalation phase and first few weeks of the target dose phase, after which time decreased body weight gain occurred. After treatment ceased there was a significant improvement in mean body weight gain, with male groups showing a 0.28 to 0.71 kg mean increase and females showing a 0.17 to 0.67 kg mean increase during the 4-week recovery period.

Table 39. Body weight, monkeys (Applicant's table)

Dose (mg/kg/d)	0		0.01		0.06		0.36	
Sex	M	F	M	F	M	F	M	F
Dose Escalation Phase								
Weight (kg) - Day 1	2.62	2.41	2.78	2.46	2.80	2.46	2.78	2.42
Weight (kg) - Day 22	2.67	2.49	2.75	2.45	2.65	2.29	2.50	2.19
Weight gain (g)	0.05	0.08	-0.03	-0.01	-0.15	-0.17	-0.28	-0.23
Diff from control (g)			-0.08	-0.09	-0.20	-0.25	-0.33	-0.31
% diff from control			↓160%	↓113%	↓400%	↓313%	↓660%	↓388%
Constant Dose Phase								
Weight (kg) - Day 365	4.40	3.50	4.38	3.66	4.04	2.71	3.83	2.75
Diff from control (g)			-0.02	0.16	-0.36	-0.79	-0.57	-0.75
% diff from control			-	↑5%	↓8%	↓23%*	↓13%	↓21%

*p<0.05; **p<0.01; F = female; M = male.

Feed Consumption

Mean food intake was reduced for all treated groups compared with control values. The effects on food consumption were most noteworthy during the early weeks of treatment, which is consistent with the effects on body weight. When observed, food intake was generally affected for up to 2 days after each dose administration and tended to return to normal until the next dose administration. There was no effect on food consumption during the recovery period.

Ophthalmoscopy

No treatment-related effects were noted during ophthalmic exams.

ECG

There were no toxicologically meaningful effects on heart rate or ECG intervals. One female receiving 0.36 mg/kg showed a bigeminal rhythm (normal heartbeat followed by a premature beat), with two episodes of sinus tachycardia in Week 13. In Week 26, this monkey experienced a continuous Left-Branch-Bundle-Block-like recording that persisted to Week 52. The study director stated that this is a rare finding in Cynomolgus monkeys and the conditions may result in occasional missed beats. A relationship to treatment could not be excluded.

During Week 52, one HD male had a 15-second section that appeared to have an interventricular conduction defect in which the beats were sinus but were not conducted as expected. One MD male had two premature aberrant sinus beats. These observations were both transient and of a type that are occasionally encountered in Cynomolgus monkeys, and therefore, were not considered to be toxicologically meaningful. Because of the isolated nature of these findings, they could not be conclusively attributed to treatment.

Hematology

Mean reticulocyte counts were decreased for treated males at Weeks 13, 26, and 52, with no meaningful decrease in erythrocyte parameters. In females, decreases in erythrocyte parameters were noted, although the decrease in reticulocytes did not occur in a dose-related manner. Treated animals tended to have an increase in mean neutrophil counts and decreases in the other white blood cell subtype counts. There were no toxicologically meaningful effects on coagulation parameters.

Table 40. Hematology, monkey, week 52 (Applicant's table)

Group/Sex	1M	2M	3M	4M
Dose level (mg/kg)	**0**	**0.01**	**0.06**	**0.36**
Hct	0.437	0	-8	-9
Hb	14.0	3	-4	-7
RBC	5.84	+2	-6	-7
Retic	0.875	-31	-35	-8
MCH	24.0	0	+1	+1
MCHC	32.1	+3	+4	+2
MCV	74.7	-2	-3	-1
WBC	14.4	-26	-32	-18
N	3.20	9	33	76
L	10.26	-38**	-51***	-46***
E	0.223	38	-39	-15
B	0.0650	-33	-58*	-64*
M	0.523	-36	-47	-31
LUC	0.1125	-36	-49	-44
Plt	464	0	-3	+22

Group/Sex	1F	2F	3F	4F
Dose level (mg/kg)	0	0.01	0.06	0.36
Het	0.434	-3	-12*	-19**
Hb	14.2	-3	-13*	-18**
RBC	5.73	-1	-10*	-17**
Retic	0.795	-22	-9	-1
MCH	24.7	-2	-2	0
MCHC	32.6	+1	0	+1
MCV	75.7	-3	-2	-2
WBC	11.9	+5	-17	+31
N	3.58	+20	-6	+195
L	7.38	+3	-19	-42
E	0.240	-30	-33	-42
B	0.0500	-27	-43	-35
M	0.568	-49*	-47	-16
LUC	0.1050	-17	-35	-38
Plt	461	-3	-7	+12

Clinical Chemistry

Changes in clinical chemistry parameters were observed at various time points during the treatment period for all treatment groups; however, the effects were typically not dose-related or observed at all of the time points. Slight decreases in mean glucose and increases in mean cholesterol (males) were the most consistent findings that appeared to be treatment related.

Table 41.Clinical chemistry, monkeys (Applicant's table)

Group/Sex	1M	2M	3M	4M
Dose level (mg/kg)	0	0.01	0.06	0.36
Gluc	3.98	-29	-14	-25
Chol	3.17	+1	+3	+19

Group/Sex	1F	2F	3F	4F
Dose level (mg/kg)	0	0.01	0.06	0.36
Gluc	3.64	-11	-9	-39**

Serum calcitonin

Mean serum calcitonin levels were similar in all study groups, including controls.

Table 42. Serum calcitonin levels-male monkeys (Applicant's table)

Time	Group	Geometric mean	p-value
D1	Control	1.33	
	NCC 0113-0217 0.01 mg/kg	1.26	0.745
	NCC 0113-0217 0.06 mg/kg	1.09	0.212
	NCC 0113-0217 0.36 mg/kg	1.20	0.503
W13	Control	1.58	
	NCC 0113-0217 0.01 mg/kg	2.10	0.392
	NCC 0113-0217 0.06 mg/kg	1.81	0.677
	NCC 0113-0217 0.36 mg/kg	1.38	0.686
W26	Control	1.97	
	NCC 0113-0217 0.01 mg/kg	1.56	0.471
	NCC 0113-0217 0.06 mg/kg	1.54	0.440
	NCC 0113-0217 0.36 mg/kg	1.40	0.292
W39	Control	1.56	
	NCC 0113-0217 0.01 mg/kg	1.48	0.858
	NCC 0113-0217 0.06 mg/kg	1.29	0.499
	NCC 0113-0217 0.36 mg/kg	1.12	0.260
W52	Control	1.64	
	NCC 0113-0217 0.01 mg/kg	1.66	0.976
	NCC 0113-0217 0.06 mg/kg	1.33	0.545
	NCC 0113-0217 0.36 mg/kg	1.50	0.802

p values for comparison with control by mixed model.

Table 43. Serum calcitonin levels-female monkeys (Applicant's table)

Time	Group	Geometric mean	p-value
D1	Control	1.25	
	NCC 0113-0217 0.01 mg/kg	1.13	0.674
	NCC 0113-0217 0.06 mg/kg	1.37	0.707
	NCC 0113-0217 0.36 mg/kg	0.94	0.239
W13	Control	1.72	
	NCC 0113-0217 0.01 mg/kg	1.13	0.181
	NCC 0113-0217 0.06 mg/kg	1.53	0.706
	NCC 0113-0217 0.36 mg/kg	1.85	0.811
W26	Control	1.26	
	NCC 0113-0217 0.01 mg/kg	1.13	0.474
	NCC 0113-0217 0.06 mg/kg	1.29	0.885
	NCC 0113-0217 0.36 mg/kg	1.21	0.768
W39	Control	1.68	
	NCC 0113-0217 0.01 mg/kg	1.13	0.116
	NCC 0113-0217 0.06 mg/kg	1.13	0.116
	NCC 0113-0217 0.36 mg/kg	0.79**	0.006
W52	Control	2.04	
	NCC 0113-0217 0.01 mg/kg	1.13*	0.045
	NCC 0113-0217 0.06 mg/kg	1.77	0.607
	NCC 0113-0217 0.36 mg/kg	1.13*	0.045

p-values for comparison with control by mixed model.
* $p<0.05$, ** $p<0.01$

Urinalysis

Effects on urine pH, sodium, potassium, and chloride were noted for treated females during Week 51. A similar trend was not observed for males.

Table 44. Urinalysis, female monkeys, week 51 (Applicant's table)

Group/Sex	1F	2F	3F	4F
Dose level (mg/kg)	**0**	**0.01**	**0.06**	**0.36**
Vol	62.0	-30	-13	-22
pH	6.70	+12	+17	+16
SG	1013	0	0	0
Prot	0.0750	-9	-29	-11
U-Na	22.5	+124	+191*	+259**
U-K	24.2	+116	+107	+110
U-Cl	23.0	+126	+191	+262

Gross Pathology

The thymus of some treated animals was noted as being small. One HD female had a pale area on the epicardial aspect of the right ventricle; this animal had an abnormal ECG waveform in Week 52. However, there were no correlating microscopic findings.

Table 45. Gross pathology, monkeys (Applicant's table)

Tissue and finding	Group/sex: / Number Examined:	1M 4	2M 4	3M 4	4M 4	1F 4	2F 4	3F 4	4F 4
Heart									
Pale area(s)		0	0	0	0	0	0	0	1
Jejunum									
Nodule(s)		0	0	0	1	0	0	0	0
Pale		0	0	0	0	0	1	1	1
Thymus									
Petechiae		0	0	0	0	0	1	0	0
Small		0	0	1	1	0	2	1	2

Organ Weights

Decreases in mean absolute and relative (to bodyweight) organ weights were observed for heart, pancreas, and thymus at all dose levels, although the effect was not always dose dependent. Some treated animals were noted as having a small thymus at necropsy. Increased mean absolute and relative thyroid weights were observed for treated females and increased mean weights for prostate and testes were observed for treated males; however, correlative microscopic findings of accelerated sexual maturity were not observed.

Table 46. Organ weights, monkey, 52 week (Applicant's table)

			Percent Change from Control					
Dose (mg/kg/d)	0		0.01		0.06		0.36	
Sex	M	F	M	F	M	F	M	F
Heart (g)	17.08	15.11	-8	-18*	-12	-38***	-17	-35***
Rel. to BW (%)	0.391	0.424	-9	-25**	-6	-17*	-12	-19**
Pancreas (g)	8.349	5.483	-25	-11	-25	-21	-32	-29
Rel. to BW (%)	0.190	0.154	-25	-16	-17	+6	-29	-10
Thymus (g)	4.21	3.23	-27	-28	-47	-10	-28	-43
Rel. to BW (%)	0.0979	0.0916	-26	-37	-40	+21	-29	-31
Thyroid (g)	0.641	0.291	-	+29	-	+9	-	+60
Rel. to BW (%)	0.0142	0.0081	-	+37	-	+50	-	+108
Prostate (g)	1.166		+10		+74		+47	
Rel. to BW (%)	0.0255		+8		+62		+67	
Testes (g)	8.397		+7		+28		+33	
Rel. to BW (%)	0.190		+1		+19		+57	

*p<0.05; **p<0.01; "-" = no noteworthy difference from control; F = female; M = male; NA = not applicable.

Histopathology

Adequate Battery: Yes

Peer Review: Yes, conducted by (b) (4) Dr. Inger Thorup, Novo Nordisk (for review of the stomach slides).

Histological Findings

Heart

One HD male showed slight multifocal myocardial vacuolation and degeneration, with karyomegaly, in the left ventricle. To assess this finding further, sections of left ventricle and interventricular septum were examined for all animals. This finding was again observed in the original left ventricle block for this monkey but not in the additional samples examined for that monkey or for any of the other animals. Moreover, no changes in myocardial cells were seen in any of the animals that had shown abnormal ECGs in Week 52.

Thymus

Minimal to slight involution/atrophy was seen in all treated male groups and in females at ≥0.06 mg/kg.

Adrenals

A slightly increased incidence of cortical vacuolation was observed for treated groups, although this did not occur in a dose-related manner.

Mandibular lymph node

An increased incidence of minimal to slight germinal center development was observed in the mandibular lymph node for HD males and all female groups. The pathologist did not feel that this was a treatment-related effect because it was the only lymphoid tissue with this finding and germinal center development in monkeys is known to be a variable feature.

Thyroid
Thyroids were first evaluated by H&E staining. For a more detailed evaluation, serial sections of thyroid in a C-cell rich region from all animals were stained for calcitonin by immunohistochemistry. No effect of treatment on the C-cells was seen.

Pancreas
There were no apparent treatment-related effects on the pancreas. Although interstitial inflammatory cells were observed for semaglutide-treated animals at a low incidence, the same finding was also observed only for a control and LD male, suggesting that this is a background finding when evaluating the genders combined.

Duodenum
Brunner's gland hypertrophy has been observed in semaglutide-treated rats. No treatment-related effects on Brunner's glands were observed in this monkey study.

Sexual maturity
The majority of males on study were sexually immature or not fully mature. Although an increase in mean testicular and prostate weights were observed for MD and HD groups, correlative microscopic findings of accelerated sexual maturity were not observed.

Table 47. Summary of histopathology findings (Applicant's table)

Tissue and Finding	Group/Sex: / Number:	1M 4	2M 4	3M 4	4M 4	1F 4	2F 4	3F 4	4F 4
Heart	Number Examined:	4	4	4	4	4	4	4	4
Focal Shrunken Myocardial Fibres - Right Ventricle		0	0	0	0	0	0	0	1
Myocardial Vacuolation and Degeneration, with Karyomegaly - Left Ventricle		0	0	0	1	0	0	0	0
Thymus	Number Examined:	4	4	4	4	4	4	4	4
Involution/Atrophy		0	1	3	2	0	0	1	4
Adrenals	Number Examined:	4	4	4	4	4	4	4	4
Cortical Hyperplasia - Focal		0	0	0	0	0	0	0	1
Cortical Vacuolation		0	1	1	0	0	1	1	1
LN Mandibular	Number Examined:	4	4	4	4	4	4	4	4
Increased Germinal Centre Development		0	0	0	3	0	1	1	1

Possible drug class targets - no treatment-related effects noted

Thyroids	Number Examined:	4	4	4	4	4	4	4	4
Ectopic Thymic Tissue		2	0	1	1	1	2	2	2
Follicular Dilatation		1	0	0	0	0	1	0	0
Interstitial Lymphoid Aggregates		2	0	2	1	0	0	0	0
Prominent Developmental Cyst(s)		0	2	3	1	0	0	1	0
Pancreas	Number Examined:	4	4	4	4	4	4	4	4
Arteritis/Periarteritis		0	0	0	0	0	1	0	0
Interstitial Inflammatory Cells		1	1	0	0	0	2	1	1
Periductal Inflammatory Cells		0	0	0	0	1	0	0	0
Testes	Number Examined:	4	4	4	4	-	-	-	-
Immature		2	1	2	1	-	-	-	-
Not Fully Mature		1	2	1	1	-	-	-	-
Seminiferous Tubular Dilatation		0	1	1	0	-	-	-	-
Seminiferous Tubular Vacuolation		2	2	1	3	-	-	-	-

Toxicokinetics

The C_{max} and AUC_{0-72h} increased with the dose for both males and females. The increase in exposure was proportional or slightly more than proportional to dose after dosing up to 0.36 mg/kg. No sex differences were observed, although the mean C_{max} and AUC_{0-72h} data suggested generally higher exposure in females compared to males but this was not entirely consistent in the individual data. Following subcutaneous administration for 52 weeks, semaglutide tended to accumulate with an average accumulation index in the range 0.9-3.7.

Table 48. Toxicokinetic parameters (Applicant's table)

Period	Dose (mg/kg)	Gender		C_{max} (nM)	T_{max} (h)	AUC_{last} * ((h)*(nM))	$AUC_{\%extra}$ (%)	Rac_{Obs}	Rac_{Pred}
Week -2	0.01	Female	Mean	16.4	24	260	NC	NC	NC
			Stdev	3.37	0.0	69.7	NC	NC	NC
			%CV	20.6	0.0	26.8	NC	NC	NC
		Male	Mean	14.8	21	240	NC	NC	NC
			Stdev	4.35	6.2	59.1	NC	NC	NC
			%CV	29.4	29	24.6	NC	NC	NC
Week -1	0.01	Female	Mean	24.0	24	449	NC	NC	NC
			Stdev	4.52	0.0	66.5	NC	NC	NC
			%CV	18.9	0.0	14.8	NC	NC	NC
		Male	Mean	21.8	24	424	NC	NC	NC
			Stdev	5.83	0.0	106	NC	NC	NC
			%CV	26.7	0.0	25.1	NC	NC	NC
	0.06	Female	Mean	149	21	2810	NC	NC	NC
			Stdev	26.9	6.5	548	NC	NC	NC
			%CV	18.1	31	19.5	NC	NC	NC
		Male	Mean	142	19	2800	NC	NC	NC
			Stdev	16.7	8.3	313	NC	NC	NC
			%CV	11.7	44	11.2	NC	NC	NC
Day 1	0.01	Female	Mean	14.1	20	751	41	NC	NC
			Stdev	2.59	6.2	97.0	6.0	NC	NC
			%CV	18.4	31	12.9	15	NC	NC
		Male	Mean	13.6	23	764	50	NC	NC
			Stdev	1.22	14	65.1	27	NC	NC
			%CV	9.00	60	8.52	54	NC	NC
	0.06	Female	Mean	95.1	16	5320	59	NC	NC
			Stdev	5.54	6.2	400	12	NC	NC
			%CV	5.83	39	7.53	20	NC	NC
		Male	Mean	83.8	14	4570	49	NC	NC
			Stdev	14.3	4.9	892	7.3	NC	NC
			%CV	17.0	35	19.5	15	NC	NC
	0.36	Female	Mean	720	17	38400	52	NC	NC
			Stdev	118	7.4	5190	8.3	NC	NC
			%CV	16.4	43	13.5	16	NC	NC
		Male	Mean	663	13	36400	46	NC	NC
			Stdev	128	5.9	5020	7.2	NC	NC
			%CV	19.3	46	13.8	16	NC	NC

Period	Dose (mg/kg)	Gender		C_{max} (nM)	T_{max} (h)	AUC_{last} * ((h)*(nM))	$AUC_{\%extra}$ (%)	Rac_{Obs}	Rac_{Pred}
Week 13	0.01	Female	Mean	24.9	13	1340	45	1.8	1.7
			Stdev	4.82	5.5	166	5.2	0.12	0.15
			%CV	19.4	41	12.4	11	6.7	8.8
		Male	Mean	21.9	14	1200	41	1.6	1.7
			Stdev	2.25	4.9	128	4.0	0.15	0.10
			%CV	10.3	35	10.7	9.6	9.6	6.1
	0.06	Female	Mean	180	8.7	9630	49	1.8	2.0
			Stdev	21.1	1.6	1060	7.2	0.18	0.28
			%CV	11.7	19	11.0	15	10	14
		Male	Mean	166	15	9160	56	2.1	2.3
			Stdev	30.7	6.9	1570	4.4	0.51	0.30
			%CV	18.5	45	17.2	7.9	25	13
	0.36	Female	Mean	1210	12	58500	45	1.5	1.8
			Stdev	179	6.2	7040	6.3	0.22	0.28
			%CV	14.8	52	12.0	14	15	16
		Male	Mean	1100	10	56400	48	1.6	2.0
			Stdev	127	2.2	4210	7.8	0.10	0.52
			%CV	11.6	22	7.47	16	6.6	26
Week 26	0.01	Female	Mean	23.1	13	1300	53	1.8	2.1
			Stdev	1.60	5.9	83.8	7.1	0.20	0.33
			%CV	6.91	46	6.43	13	11	16
		Male	Mean	23.9	17	1260	52	1.7	2.0
			Stdev	7.55	7.4	220	4.0	0.22	0.20
			%CV	31.6	43	17.4	7.6	13	9.6
	0.06	Female	Mean	165	13	8870	42	1.7	1.7
			Stdev	23.9	5.5	1160	7.7	0.17	0.30
			%CV	14.5	41	13.0	18	10	17
		Male	Mean	153	13	8040	51	1.8	2.1
			Stdev	38.6	5.5	906	12	0.32	0.50
			%CV	25.3	41	11.3	23	17	24
	0.36	Female	Mean	985	13	46200	44	1.2	1.6
			Stdev	117	5.9	6600	6.0	0.26	0.14
			%CV	11.8	46	14.3	14	21	8.8
		Male	Mean	983	9.3	48700	54	1.4	2.3
			Stdev	177	2.1	4350	11	0.20	0.84
			%CV	18.0	22	8.94	20	14	37

Period	Dose (mg/kg)	Gender		C_{max} (nM)	T_{max} (h)	AUC_{last} * ((h)*(nM))	$AUC_{\%extra}$ (%)	Rac_{Obs}	Rac_{Pred}
Week 39	0.01	Female	Mean	25.8	25	1370	41	1.9	1.7
			Stdev	2.75	18	64.1	6.3	0.25	0.21
			%CV	10.6	72	4.66	16	13	13
		Male	Mean	20.2	16	1190	49	1.6	1.9
			Stdev	3.32	6.2	178	5.5	0.17	0.15
			%CV	16.4	39	14.9	11	11	8.2
	0.06	Female	Mean	152	24	8910	NC	1.7	NC
			Stdev	22.1	0.0	1170	NC	0.20	NC
			%CV	14.6	0.0	13.1	NC	12	NC
		Male	Mean	139	18	8230	46	1.9	1.8
			Stdev	11.2	16	683	3.6	0.53	0.15
			%CV	8.03	89	8.30	7.7	28	8.2
	0.36	Female	Mean	1040	8.7	55300	46	1.5	1.8
			Stdev	123	3.9	6950	5.7	0.28	0.20
			%CV	11.8	45	12.6	12	19	11
		Male	Mean	911	20	51700	40	1.4	1.7
			Stdev	102	16	6220	8.5	0.25	0.29
			%CV	11.2	79	12.0	22	17	17
Week 52	0.01	Female	Mean	29.3	14	1540	42	2.1	1.7
			Stdev	3.05	4.9	94.1	7.6	0.28	0.27
			%CV	10.4	35	6.11	18	13	16
		Male	Mean	24.9	18	1380	40	1.8	1.6
			Stdev	2.63	6.6	155	2.2	0.18	0.087
			%CV	10.5	37	11.2	5.6	9.9	5.3
	0.06	Female	Mean	172	12	9210	47	1.7	1.7
			Stdev	28.9	0.0	1380	9.7	0.24	0.16
			%CV	16.8	0.0	15.0	21	14	9.6
		Male	Mean	166	12	9260	48	2.2	1.9
			Stdev	14.0	0.0	1310	8.1	0.77	0.34
			%CV	8.44	0.0	14.2	17	36	17
	0.36	Female	Mean	1150	11	56900	46	1.5	1.9
			Stdev	131	3.3	6530	8.5	0.10	0.30
			%CV	11.4	31	11.5	19	7.0	16
		Male	Mean	1080	11	52500	43	1.4	1.7
			Stdev	190	1.6	8970	7.3	0.20	0.21
			%CV	17.6	14	17.1	17	14	12

* AUC_{last}: For the dose escalation (adaptation) period (Week -2 and Week -1) $AUC_{last}=AUC_{0\text{-}24\ hr}$
Remaining study days; Day 1, and Weeks 26, 39 and 52: $AUC_{last}=AUC_{0\text{-}72\ hr}$

Anti-Drug Antibody Analysis

No samples were found to be positive for anti-semaglutide antibody formation.

Dosing Solution Analysis

The mean concentrations of test formulations were generally within ±10% of the nominal concentrations. Samples for all dose groups taken during Week 28 were between 68% and 79% of nominal, and the LD formulation samples taken during Weeks 39 and 52 were 85% and 86% of nominal, respectively.
Semaglutide was detected in the vehicle control formulation sampled during Week 28; it was established that this result was due to carry-over from the analysis of the previous sample rather than contamination of the dosing formulation administered to the control animals. These deviations did not negatively impact the interpretability of the study results.

Other studies

Semaglutide drug substance was initially manufactured by a (b) (4) process and the majority of toxicology studies were conducted with drug substance from this process. Before initiation of Phase 3 clinical studies, the manufacturing process was changed to a (b) (4) process where the semaglutide peptide backbone is produced by fermentation in yeast. To qualify the impurity profile related to the new (b) (4) process, a 13-week comparative rat toxicity study with (b) (4) semaglutide was conducted. Furthermore, to qualify the semaglutide end-of-shelf life drug product, a 4-week toxicity study was conducted in rats, comparing aged drug product with fresh drug product.

Study title: Comparative toxicity study by subcutaneous administration of fresh or aged forms of the test material to Sprague Dawley rats for 4 weeks (after an initial 2-week dose escalation period) followed by a 2 week recovery period

Study no.:	209159
Study report location:	Module 4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 8, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Aged NNC 0113-0217, batch 412_N09051A, 85.5% pure Fresh NNC 0113-0217, batch # 412_N09051, 95.8% pure.

Key Study Findings

The fresh and aged semaglutide formulations showed a similar toxicological and toxicokinetic profile. Treatment related findings with both formulations included decrease in body weight gain and food consumption, and histopathological findings in the duodenum (minimal Brunner's gland hypertrophy).

Methods

Doses:

Group	Treatment	Weeks	Dose (mg/kg/day)
1	Control	1-6	0
2	Aged NNC 0113-0217	1	0.01
2	Aged NNC 0113-0217	2	0.1
2	Aged NNC 0113-0217	3-6	0.86
3	Fresh NNC 0113-0217	1	0.01
3	Fresh NNC 0113-0217	2	0.1
3	Fresh NNC 0113-0217	3-6	0.86

Frequency of dosing: Once daily
Route of administration: Subcutaneous injection
Dose volume: 0.5 mL/kg
Formulation/Vehicle: disodium (b) (4) phosphate, dihydrate, propylene glycol and phenol to (b) (4)% of the final volume of water for injection
Species/Strain: Sprague Dawley rats
Number/Sex/Group: 12
Age: 49-55 days old
Weight: 264-329 g for males and 194-246 g for females.
Satellite groups: 6/sex/group for recovery
Unique study design: No
Deviation from study protocol: There were no deviations that affected the integrity or validity of the study

Observations and Results

Mortality

None

Clinical Signs

There were no treatment-related adverse clinical signs.

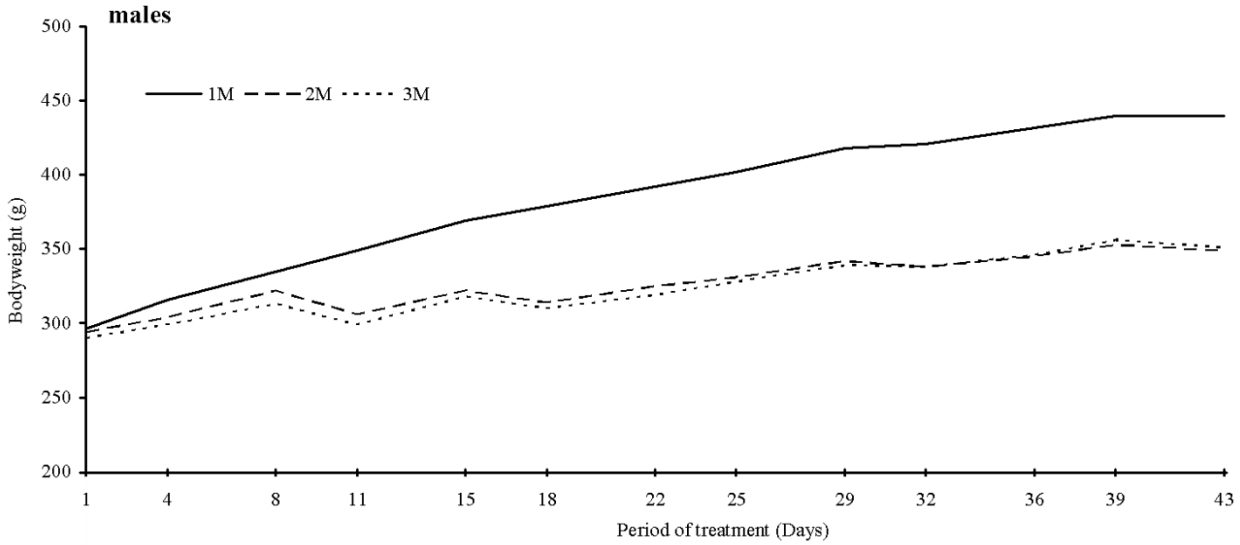
Body Weights

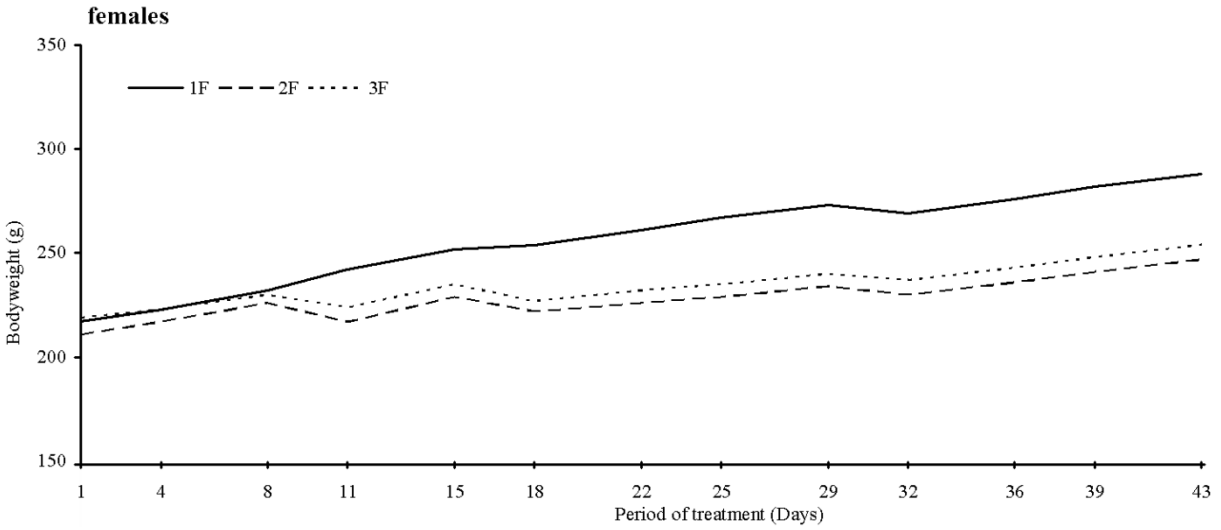
Similar decrease in body weight gain was observed in animals treated with the aged or fresh formulations.

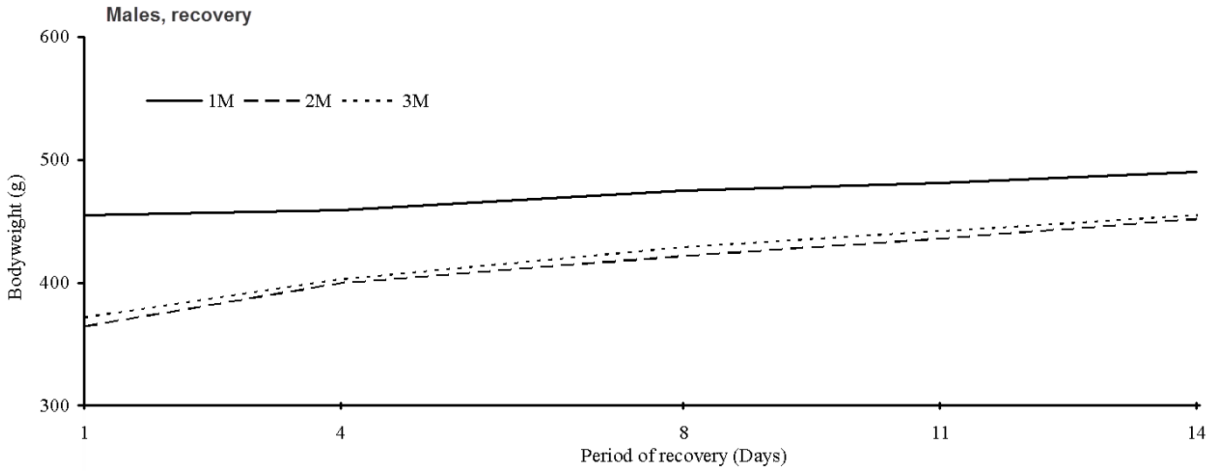
Table 49. Body weight change, percent change from control (Applicant's table)

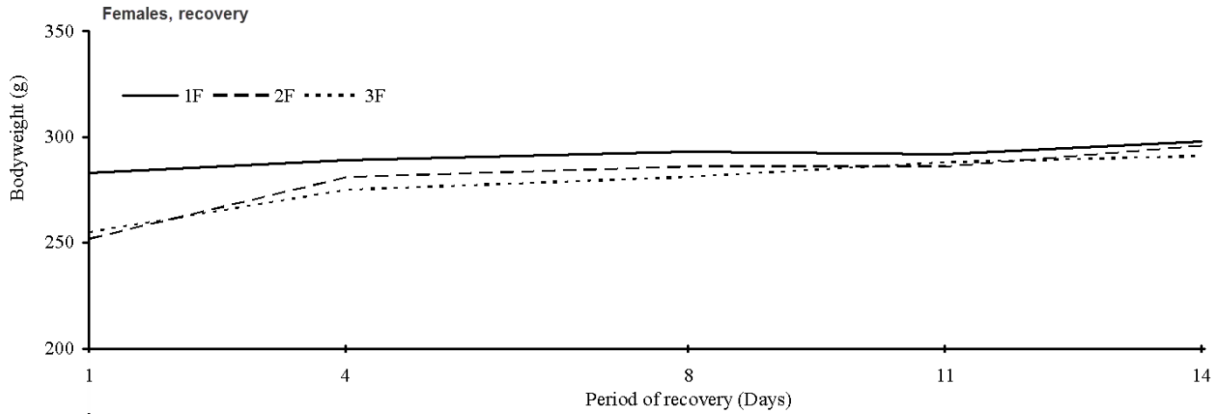
Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	**0**	**0.86**	**0.86**	**0**	**0.86**	**0.86**
Day 1-4	20	-50**	-55**	7	-14	-43
Day 4-8	19	-5	-26*	9	0	-22
Day 8-11	14	-214**	-200**	10	-190**	-170**
Day 11-15	20	-20	-5	10	+20	+20
Day 15-18	10	-180**	-180**	2	-450**	-550**
Day 18-22	13	-15	-31*	7	-43	-29
Day 18-43	61	-43**	-33**	34	-26**	-21*
Day 1-43	144	-62**	-58**	71	-49**	-51**
Day R1-R4	4	+750**	+675**	5	+460**	+300**
Day R4-R8	15	+47	+80*	4	+25	+50
Day R8-R11	7	+100*	+86*	-1	-100	-800**
Day R11-R14	9	+78	+44	6	+50	-67
Day R1-R14	36	+139**	+131**	15	+187**	+133**

Figure 17. Body weight (Applicant's figure)









Feed Consumption

Similar decrease in food consumption was observed in animals treated with the aged or fresh formulations.

Group /Sex		Mean 1-42	As % of Control	Mean R1-R14	As % of Control
1M	Mean	29	-	28	-
	SD	1.4		2.3	
	N	6		2	
2M	Mean	23	80	33	117
	SD	1.3		1.9	
	N	6		2	
3M	Mean	23	78	31	111
	SD	0.8		0.6	
	N	6		2	

Group /Sex		Mean 1-42	As % of Control	Mean R1-R14	As % of Control
1F	Mean	22	-	20	-
	SD	0.8		0.0	
	N	6		2	
2F	Mean	19	85	26	129
	SD	0.6		2.3	
	N	6		2	
3F	Mean	19	86	24	124
	SD	1.3		0.8	
	N	6		2	

Ophthalmoscopy

There were no treatment-related ophthalmoscopic findings.

Hematology

There were no toxicologically significant findings for animals that received either the aged or fresh forms of semaglutide. The small reduction in reticulocyte counts in males was considered of no toxicological significance given the absence of any adverse effect upon related erythrocytic parameters (the erythrocyte counts, hematocrit and hemoglobin concentrations for these animals tended to be higher than controls). Higher platelet count in treated females was attributed to lower than expected values for the control females.

Table 50. Hematology, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	0	0.86	0.86	0	0.86	0.86
Hct	0.418	+5**	+5**	0.403	+1	+1
Hb	14.9	+7**	+6**	14.6	+3	+1
RBC	7.87	+8**	+7**	7.53	+3	+3
Retic	4.29	-29**	-18*	3.70	-4	-6
MCH	19.0	-2	-1	19.4	-1	-2
MCHC	35.7	+1	+1	36.2	+2**	+1
MCV	53.3	-3*	-2	53.5	-2	-2
WBC	10.65	+2	+20	8.73	+11	+10
N	2.06	-17	+34	1.23	+3	-9
L	8.10	+8	+17	7.08	+13	+14
E	0.13	-8	+15	0.12	0	+17
B	0.03	+33	+67*	0.02	+50	+50
M	0.27	-7	+15	0.20	0	-5
LUC	0.07	-14	0	0.09	0	-22
Plt	1117	+7	+2	965	+32**	+30**
PT	14.1	+4	+9*	14.8	+1	+1
APTT	18.7	+1	+5	17.7	-1	+6

Group : 1 2 3
Compound : Control Aged NNC 0113-0217 Fresh NNC 0113-0217
Dose (mg/kg/day) : 0 0.86 0.86

Table 51. Hematology, recovery, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	0	0.86	0.86	0	0.86	0.86
Hct	0.426	+3	+2	0.420	0	+4*
Hb	15.1	+1	+1	15.1	0	+2
RBC	8.56	-1	-1	8.05	-1	+4
Retic	2.64	+28*	+13	1.83	+22	+7
MCH	17.7	+3	+1	18.8	+1	-2
MCHC	35.6	-1*	-2*	36.0	0	-2*
MCV	49.8	+4*	+3	52.3	+1	0
WBC	13.65	-13	-20	10.41	-14	-27**
N	2.65	-28	-24	1.32	-7	-42
L	10.28	-9	-19	8.50	-14	-24*
E	0.15	-7	-27	0.15	-33	-33
B	0.05	-40	-40	0.03	-33	-33*
M	0.41	-17	-17	0.30	-37*	-27
LUC	0.11	+18	-9	0.13	-23	-31*
Plt	1169	+9	0	1129	+14	+5
PT	15.1	-1	+1	16.6	-1	-10
APTT	22.9	-6	-13	17.5	-7	+11

Group : 1 2 3
Compound : Control Aged NNC 0113-0217 Fresh NNC 0113-0217
Dose (mg/kg/day) : 0 0.86 0.86

Clinical Chemistry

There were no toxicologically significant differences between animals which received the aged or fresh forms of semaglutide. Reduction in triglycerides and glucose were observed with either formulation at the end of dosing. No changes were observed two weeks after dose cessation.

Table 52. Clinical chemistry, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	0	0.86	0.86	0	0.86	0.86
ALP	128	+13	+13	76	+18	+22
ALT	51	+12	-6	35	+6	-3
AST	77	-5	-10*	65	+5	+2
Bili	2	0	0	2	0	0
Urea	6.10	+1	0	6.36	-2	-2
Creat	32	-9	-3	38	-11	-8
Gluc	8.61	-13	-8	7.63	-21**	-12*
Chol	1.74	+1	-8	2.23	+7	0
Trig	0.75	-39**	-52**	0.51	-20	-22*
Na	142	+1	+1	142	+1**	+1**
K	4.8	-2	-2	4.2	-2	+5
Cl	103	0	-1	101	+1	+2**
Ca	2.60	0	+1	2.74	-2	-3*
Phos	2.30	-5	-6	2.19	-3	-1
Total Prot	66	+2	+2	73	-4	-7**
Alb	31	+6**	+3*	38	-5	-5*
a1	13	-8	-15*	11	0	-9
a2	5	0	0	5	-20	0
Beta	15	-7*	0	15	-7	-7
Gamma	3	0	+33	5	0	-20
A/G Ratio	0.87	+11**	+8*	1.09	-3	0

Group : 1 2 3
Compound : Control Aged NNC 0113-0217 Fresh NNC 0113-0217
Dose (mg/kg/day) : 0 0.86 0.86

Table 53. Clinical chemistry, recovery, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	**0**	**0.86**	**0.86**	**0**	**0.86**	**0.86**
ALP	92	+20	+24	56	+18	+4
ALT	34	-3	-6	31	-23	-16
AST	71	-4	-1	69	-3	-1
Bili	2	0	0	2	0	0
Urea	5.33	+10	+8	6.30	-1	+4
Creat	31	+3	+19**	48	-15	-13
Gluc	7.17	-4	-6	6.99	+2	-7
Chol	1.42	+20	-6	2.26	+1	-9
Trig	0.43	+28	+16	0.42	+10	-7
Na	143	+1	+1	143	0	0
K	5.0	-6	-8	4.5	-7	0
Cl	102	+2*	+1	102	+1	+2
Ca	2.64	-2	-2	2.71	-5**	-1
Phos	2.58	0	2	2.32	-12	+6
Total Prot	67	-1	-3	71	0	0
Alb	31	0	0	37	-5	0
a1	12	+8	0	11	+9	+9
a2	4	0	0	4	0	0
Beta	15	-7	-7	14	+7	0
Gamma	5	-40*	-20	5	+20	-20
A/G Ratio	0.88	+5	+5	1.10	-10	+1

Group : 1 2 3
Compound : Control Aged NNC 0113-0217 Fresh NNC 0113-0217
Dose (mg/kg/day) : 0 0.86 0.86

Urinalysis

Similar changes were observed in animals receiving the fresh or ages drug formulation, including increase in urinary volume, sodium, and chloride output, and decrease in protein concentration.

Table 54. Urinalysis, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	**0**	**0.86**	**0.86**	**0**	**0.86**	**0.86**
Vol	6.4	+36*	+31*	4.4	+73**	+66**
pH	7.0	0	0	6.3	+6	+8*
SG	1038	-1**	-1**	1038	-1**	-1*
Prot	0.87	-34**	-40**	0.19	-37**	-26*
U-Na	75.8	+22*	+22*	76.9	+13	+22*
U-K	179.9	-40**	-39**	173.9	-36**	-31**
U-Cl	57.6	+22*	+14	57.3	+14*	+24**

Group : 1 2 3
Compound : Control Aged NNC 0113-0217 Fresh NNC 0113-0217
Dose (mg/kg/day) : 0 0.86 0.86

Table 55.Urinalysis, recovery, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	0	0.86	0.86	0	0.86	0.86
Vol	7.6	+29	+12	4.0	+75*	+60
pH	7.6	+3	0	6.6	+3	+3
SG	1032	-1**	-1*	1036	-1	-1
Prot	0.87	-29	-11	0.17	-18	-29
U-Na	82.7	-5	-31	78.7	-18	-1
U-K	182.8	-26*	-19	155.9	-11	-22*
U-Cl	82.2	-18	-36**	56.5	+4	+13

Group	: 1	2	3
Compound	: Control	Aged NNC 0113-0217	Fresh NNC 0113-0217
Dose (mg/kg/day)	: 0	0.86	0.86

Gross Pathology

No toxicologically significant differences were observed between animal given the aged or fresh formulation.

Organ Weights

Overall, no toxicologically significant differences in organ weight were seen between animals administered the aged or fresh forms of semaglutide. All changes were considered secondary to the reduced terminal body weights and had no histological correlates.

Table 56. Organ weight, percent change vs. control (Applicant's table)

	Dosing, week 4			Recovery, week 2		
Group/Sex	1M	2M	3M	1M	2M	3M
Dose (mg/kg/day)	0	0.86	0.86	0	0.86	0.86
Adrenals	0.061	-16	-15	0.065	-17	-6
Brain	2.111	-2	-3	2.112	-2	-2
Epididymides	1.154	-5	-10	1.276	-9	-6
Heart	1.354	-20	-21	1.500	-7	-9
Kidneys	2.792	-12	-8	3.053	-14*	-14*
Liver	12.591	-23	-23	13.438	-10	-12
Lungs + Bronchi	1.601	-14	-12	1.587	-2	-4
Pituitary	0.013	-15	-15	0.014	-14	-7
Prostate	0.958	-28	-27	0.967	-11	-16
Salivary Glands	0.653	-15	-17	0.658	-6	+8**
Seminal Vesicles	1.784	-31*	-32*	1.912	-4	-10
Spleen	0.744	-28	-26	0.775	-6	-4
Testes	3.445	-4	-4	3.581	-9	+1
Thymus	0.345	-18	-14	0.314	-6	-10
Thyroids + Paras	0.017	-12	-12	0.019	-21	-21

* Indicates statistical significance

	Dosing, week 4			Recovery, week 2		
Group/Sex	**1F**	**2F**	**3F**	**1F**	**2F**	**3F**
Dose (mg/kg/day)	**0**	**0.86**	**0.86**	**0**	**0.86**	**0.86**
Adrenals	0.072	-11	-8	0.071	-1	+6
Brain	1.979	-2	0	1.970	+2	+2
Heart	0.957	-17	-11	0.961	+1	+5*
Kidneys	1.904	-9*	-8	1.773	+7	-4
Liver	8.952	-20	-18	8.132	+17**	-2
Lungs + Bronchi	1.364	-13	-5	1.297	+4	-1
Ovaries	0.102	-8	-15	0.094	+2	-13
Pituitary	0.018	-17	-17	0.017	0	+6
Salivary Glands	0.467	-18	-12	0.428	+2	+1
Spleen	0.543	-15*	-14*	0.522	+6	-5
Thymus	0.327	-6*	-9	0.273	+14	+11
Thyroids + Paras	0.014	0	-7	0.014	0	+7
Uterus + Cervix	0.568	+43	+15	0.597	-16	+5

* Indicates statistical significance

Group	:	1	2	3
Compound	:	Control	Aged NNC 0113-0217	Fresh NNC 0113-0217
Dose (mg/kg/day)	:	0	0.86	0.86

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

Similar changes in the duodenum (Brunner's gland hypertrophy) were observed with the aged and fresh formulation.

Table 57. Summary of histopathology findings (Applicant's table)

Dosing phase

Group/sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	**0**	**0.86**	**0.86**	**0**	**0.86**	**0.86**
Brunner's gland hypertrophy						
Minimal	0	10	11	0	11	10
Total	0	10	11	0	11	10
Number of animals examined	12	12	12	12	12	12

Recovery period, week 2

Group/Sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	0	0.86	0.86	0	0.86	0.86
Brunner`s gland hypertrophy						
Minimal	0	0	1	0	2	1
Total	0	0	1	0	2	1
Number of animals examined	6	6	6	6	6	6

Toxicokinetics

There were no major differences in exposure and no evidence of antibody formation between aged and fresh batches of semaglutide.

Table 58. Estimated toxicokinetic parameters on Day 15 and Week 6 (Applicant's table)

Period	Treatment	Gender	C_{max} (nM)	T_{max} (h)	AUC ((h)*(nM))	AUC_{tau} * ((h)*(nM))	$AUC_{\%extra}$ (%)	Rac_{Pred}	Rac_{Obs}
Day 15	0.86 mg/kg/day Aged	Female	1041	6	NC	15250	NC	NC	NC
		Male	1263	10	NC	20330	NC	NC	NC
	0.86 mg/kg/day Fresh	Female	843.5	10	NC	14670	NC	NC	NC
		Male	1032	10	NC	17890	NC	NC	NC
Week 6	0.86 mg/kg/day Aged	Female	1456	3	NC	16530	34.1	1.54	1.08
		Male	1159	3	NC	20590	54.4	2.15	1.01
	0.86 mg/kg/day Fresh	Female	1087	3	NC	19300	37.9	1.59	1.32
		Male	1197	3	NC	21370	55.8	2.21	1.20

* AUC_{tau}=$AUC_{0\text{-}24\ hr}$

Dosing Solution Analysis

All concentrations were between 95 and 100% of nominal concentrations.

Study title: (b) (4) NNC 0113-0217. Comparative toxicity study by subcutaneous administration to SD rats for 13 weeks

Study no.:	210195
Study report location:	Module 4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 22, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) NNC 0113-0217, batch # LP0217K2x12, 96.1% pure (b) (4) NNC 0113-0217, batch # MP0217SDY002, 90.4% pure

Key Study Findings

The aim of this study was to compare the toxicology and toxicokinetics of semaglutide manufactured by a fully (b) (4) process versus semaglutide manufactured by a (b) (4) process.

Generally, the range and extent of the treatment-related findings were similar for both the (b) (4) forms of semaglutide. Treatment related findings included reduced food intake and body weight and histopathological changes in the duodenum (Brunner's gland hypertrophy) and pancreas (acinar atrophy), both minimal to slight in severity.

Methods

Doses:	Week 1: 0.01 mg/kg/day Week 2: 0.1 mg/kg/day Week 3-15: 0.6 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Subcutaneous injection
Dose volume:	0.5 mL/kg
Formulation/Vehicle:	Aqueous solution of 1.42 mg/mL disodium (b) (4) phosphate dihydrate and 14.0 mg/mL propylene glycol in water for injection
Species/Strain:	SD rats
Number/Sex/Group:	15
Age:	49-55 days old at the start of treatment
Weight:	250-325 g (males), 184-234 g (females)
Satellite groups:	5 sex/group for ADAs analysis
Unique study design:	NA
Deviation from study protocol:	No correction factor for test material content had been applied during the preparation of the dose formulations, resulting in the actual doses being reduced from the intended doses by approximately 20-25%. As the doses for both forms of the test material were similar, and there was a similar pharmacological effect on body weight, the findings for both forms of semaglutide could still be compared and the aim of the study could be achieved. Several other deviations occurred but did not affect the validity of the study.

Observations and Results

Mortality

None

Clinical Signs

Hunched posture and thin appearance was observed in one female given (b) (4) NNC 0113-0217 and two females given (b) (4) NNC 0113-0217 after the dose increase from 0.1 to 0.6 mg/kg/day. These animals showed full recovery after two or three days at this dose level. However, one of these females given (b) (4) NNC 0113-0217 was recorded as being thin again from Week 5 until the end of the treatment period (Week 15).

Body Weights

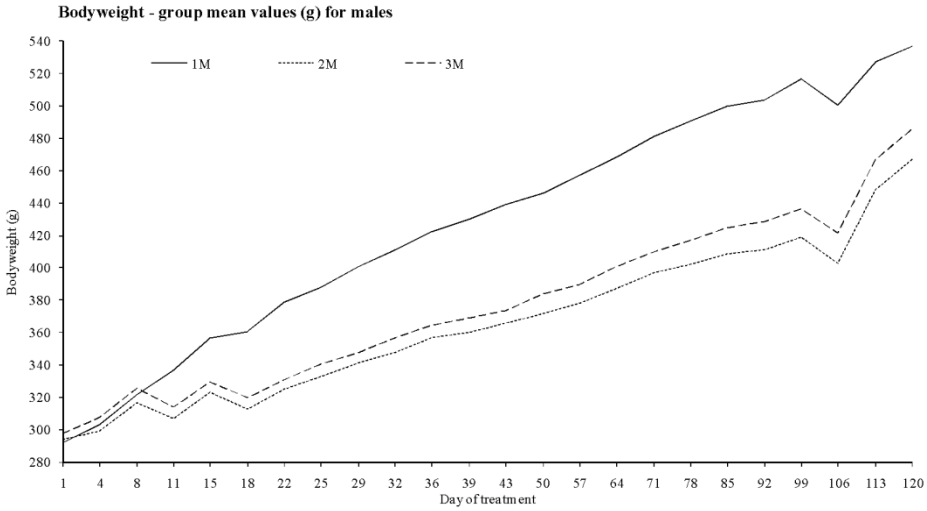
Similar changes in body weight gain and body weight were observed in animals administered the [redacted] drug product.

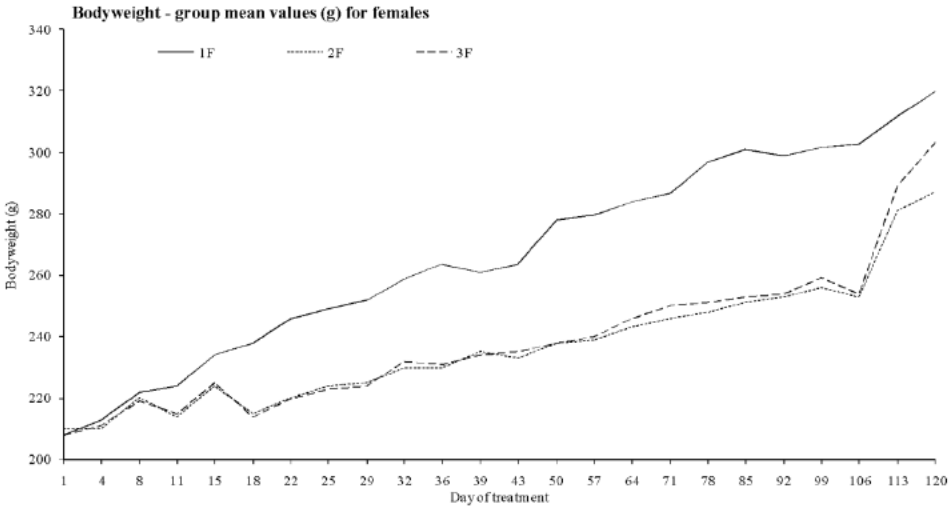
Table 59. Body weight

Males				
	BW gain D1-106		BW	
			D106	D120
	g	% vs. Ctrl	% vs. Ctrl	% vs. Ctrl
Gr 1-Control	209			
Gr 2- [redacted] DP	109	-48	-20	-13
Gr 3 [redacted] DP	124	-41	-16	-9

Females				
	BW gain D1-106		BW	
			D106	D120
	g	% vs. Ctrl	% vs. Ctrl	% vs. Ctrl
Gr 1-Control	95			
Gr 2- [redacted] DP	43	-55	-17	-10
Gr 3- [redacted] DP	46	-52	-16	-5

Figure 18. Body weight

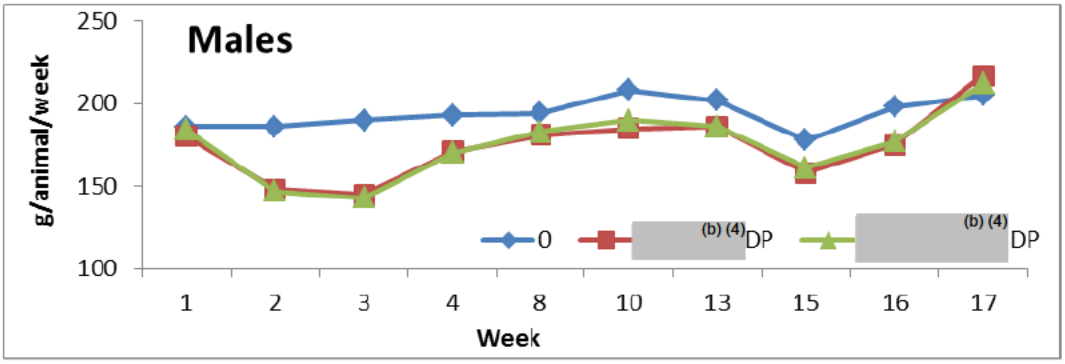


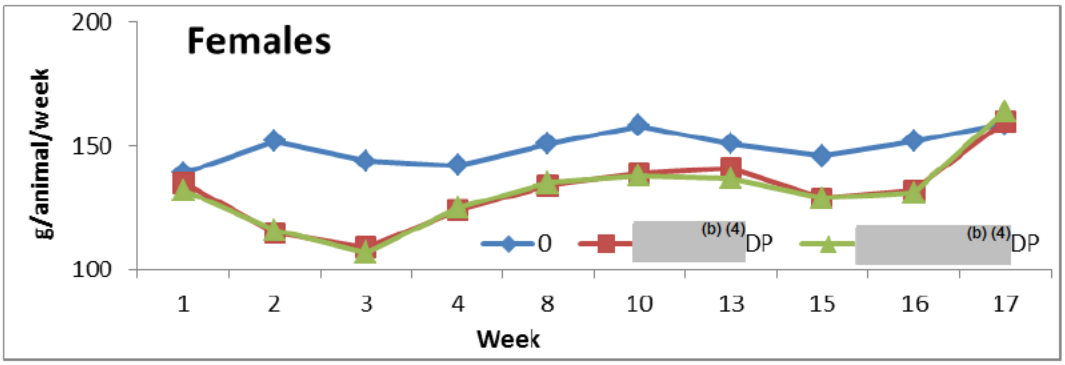


Feed Consumption

Similar decrease in food consumption was observed in animal administered the [redacted] (b) (4) drug product.

Figure 19. Food consumption





Ophthalmoscopy

There were no treatment-related ophthalmoscopy findings.

Hematology

Overall, there were no major differences between animals administered [redacted] semaglutide. Decrease in reticulocyte count was observed in both sexes and of a similar magnitude for those given either [redacted] drug product. There was a slight increase of hematocrit, hemoglobin concentration and erythrocyte count in females with both forms of semaglutide.

Table 60. Hematology, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose level (mg/kg/day)	0	0.6	0.6	0	0.6	0.6
Hct	0.433	+3	0	0.405	+7**	+5**
Hb	14.6	+3	+2	13.8	+8**	+7**
RBC	8.79	+1	-2	7.77	+8**	+7**
Retic	2.34	-29**	-30**	2.34	-26**	-32**
MCH	16.7	+2	+4**	17.8	0	0
MCHC	33.8	+1	+2**	34.1	+1	+1**
MCV	49.3	+1	+2	52.2	-1	-1
WBC	8.79	+12	-9	7.52	-7	-18
N	1.61	-2	-12	0.77	27	+10
L	6.76	+15	-9	6.42	-11	-21
E	0.16	0	-6	0.13	-31*	-31*
B	0.03	+33	-33	0.02	0	0
M	0.18	+6	-11	0.12	+17	+8
LUC	0.06	+17	-17	0.06	-33	-33
Plt	1116	-2	+1	1081	+11	+3
PTP	19.8	+2	-1	19.6	+4	+2
APTT	14.5	+30*	+14	13.6	+9	+6

Clinical Chemistry

Overall, similar changes were observed in animals administered the [redacted] drug product. The most notable change was a reduction in triglycerides.

Table 61. Clinical chemistry, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose level (mg/kg/day)	0	0.6	0.6	0	0.6	0.6
ALP	85	-2	-1	38	+45**	+11^
ALT	61	-3	-3	32	-3	+34
AST	86	0	+7	74	+1	+16
Bili	2	+50	0	2	0	+50
Urea	5.8	-13*	-11	5.31	+11	+5
Creat	29	-17*	-17*	34	-9	-15
Gluc	8.52	-6	-9	6.93	0	+2
Chol	1.83	0	+13	2.63	-15	+2^
Trig	0.9	-59**	-56**	0.65	-40**	-43**
Na	144	0	0	142	+1**	+1*
K	4.9	-4	-4	4.2	+2	0
Cl	102	+1	+1	102	0	-1
Ca	2.6	0	-1	2.6	0	+2
Phos	1.82	-7	-10*	1.42	+6	+8
Total Prot	65	+2	-2	67	0	-3
Alb	28	0	0	33	-6	-3
a1	13	-8	0	11	-9	0
a2	5	0	0	4	0	0
Beta	15	0	0	15	-7	-13
Gamma	4	+25	+25	5	+20	+20
A/G	0.77	-1	+1	0.94	-4	+2

Urinalysis

No major differences were observed between animals receiving (b) (4) semaglutide.

Increase in sodium output which was associated with slightly low potassium output with both forms of semaglutide. High chloride output for males given the (b) (4) form and females given both forms was noted, though statistical significance was not always attained. Urinary volume was increased and specific gravity was reduced compared to controls in males receiving (b) (4) semaglutide and urinary protein levels were reduced in males receiving both forms. The apparent reduction of urinary protein in females was attributed to a high control group mean value due to a grossly high value of 5.4 g/L in one control female.

The changes in the urinary parameters were related to the pharmacological action of semaglutide as GLP-1 agonists have been shown to reduce sodium re-absorption in the proximal tubule (Larsen et al. 2001; Gutzwiller et al. 2004).

Table 62. Urinalysis, percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	1F	2F	3F
Dose level (mg/kg/day)	0	0.6	0.6	0	0.6	0.6
Vol	9.8	+3	+26*	7.1	17	10
pH	6.8	+3	+1	6.4	0	0
SG	1030	0	-1**	1030	0	0
Prot	0.98	-32*	-39**	0.65	-83	-86*
T-Na	0.838	24	+52**	0.59	49**	28
T-K	1.356	-15**	-16**	0.963	-6	-16
T-Cl	0.501	-3	+42*	0.375	54*	23

Gross Pathology

No major differences were observed between animals receiving (b) (4) drug product.

Organ Weights

No toxicologically significant differences were observed between animals receiving (b) (4) drug product. The reduction in organ weights as compared to controls was generally considered secondary to the reduced terminal body weight. The slight reduction in relative heart weight was considered most likely to be incidental and of no biological significance as it was small (7% for males and 5% for females) and not associated with histopathological changes.

Table 63. Organ weights, percent change vs. control (Applicant's table)

	group mean values (g)			relative to bodyweight (%)		
Group/Sex	**1M**	**2M**	**3M**	**1M**	**2M**	**3M**
Dose level (mg/kg/day)	**0**	**0.6**	**0.6**	**0**	**0.6**	**0.6**
Terminal Bodyweight	494	-19**	-15**	494	-19**	-15**
Adrenals	0.055	-9	-7	0.0112	+12*	+9
Brain	2.185	-2	-1	0.448	+21**	+16**
Epididymides	1.275	-6	-1	0.261	+16**	+16**
Heart	1.605	-23**	-21**	0.325	-4	-7*
Kidneys	3.344	-6	-7	0.678	+17**	+9*
Liver	14.813	-22**	-21**	2.99	-4	-6
Lungs + Bronchi	1.896	-16*	-11	0.391	+3	+3
Pituitary	0.013	-8	-8	0.0027	+11	+7
Prostate	0.895	-15*	-5	0.184	+4	+10
Salivary Glands	0.732	-16**	-12**	0.149	+5	+3
Seminal Vesicles	2.006	-15**	-19**	0.408	+6	-4
Spleen	0.728	-22**	-16*	0.148	-3	-1
Testes	3.539	-2	1	0.724	+21**	18**
Thymus	0.194	-11	-9	0.0391	+12	+9
Thyroids + Paras	0.018	-11	-11	0.0035	+11	+11

	group mean values (g)			relative to bodyweight (%)		
Group/Sex	**1F**	**2F**	**3F**	**1F**	**2F**	**3F**
Dose level (mg/kg/day)	**0**	**0.6**	**0.6**	**0**	**0.6**	**0.6**
Terminal Bodyweight	281	-18**	-19**	281	-18**	-19**
Adrenals	0.061	-2	-8	0.0219	+19**	+11
Brain	2.015	0	-3	0.723	+22**	+19**
Heart	1.003	-17**	-22**	0.357	+1	-5*^^
Kidneys	1.916	-4	-7	0.681	+17**	+14**
Liver	8.346	-18**	-21**	2.98	0	-4
Lungs + Bronchi	1.291	-3	-8	0.46	+18**	+13**
Ovaries	0.091	-20**	-26**	0.0323	-2	-11
Pituitary	0.02	-20**	-20*	0.0071	-3	+1
Salivary Glands	0.436	-10*	-11*	0.157	+9	+8
Spleen	0.501	-14*	-22**	0.178	+6	-4
Thymus	0.214	-14	-18	0.0756	+7	+2
Thyroids + Paras	0.015	-20*	-20	0.0052	-4	+2
Uterus + Cervix	0.886	-3	-4	0.323	+17	+16

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings
There was no significant difference in animals given [redacted] (b) (4) forms of semaglutide.

Duodenum
Minimal to slight hypertrophy of the Brunner's glands was observed in all treated male and female groups.

Pancreas
Minimal to slight focal acinar atrophy and minimal to moderate mononuclear inflammatory cells was observed in all treated male and female groups.

Group/sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	0	0.6	0.6	0	0.6	0.6
Brunners Glands Hypertrophy						
Minimal	0	8	5	0	4	5
Slight	0	0	1	0	0	0
Total	0	8	6	0	4	5
Number of tissues examined	10	10	10	10	10	10

Group/sex	1M	2M	3M	1F	2F	3F
Dose (mg/kg/day)	0	0.6	0.6	0	0.6	0.6
Acinar Atrophy, Focal						
Minimal	1	2	2	0	2	3
Slight	0	0	0	0	1	0
Total	1	2	2	0	3	3
Inflammatory Cells – Mononuclear Cells, Exocrine Pancreas						
Minimal	0	3	3	2	0	2
Moderate	0	0	0	0	1	0
Total	0	3	3	2	1	2
Number of tissues examined	10	10	10	10	10	10

Toxicokinetics

No major differences in toxicokinetic parameters were observed between [redacted] (b) (4) semaglutide.

Table 64. Toxicokinetic parameters, [redacted] NNC0113-0217 (Applicant's table)

Day	Treatment	Sex	C_{max} (nM)	t_{max} (h)	AUC_{0-24h} (h*nM)	Rac_{obs}
15	[redacted]	Male	471	6.00	8280	NC
		Female	580	6.00	8990	NC
	[redacted]	Male	445	6.00	7420	NC
		Female	474	6.00	8220	NC
Week 15	[redacted]	Male	857	6.00	14100	1.70
		Female	1040	6.00	15600	1.73
	[redacted]	Male	736	3.00	13700	1.84
		Female	868	3.00	15400	1.88

Antibody analysis

Two males (No. 41 and 42) receiving [redacted] NNC 0113-0217 developed a very low level anti-NNC 0113-0217 antibody response. These antibodies were not neutralizing or cross reactive to endogenous GLP-1.

Table 65. Antidrug antibody (Applicant's table)

Group	Treatment	Screening positive/Total	Neutralising positive/Total
1	Vehicle	0/10	NA
2	[redacted] NNC 0113 -0217	0/10	NA
3	[redacted] NNC 0113-0217	2/10	0/10

Dosing Solution Analysis

The conversion factor that should have been used to allow for the low NNC 0113-0217 content of both test materials had not been applied, and, as a consequence, the theoretical concentrations of NNC 0113-0217 were 0.96 and 0.90 mg/mL for [redacted], respectively. Formulation analysis revealed that the mean actual concentrations of NNC 0113-0217, [redacted], respectively, in Weeks 3 and 15 were between 104 and 105% of the theoretical value for [redacted] NNC 0113-0217 and were between 103 and 104% of the theoretical value for [redacted] NNC 0113-0217. This demonstrated that except for the correction factor error, accurate formulation had occurred.

Treatment	Week	Stated concentration (mg/mL)	Theoretical concentration (mg/mL)	Measured concentration (mg/mL)	Recovery (%) from stated concentration	Recovery (%) from theoretical concentration
Control	3	0		0	ND	ND
Control	3 (backup)	0		0	ND	ND
Control	15	0		0	ND	ND
Control	15 (backup)	0		0	ND	ND
(b) (4)	3	1.20	0.96	1.01	84	105
	3 (backup)	1.20	0.96	1.00	83	104
	15	1.20	0.96	1.01	84	105
	15 (backup)	1.20	0.96	1.01	83	105
	3	1.20	0.90	0.94	78	104
	3 (backup)	1.20	0.90	1.20	100	EX
	15	1.20	0.90	0.93	78	103
	15 (backup)	1.20	0.90	0.93	78	103

ND Not detected
EX Excluded

7 Genetic Toxicology

Semaglutide was tested in a standard battery of genotoxicity tests consisting of Ames test, human peripheral blood lymphocyte chromosome aberration test, and *in vivo* micronucleus test in bone marrow from treated rats. Following changes in the manufacturing process before Phase 3 ((b) (4) semaglutide), the Ames and chromosomal aberration tests were repeated with the (b) (4) semaglutide drug product. Both (b) (4) semaglutide showed no evidence of genetic toxicity. In the following section, data from the in vitro studies with the (b) (4) semaglutide and from the in vivo study with the (b) (4) semaglutide are reported.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Reverse mutation in four histidine-requiring strains of Salmonella typhimurium and two tryptophan-requiring strains of Escherichia coli using a treat and plate methodology

Study no.: 210193
Study report location: Module 4.2.3.3
Conducting laboratory and location: (b) (4)
Date of study initiation: July 12, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) NNC 0113-0217, batch # MP0217SDY002

Key Study Findings

(b) (4) semaglutide was not mutagenic in the absence and in the presence of a rat liver metabolic activation system (S-9) using a treat and plate methodology.

Methods

Strains: TA98, TA100, TA1535, TA1537, WP2 pKM101 and WP2 uvrA pKM101

Concentrations in definitive study: 312.5, 625, 1250, 2500, 5000 ug/plate

Basis of concentration selection: Preliminary assay

Negative control: Purified water

Positive control:

Chemical	Final concentration (μg/mL)	Use Strain(s)	S-9
2-nitrofluorene (2NF)	25	TA98	–
4-nitroquinoline 1-oxide (NQO)	1	TA100	–
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	2.5	TA1535	–
	7.5	WP2 uvrA pKM101	–
		WP2 pKM101**	–
ICR-191	1	TA1537	–
2-aminoanthracene (AAN)	20	WP2 uvrA pKM101	+
	5	TA1535	+
	2.5	TA98	+
	2.5	TA100	+
	2.5	TA1537	+

Formulation/Vehicle: For Test Article: Water for Injection
For Positive Controls: Water/DMSO

Incubation & sampling time: 37±1°C in the dark 2 days (all viability [nutrient agar] plates) or for 2 to 3 days (all mutagenicity [Vogel-Bonner E agar] plates).

Method: The 'Treat and plate' method, in which the agent is incubated with the microbial cells prior to plating, was used in this study. This method is recommended in preference to standard plate-incorporation tests for compounds which may cause artefacts due to growth stimulation [Mahon et al, 1989]. This method allows the separation of treatment and selective systems, and determines total viable cells as well as numbers of mutants.

Study Validity

The number of revertant colonies on negative control plates fell within acceptable ranges and was significantly elevated by positive control treatments. Less than 5% of plates were lost, leaving adequate numbers of plates at all treatments. The study was considered valid.

Results

No increase in revertant colonies was observed following semaglutide treatment in any of the test strains, in the absence and presence of S-9.

Results of formulation analyses demonstrated achieved concentrations within 100±10% of the nominal test article concentrations and were therefore considered acceptable.

Table 66. Summary of mutagenicity data (Applicant's table)

Experiment 1: Viable bacteria counts for (b) (4) NNC 0113-0217 and control treatments in the absence of S-9

Strain	Compound	Conc. Level (µg/mL)	Mean	Standard Deviation	Fold Increase	Revertant numbers per plate
TA98	(b) (4) NNC 0113-0217	8	97.5	0.7	0.9	97, 98
		40	99.0	11.3	0.9	91, 107
		200	78.5	9.2	0.7	85, 72
		1000	116.0	1.4	1.1	115, 117
		5000	103.0	2.8	1.0	101, 105
	Purified Water		106.0	1.4		105, 107
TA100	(b) (4) NNC 0113-0217	8	61.0	7.1	0.6	66, 56
		40	75.5	6.4	0.7	71, 80
		200	82.0	4.2	0.8	85, 79
		1000	80.0	1.4	0.8	81, 79
		5000	96.5	4.9	0.9	100, 93
	Purified Water		105.0	4.2		108, 102
TA1535	(b) (4) NNC 0113-0217	8	84.0	0.0	1.0	84, 84
		40	66.0	1.4	0.8	67, 65
		200	84.5	4.9	1.0	88, 81
		1000	76.5	14.8	0.9	87, 66
		5000	101.5	0.7	1.2	101, 102
	Purified Water		85.5	16.3		97, 74
TA1537	(b) (4) NNC 0113-0217	8	128.0	24.0	0.9	145, 111
		40	114.5	9.2	0.8	108, 121
		200	139.0	8.5	1.0	145, 133
		1000	134.0	9.9	0.9	127, 141
		5000	128.0	2.8	0.9	130, 126
	Purified Water		144.5	2.1		143, 146
WP2 pKM101	(b) (4) NNC 0113-0217	8	210.5	17.7	1.0	223, 198
		40	198.0	26.9	1.0	179, 217
		200	165.0	4.2	0.8	168, 162
		1000	183.5	7.8	0.9	189, 178
		5000	198.5	27.6	1.0	179, 218
	Purified Water		207.0	7.1		212, 202
WP2 uvrA pKM101	(b) (4) NNC 0113-0217	8	213.0	26.9	1.2	232, 194
		40	200.5	40.3	1.2	172, 229
		200	238.5	7.8	1.4	233, 244
		1000	194.5	17.7	1.1	207, 182
		5000	229.5	10.6	1.3	237, 222
	Purified Water		172.0	25.5		154, 190
TA98	2NF	25	71.5	0.7	0.7	71, 72
TA100	NQO	1	98.5	3.5	0.9	96, 101
TA1535	MNNG	2.5	46.0	8.5	0.5	40, 52
TA1537	ICR-191	1	87.5	0.7	0.6	87, 88
WP2 pKM101	MNNG	7.5	137.0	2.8	0.7	139, 135
WP2 uvrA pKM101	MNNG	7.5	129.0	22.6	0.8	113, 145

Experiment 1: Viable bacteria counts for (b) (4) NNC 0113-0217 and control treatments in the presence of S-9

Strain	Compound	Conc. Level (µg/mL)	Mean	Standard Deviation	Fold Increase	Revertant numbers per plate
TA98	(b) (4) NNC 0113-0217	8	90.5	0.7	0.9	90, 91
		40	89.0	1.4	0.9	90, 88
		200	100.5	3.5	1.0	103, 98
		1000	85.0	11.3	0.8	77, 93
		5000	75.0	8.5	0.7	81, 69
	Purified Water		104.5	16.3		93, 116
TA100	(b) (4) NNC 0113-0217	8	99.5	3.5	0.9	97, 102
		40	103.5	10.6	0.9	111, 96
		200	80.0	14.1	0.7	90, 70
		1000	146.5	34.6	1.3	171, 122
		5000	102.5	10.6	0.9	110, 95
	Purified Water		114.0	1.4		115, 113
TA1535	(b) (4) NNC 0113-0217	8	100.5	6.4	1.3	96, 105
		40	102.0	12.7	1.3	111, 93
		200	124.0	8.5	1.6	118, 130
		1000	102.0	15.6	1.3	113, 91
		5000	134.0	11.3	1.7	142, 126
	Purified Water		78.0	25.5		96, 60
TA1537	(b) (4) NNC 0113-0217	8	32.0	11.3	0.9	40, 24
		40	50.0	14.1	1.4	60, 40
		200	45.5	6.4	1.3	41, 50
		1000	58.5	3.5	1.7	61, 56
		5000	52.5	12.0	1.5	61, 44
	Purified Water		35.0	5.7		31, 39
WP2 pKM101	(b) (4) NNC 0113-0217	8	176.0	26.9	0.9	157, 195
		40	182.0	4.2	0.9	179, 185
		200	173.5	6.4	0.9	169, 178
		1000	169.5	21.9	0.9	154, 185
		5000	204.0	5.7	1.0	208, 200
	Purified Water		199.0	26.9		218, 180
WP2 uvrA pKM101	(b) (4) NNC 0113-0217	8	195.0	29.7	1.0	216, 174
		40	172.5	20.5	0.9	158, 187
		200	166.5	3.5	0.8	164, 169
		1000	220.5	2.1	1.1	219, 222
		5000	204.0	1.4	1.0	205, 203
	Purified Water		198.5	2.1		197, 200
TA98	AAN	2.5	70.5	7.8	0.7	76, 65
TA100	AAN	2.5	96.0	1.4	0.8	97, 95
TA1535	AAN	5	82.5	29.0	1.1	62, 103
TA1537	AAN	2.5	44.5	7.8	1.3	39, 50
WP2 uvrA pKM101	AAN	20	174.5	21.9	0.9	159, 190

Experiment 2: Viable bacteria counts for (b) (4) NNC 0113-0217 and control treatments in the absence of S-9

Strain	Compound	Conc. Level (µg/mL)	Mean	Standard Deviation	Fold Increase	Revertant numbers per plate
TA98	(b) (4) NNC 0113-0217	312.5	253.0	0.0	0.8	253, 253
		625	110.5	14.8	0.3	100, 121
		1250	204.5	19.1	0.6	191, 218
		2500	253.0	7.1	0.8	248, 258
		5000	296.0	4.2	0.9	299, 293
	Purified Water		331.5	51.6		368, 295
TA100	(b) (4) NNC 0113-0217	312.5	95.5	7.8	0.5	90, 101
		625	154.0	15.6	0.8	165, 143
		1250	190.5	17.7	1.0	178, 203
		2500	248.0	8.5	1.3	242, 254
		5000	242.5	14.8	1.2	232, 253
	Purified Water		197.0	19.8		211, 183
TA1535	(b) (4) NNC 0113-0217	312.5	62.5	4.9	0.3	66, 59
		625	221.5	6.4	0.9	226, 217
		1250	277.0	28.3	1.2	297, 257
		2500	230.5	16.3	1.0	242, 219
		5000	129.0	12.7	0.5	138, 120
	Purified Water		237.0	14.1		247, 227
TA1537	(b) (4) NNC 0113-0217	312.5	193.5	129.4	0.9	285, 102
		625	246.5	7.8	1.2	241, 252
		1250	244.0	22.6	1.2	228, 260
		2500	554.5	19.1	2.7	541, 568
		5000	252.0	18.4	1.2	265, 239
	Purified Water		204.0	7.1		209, 199
WP2 pKM101	(b) (4) NNC 0113-0217	312.5	325.5	23.3	2.0	342, 309
		625	207.0	35.4	1.3	232, 182
		1250	314.5	57.3	1.9	274, 355
		2500	522.5	19.1	3.2	509, 536
		5000	694.0	53.7	4.2	732, 656
	Purified Water		165.5	21.9		181, 150
WP2 uvrA pKM101	(b) (4) NNC 0113-0217	312.5	721.0	113.1	1.8	801, 641
		625	673.5	91.2	1.6	738, 609
		1250	756.5	12.0	1.8	748, 765
		2500	501.0	1.4	1.2	500, 502
		5000	928.0	87.7	2.3	866, 990
	Purified Water		411.5	74.2		359, 464
TA98	2NF	25	103.5	3.5	0.3	101, 106
TA100	NQO	1	214.5	16.3	1.1	203, 226
TA1535	MNNG	2.5	156.5	21.9	0.7	141, 172
TA1537	ICR-191	1	421.5	74.2	2.1	369, 474
WP2 pKM101	MNNG	7.5	184.0	9.9	1.1	177, 191
WP2 uvrA pKM101	MNNG	7.5	1589.0	33.9	3.9	1565, 1613

Experiment 2: Viable bacteria counts for (b) (4) NNC 0113-0217 and control treatments in the presence of S-9

Strain	Compound	Conc. Level (µg/mL)	Mean	Standard Deviation	Fold Increase	Revertant numbers per plate
TA98	(b) (4) NNC 0113-0217	312.5	267.5	20.5	1.1	253, 282
		625	220.5	47.4	0.9	254, 187
		1250	185.5	16.3	0.8	197, 174
		2500	114.5	33.2	0.5	138, 91
		5000	229.5	12.0	1.0	238, 221
	Purified Water		240.0	15.6		229, 251
TA100	(b) (4) NNC 0113-0217	312.5	352.5	3.5	1.0	350, 355
		625	333.5	29.0	1.0	313, 354
		1250	276.0	1.4	0.8	275, 277
		2500	136.0	0.0	0.4	136, 136
		5000	285.0	31.1	0.8	307, 263
	Purified Water		343.0	28.3		363, 323
TA1535	(b) (4) NNC 0113-0217	312.5	143.5	30.4	0.9	122, 165
		625	367.0	59.4	2.4	325, 409
		1250	399.5	14.8	2.6	389, 410
		2500	299.0	91.9	1.9	364, 234
		5000	189.5	19.1	1.2	176, 203
	Purified Water		156.0	70.7		106, 206
TA1537	(b) (4)t NNC 0113-0217	312.5	144.0	2.8	1.5	142, 146
		625	191.5	10.6	1.9	184, 199
		1250	306.0	8.5	3.1	312, 300
		2500	170.5	29.0	1.7	150, 191
		5000	249.0	21.2	2.5	264, 234
	Purified Water		99.0	38.2		72, 126
WP2 pKM101	(b) (4) NNC 0113-0217	312.5	392.5	44.5	0.5	424, 361
		625	450.0	22.6	0.5	434, 466
		1250	436.5	50.2	0.5	401, 472
		2500	1616.5	62.9	1.9	1661, 1572
		5000	701.0	104.7	0.8	627, 775
	Purified Water		842.0	67.9		794, 890
WP2 uvrA pKM101	(b) (4) NNC 0113-0217	312.5	803.5	129.4	1.5	895, 712
		625	708.5	71.4	1.3	759, 658
		1250	824.0	15.6	1.5	813, 835
		2500	597.0	14.1	1.1	587, 607
		5000	464.0	49.5	0.9	499, 429
	Purified Water		540.5	31.8		563, 518
TA98	AAN	2.5	129.0	9.9	0.5	122, 136
TA100	AAN	2.5	257.5	13.4	0.8	267, 248
TA1535	AAN	5	225.0	5.7	1.4	221, 229
TA1537	AAN	2.5	55.5	9.2	0.6	62, 49
WP2 uvrA pKM101	AAN	20	667.0	1.4	1.2	666, 668

Table 67. Historical negative controls (Applicant's table)

Strain	S-9	No. of studies	No. of plates	Mean	99% reference range[1]	99% confidence interval for group mean of: 4 values [2]	5 values [2]	6 values [2]
		Revertant numbers for individual plates						
TA98	-	50	503	25	10.0-43.0	16.4-33.3	17.1-32.3	17.7-31.5
TA98	+	50	525	35	15.0-56.0	24.7-46.2	25.6-44.9	26.4-44.0
TA100	-	50	572	111	72.0-160.0	88.8-134.1	90.9-131.5	92.6-129.6
TA100	+	50	588	119	77.0-178.0	92.9-145.4	95.4-142.4	97.2-140.1
TA1535	-	50	505	17	5.0-33.0	9.8-24.8	10.4-23.9	10.9-23.2
TA1535	+	50	524	19	6.0-35.0	11.6-26.8	12.2-25.9	12.7-25.2
TA1537	-	50	512	11	2.0-27.0	5.4-17.7	5.9-16.9	6.2-16.3
TA1537	+	50	534	15	4.0-32.0	8.3-22.9	8.9-21.9	9.4-21.2
WP2 pKM101	-	14	140	52	20.0-76.0	38.0-66.0	39.3-64.4	40.2-63.2
WP2 pKM101	+	14	140	61	21.0-93.0	41.7-82.6	43.5-80.1	44.9-78.3
WP2 uvrA pKM101	-	32	359	154	71.0-255.0	111.9-199.5	115.9-194.2	118.9-190.4
WP2 uvrA pKM101	+	32	360	186	94.0-294.0	139.3-235.3	143.7-229.6	147.0-225.4

[1] Reference ranges are calculated from percentiles of the observed distributions.
[2] Calculated from square-root transformed data.

Table 68. Historical positive controls (Applicant's table)

Strain	± S-9	Positive control	Mean No of revertants	SD	Range* lower	Range* upper
TA98	-	2NF	612.5	439.5	-520	1745
	+	AAN	376.4	172.3	-67	820
TA100	-	NQO	593.5	245.4	-39	1226
	+	AAN	281.9	120.6	-29	593
TA1535	-	MNNG	1652.2	631.3	26	3278
	+	AAN	45.6	27.1	-24	115
TA1537	-	ICR-191	590.9	484.3	-657	1838
	+	AAN	31.5	19.2	-18	81
WP2 pKM101	-	MNNG	374.2	120.9	63	686
	+	-	NE	NE	NE	NE
WP2 uvrA pKM101	-	MNNG	329.7	145.6	-45	705
	+	AAN	408.7	205.9	-122	939

* 99% confidence limits about the mean

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes

Study no.: 210194
Study report location: Module 4.2.3.3
Conducting laboratory and location: (b) (4)
Date of study initiation: April 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) NNC 0113-0217, batch # MP0217SDY002, 90.4% pure

Key Study Findings

(b) (4) semaglutide did not induce chromosome aberrations in cultured human peripheral blood lymphocytes in the absence or presence of a rat liver metabolic activation system (S-9) when tested up to 5000 µg/mL.

Methods

Cell line:	Blood from three healthy, non-smoking male volunteers was used.
Concentrations in definitive study:	3000 to 5000ug/mL
Basis of concentration selection:	Preliminary cytotoxicity range-finder experiment
Negative control:	Purified water
Positive control:	

Chemical	Final concentration (μg/mL)	S-9
4-Nitroquinoline 1-oxide (NQO)	2.50	-
	5.00	-
Cyclophosphamide (CPA)	6.25	+
	12.5	+

Formulation/Vehicle:	For Test Article: Water for Injection For Positive Controls: DMSO
Incubation & sampling time:	Experiment 1: 3-hour exposure +/- S-9, 17 hour recovery, harvest 20-hour after start of exposure. Experiment 2: 20-hour exposure - S-9, 3-hour exposure + S-9, 17 hour recovery, harvest 20-hour after start of exposure.

Study Validity

The study was considered valid as:

1. The binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures.
2. The proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the normal range.
3. At least 160 cells out of an intended 200 were suitable for analysis at each concentration, unless 10 or more cells showing structural aberrations (per slide) other than gaps only were observed during analysis.
4. The positive controls induced statistically significant increases in the proportion of cells with structural aberrations.

Results

There was no increase in the frequencies of cells with structural aberrations.

Results of formulation analyses demonstrated achieved concentrations within 100±10% of the nominal test article concentrations and were therefore considered acceptable.

Table 69. Chromosome aberrations assay, Experiment 1 (Applicant's table)

Treatment	Concentration (µg/mL)	Cytotoxicity (%)	% Cells with Chromosome Aberrations (Excluding Gaps)	Historical Control Range (%) [#]	Statistical significance
3+17 hour -S-9	Vehicle [a]	-	1.50	0-3	-
	3500	0	0.00		NC
	4500	16	0.50		NC
	5000	23	0.00		NC
	*NQO, 2.50	ND	23.38		$p \leq 0.001$
3+17 hour +S-9	Vehicle [a]	-	0.50	0-3	-
	4000	0	0.00		NC
	4500	0	0.00		NC
	5000	0	0.50		NC
	*CPA, 12.5	ND	54.05		$p \leq 0.001$

[a] Vehicle control was purified water
* Positive control
[#] 95th percentile of the observed range
NC = Not calculated
ND = Not determined

Table 70. Chromosome aberrations assay, Experiment 2 (Applicant's table)

Treatment	Concentration (µg/mL)	Cytotoxicity (%)	% Cells with Chromosome Aberrations (Excluding Gaps)	Historical Control Range (%) [#]	Statistical significance
20+0 hour -S-9	Vehicle [a]	-	0.50	0-3	-
	3000	0	0.00		NC
	4000	0	0.00		NC
	5000	0	1.00		NC
	*NQO, 2.50	ND	25.66		$p \leq 0.001$
3+17 hour +S-9	Vehicle [a]	-	0.50	0-3	-
	3000	0	1.00		NC
	4000	0	0.50		NC
	5000	0	1.00		NC
	*CPA, 6.25	ND	33.61		$p \leq 0.001$

[a] Vehicle control was purified water
* Positive control
[#] 95th percentile of the observed range
NC = Not calculated
ND = Not determined

Table 71. Historical vehicle control ranges – Male donors (Applicant's table)

		Structural aberrations observed on 100 cells scored		Numerical aberrations observed during scoring of structural aberrations	
		Structural aberrations including gaps	Structural aberrations excluding gaps	Polyploid cells	Numerical aberrations (H+E+P)
-S9	Number of studies	27	27	27	27
	Number of cultures	102	102	102	102
	Median	1	0	0	0
	Mean	0.94	0.61	0.26	0.35
	SD	1.00	0.80	0.53	0.61
	Observed range	**0 – 4**	**0 – 3**	**0 – 2**	**0 – 3**
	95% reference range	**0 – 3**	**0 – 3**	**0 – 2**	**0 – 2**
+S9	Number of studies	26	26	26	26
	Number of cultures	98	98	98	98
	Median	0	0	0	0
	Mean	0.74	0.58	0.27	0.34
	SD	0.93	0.81	0.53	0.62
	Observed range	**0 – 3**	**0 – 3**	**0 – 2**	**0 – 3**
	95% reference range	**0 – 3**	**0 – 3**	**0 – 2**	**0 – 2**

H = Hyperdiploid, E=Endoreduplicated, P = Polyploid
Reference ranges are calculated from percentiles of the observed distributions.

Calculated in April 2010 by (b) (4) from audited report data of studies started between April 2008 and July 2009.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Induction of Micronuclei in the Bone Marrow of Treated Rats

Study no:	665/793 (Sponsor Report #NN206409)
Study report location:	Module 4.2.3.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 22, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, batch # 412_N06314, 92.21% pure

Key Study Findings

- Semaglutide was not clastogenic in the in vivo micronucleus assay in male rats at doses up to 6.9 mg/kg.

Methods

Doses in definitive study:

Treatment	Dose level (mg/kg)	Dose volume (mL/kg)	Number of animals treated	Number of animals sampled	
				24 hours after administration	48 hours after administration
UTC	-	-	6M	6M	-
Vehicle	0	1.0	12M	6M	6M
	1.875	1.0	6M	6M	-
NNC 0113-0217	3.75	1.0	6M	6M	-
NNC 0113-0217	7.5	1.0	12M	6M	6M
CPA (subcutaneous)	25	10	6M	6M	-
CPA (oral)	20	10	6M	6M	-

M Male
UTC Untreated controls

Frequency of dosing: Single administration
Route of administration: Subcutaneous injection
Dose volume: 1 mL/kg
Formulation/Vehicle: Disodium [redacted] (b) (4) phosphate dihydrate 1.42 mg/mL, propylene glycol 14 mg/mL, phenol 5.5 mg/mL, water for injection [redacted] (b) (4), pH 7.4
Species/Strain: Rat/Sprague-Dawley
Number/Sex/Group: 6 males/group
Satellite groups: None
Basis of dose selection: Toxicity range-finding studies.
Negative control: Disodium [redacted] (b) (4) phosphate dihydrate 1.42 mg/mL, propylene glycol 14 mg/mL, phenol 5.5 mg/mL, water for injection [redacted] (b) (4), pH 7.4
Positive control: 20 mg/kg (oral) or 25 mg/kg (subcutaneous) cyclophosphamide (CPA) dissolved in saline. A second route of administration was included because of failure to achieve an adequate mutation frequency with the positive control article in an initial micronucleus study.

Study Validity

The study met the acceptance criteria, as positive control animals in both dosing groups (subcutaneous and oral gavage administrations) exhibited a statistically significant increase in the number of MN PCE when compared with the concurrent control groups, and negative controls were within (48 hour sample) or slightly below (24 hour sample) the historical vehicle control ranges.

Results

Clinical signs

Chromodacryorrhea and lethargy were observed in all treated groups, with higher incidence at the high-dose.

Table 72. Clinical signs (Applicant's table)

24 hour sample time:

Dose (mg/kg)	Animals Treated	Deaths	Observations
UTC	6M	0	Days 1 & 2: Normal (6M)
Vehicle	5M*	0	Day 1: Normal (5M) Day 2: Normal (5M)
1.875	6M	0	Day 1: Normal (6M) Day 2: Normal (3M), chromodacryorrhea (3M), areas of red/brown staining around the snout (1M)
3.75	6M	0	Day 1: Normal (6M) Day 2: Normal (2M), chromodacryorrhea (3M), lethargy (4M)
7.5	6M	0	Day 1: Normal (6M) Day 2: Chromodacryorrhea (2M), lethargy (6M), areas of red/brown staining around the snout (2M)
CPA, 25 (subcutaneous)	6M	0	Days 1 & 2: Normal (6M)
CPA, 20 (oral)	6M	0	Days 1 & 2: Normal (6M)

M Male
UTC Untreated controls
* 6M assigned to vehicle group, however 1M was incorrectly dosed and subsequently killed and discarded

48 hour sample time:

Dose (mg/kg)	Animals Treated	Deaths	Observations
Vehicle	6M	0	Days 1, 2 & 3: Normal (6M)
7.5	6M	0	Day 1: Normal (6M) Day 2: Normal (1M), chromodacryorrhea (4M), lethargy (5M), areas of red/brown staining around the snout (1M)

M Male

Body weight

Decrease in body weight gain was observed in all treated groups, without dose relationship.

Table 73. Body weight gain, percent change (Applicant's table)

Treatment	Body Weight Gain (% increase) (Day 1 to Day 2)
Untreated control	+3.8%
Vehicle	+2.8%
1.875 mg/kg NNC 0113-0217	-10.2%
3.75 mg/kg NNC 0113-0217	-8.5%
7.50 NNC 0113-0217	-12.8%
25 mg/kg CPA	+5.0%
20 mg/kg CPA (oral)	+4.1%
48-hour time point (Day 1 to Day 3)	
Vehicle	+5.6%
7.50 NNC 0113-0217	-11.7%

Micronucleus assessment

Rats treated with NNC 0113-0217 at all doses exhibited group mean %PCE that were within historical vehicle control values and were either similar to or slightly higher than those observed in the concurrent vehicle controls. As such, these data indicated no evidence of test article related bone marrow toxicity (as may be observed as a reduction in %PCE in treated versus control or dose dependent decrease).

Group mean frequencies of MN PCE were similar to and not statistically different from those seen in concurrent vehicle controls for all NNC 0113-0217 dose groups (at both sample times). Individual frequencies of MN PCE for all treated animals were consistent with historical vehicle distribution data and similar to frequencies observed in the concurrent controls.

On the basis of these results, it was concluded that NNC 0113-0217 did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats treated with up to an achieved dose of 6.9 mg/kg NNC 0113-0217. At 6.9 mg/kg individual plasma concentrations of NNC 0113-0217 ranged from 3,414 to 5,685 nmol/L. A summary of the micronucleus data and TK data is shown in the sponsor-generated tables below.

Table 74. Micronucleus assay, 24h sample time (Applicant's table)

Treatment group (mg/kg)	Kill Time (hours)	Sex	% PCE	Group mean % micronucleated PCE per treatment group (±sd)
Vehicle Control	24	M	42.52	0.06 ± 0.07
UTC	24	M	51.00	0.10 ± 0.10
1.875	24	M	51.30	0.08 ± 0.09
3.75	24	M	47.83	0.11 ± 0.09
7.5	24	M	46.17	0.04 ± 0.06
CPA, 25+	24	M	41.83	2.48 ± 0.35
CPA, 20++	24	M	45.75	2.97 ± 0.44

UTC Untreated control
\+ Administered as a single dose (subcutaneous injection)
++ Administered as a single dose (oral by gavage)

Table 75. Micronucleus assay, 48h sample time (Applicant's table)

Treatment group (mg/kg)	Kill Time (hours)	Sex	% PCE	Group mean % micronucleated PCE per treatment group (±sd)
Vehicle Control	48	M	46.22	0.07 ± 0.06
7.5	48	M	46.60	0.07 ± 0.07

Table 76. Toxicokinetics (Applicant's table)

	Subject	Treatment Description	Study Day	Hour Nominal	Run ID	Dilution Factor	Concentration (nmol/L)	LLOQ	Result Comment
1	55	0mg/kg	1	4	1	1	<0.500	0.5nmol/L	Single determination
2	56	0mg/kg	1	4	1	1	<0.500	0.5nmol/L	Single determination
3	57	0mg/kg	1	4	1	1	0.662	0.5nmol/L	
4	58	7.5 mg/kg	1	4	1	1000	4502	0.5nmol/L	
5	59	7.5 mg/kg	1	4	1	1000	3414	0.5nmol/L	
6	60	7.5 mg/kg	1	4	1	1000	5685	0.5nmol/L	

8 Carcinogenicity

Study title: Carcinogenicity Study by Subcutaneous Administration to CD rats for 104 Weeks

Study no.:	207363
Study report location:	Module 4.2.3.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 6, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, batch # LP217G1T01, LP217G1V04, LP0217G1V04, LP0217G1V06
CAC concurrence:	No. The ECAC had insufficient data on which to base dose recommendations. The Committee also recommended that a water injected control should be added. Lower doses were used in the study; a water-injected control was not included. ECAC was not re-consulted before study initiation.

Key Study Findings

Statistically significant neoplastic findings:

- Statistically significant dose-related increase in the incidence of thyroid C-cell adenomas and combined C-cell adenoma and carcinoma was observed in males and females at ≥0.01 mg/kg/day (0.4X MRHD). Dose-related increase in the incidence of C-cell carcinoma was noted in males at ≥0.025 mg/kg/day (0.7X MRHD).

Non-Neoplastic findings:

- There was no treatment-related effect on survival.
- Reduced feed consumption and decreased body weight gain were observed throughout dosing at ≥0.01 mg/kg/day.
- Increase in minimal to moderate focal and diffuse thyroid C-cell hyperplasia was observed in males at all dose levels, with dose-related incidence.
- Increased incidence of minimal to slight Brunner's gland hypertrophy at all doses

Adequacy of Carcinogenicity Study

The study protocol was reviewed by ECAC. The sponsor's dose selection (0.025, 0.08, 0.25mg/kg) was based on a combination of clinical signs (hunched body posture) and a marked decrement in weight gain at 0.784 mg/kg/d, which was interpreted as exceeding the MTD in the 3 month study. However, excessive decrements in weight gain were

observed at all dose levels, including a 21% and 15% decrement in males and females, respectively, at the lowest dose tested (0.004mg/kg). The Committee had insufficient data on which to base dose recommendations because of the decreased weight gain at the lowest dose tested. The Committee was concerned that reduction in body weight gain would confound interpretation of tumor incidence data and suggested that the sponsor explore lower doses to find a no observed effect level regarding body weight. The Committee also recommended addition of a water-injected control group, because the final formulation containe (b) (4) mg/ml of phenol an (b) (4) mg/ml of propylene glycol (b) (4) The applicant lowered the doses, but did not re-consult ECAC prior to study initiation. A water-injected control was not included in the study. The study was considered adequate, based on the finding of tumors. The Committee concurred that the combined incidence of drug-related thyroid C-Cell adenomas and carcinomas was increased in rats at doses of ≥0.01 mg/kg/day in males and females.

Appropriateness of Test Models

The rat is a commonly used test model for carcinogenesis. The route and frequency of dosing was consistent with the intended clinical use of semaglutide.

Evaluation of Tumor Findings

The administration of semaglutide once daily by subcutaneous injection to Sprague-Dawley rats for two years resulted in thyroid C-cell tumors in males and females at ≥0.01 mg/kg/day. A statistically significant dose-related increase in the incidence of C-cell adenoma and combined C-cell adenoma and carcinoma was observed in males and females at ≥0.01mg/kg by trend test and by pairwise comparison. Dose-related increase in the incidence of C-cell carcinoma was observed in males at ≥0.025 mg/kg/day (statistically significant by pairwise and trend tests). Tumors occurred starting at below the clinical exposure.

Methods

Doses:	0.0025, 0.01, 0.025, 0.1 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	0.25 mL/kg
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	1.42 mg/mL disodium (b) (4) phosphate, dihydrate; 14.0 mg/mL propylene glycol; 5.50 mg/mL phenol in water for injection, adjusted to pH 7.4.
Basis of dose selection:	13-week rat study
Species/Strain:	Crl:CD (SD)IGS BR rats
Number/Sex/Group:	70/sex/group
Age:	36-42 days old
Animal housing:	The animals were housed five of one sex per cage in polycarbonate cages with a stainless steel mesh lid
Paradigm for dietary restriction:	NA
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	No
Deviation from study protocol:	None affecting the integrity of the study

Observations and Results

Mortality

There was no treatment related effect on survival. Statistically significant pairwise comparisons in mortality were noted in females when comparing the control group versus the mid and mid-high dose groups (p=0.0227 and p=0.0151 respectively), but without the corresponding statistically significant dose response relationship.
The most common factors contributing to death were pituitary tumors in males and females and mammary tumors in females; the incidence for both was not affected by treatment.

Figure 20. Kaplan-Meier survival functions for male rats

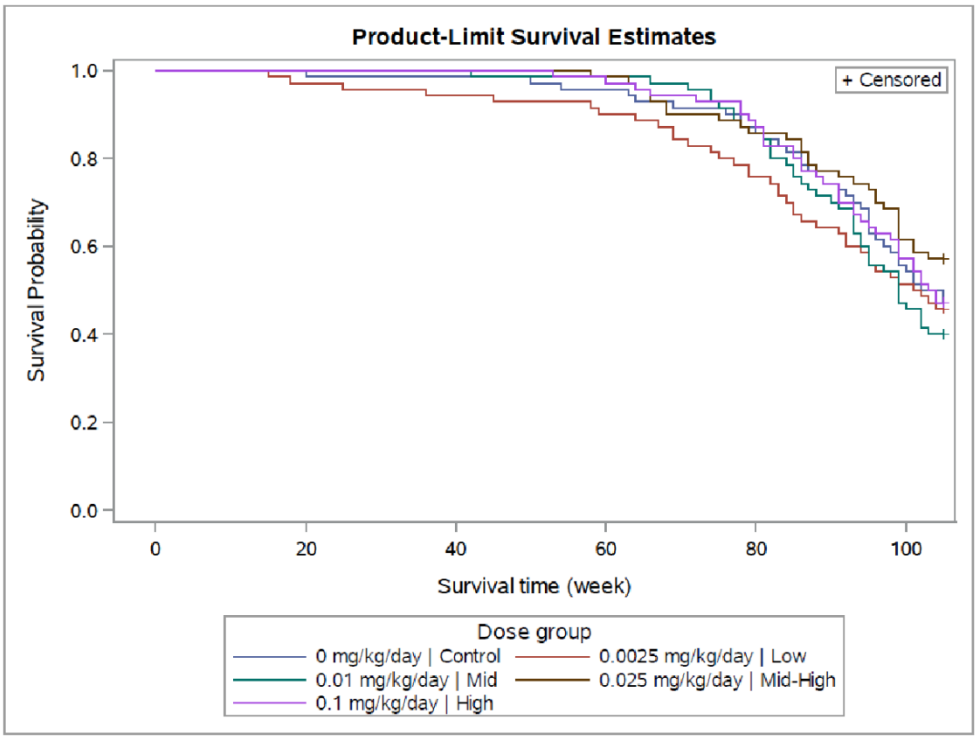


Figure 21. Kaplan-Meier survival functions for female rats

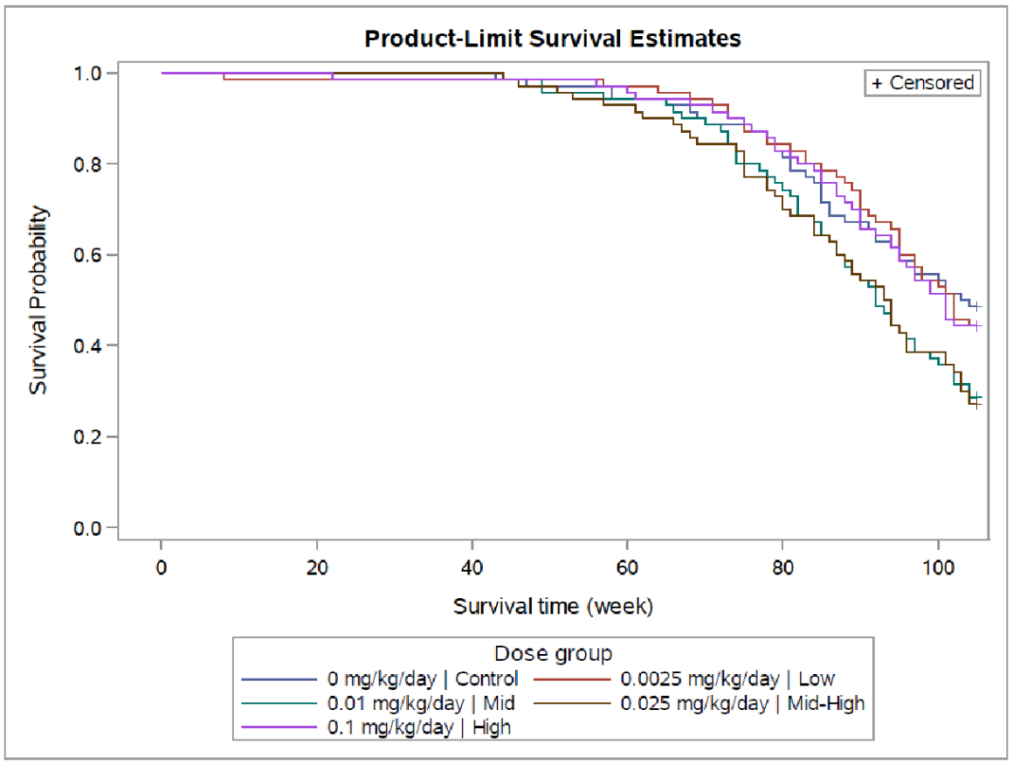


Table 77. Group distribution of mortality (Applicant's table)

Group/sex	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (mg/kg/day)	0	0.0025	0.01	0.025	0.1	0	0.0025	0.01	0.025	0.1
Number of animals/group	70	70	70	70	70	70	70	70	70	70
Number of decedents	35+	38	42	30	37	36	39	50	51	39
Number surviving	35	32	28	40	33	34	31	20	19	31
% Survival	50	46	40	57	47	49	44	29	27	44

+ Two animals died during terminal procedures and were not included in statistical analysis, in the tables decedants for Group 1 is 37 and for terminals is 33.

Table 78. Factors contributory to death (Applicant's table)

	-- Animals Affected --									
Animal sex:	-- Males --					-- Females --				
Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
No. in group:	70	70	70	70	70	70	70	70	70	70
Factors .....Number examined:	37	38	42	30	37	36	39	50	51	39
Unknown	8	6	7	2	3	0	0	1	1	0
Poor Clinical Condition	0	2	3	1	1	0	0	0	1	2
Pituitary Neoplasm	8	7	18	11	24	14	21	22	22	17
Mammary Neoplasm	0	0	0	0	0	14	12	17	17	9

Clinical Signs

Increased incidence of thin appearance and hunched posture was observed in all treated male and female groups.

Table 79. Clinical signs (Applicant's table)

Weeks -1 - 106		Number of animals affected									
	Group/sex:	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Category	Observation	Number in group: 70	70	70	70	70	70	70	70	70	70
Build Conformation	Thin	8	7	16	14	25	8	12	18	27	32
Posture	Hunched	7	11	16	14	27	17	19	27	24	27

Body Weights

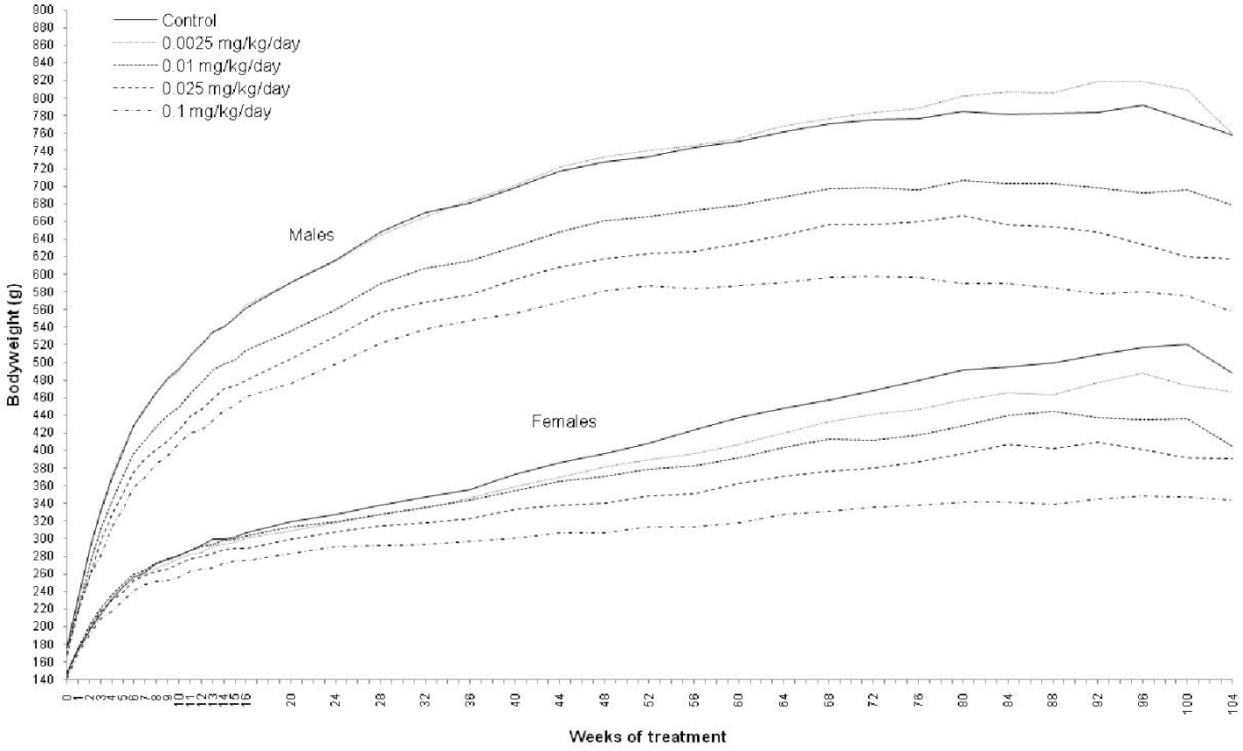
Dose-related decrease in body weight gain and body weight was observed consistently throughout the treatment period in males and females at ≥0.01 mg/kg/day.

Table 80. Body weight

Males					
	BW		BW gain		BW wk 104
	wk 0	wk 104	g	%	% vs. Ctrl
0	176	758	582		
0.0025	176	759	583	0	0
0.01	175	679	504	-23	-10
0.025	167	618	451	-23	-18
0.1	169	558	389	-33	-26

Females					
	BW		BW gain		BW wk 104
	wk 0	wk 104	g	%	% vs. Ctrl
0	147	488	341		
0.0025	146	467	321	-6	-4
0.01	149	405	256	-28	-17
0.025	145	391	246	-28	-20
0.1	141	344	203	-40	-30

Figure 22. Body weight (Applicant's figure)



Feed Consumption

Dose-related decrease in food consumption was observed in males at ≥0.01mg/kg/day and in females at ≥0.1 mg/kg/day.

Ophthalmoscopy

Conducted during Weeks 52 and 100 in control and high dose animals (20/sex)

There were no treatment-related changes.

Hematology

Blood samples were obtained from all animals after overnight withdrawal of food during Week 104 of treatment (before dosing).

No toxicology relevant findings were observed.

Clinical chemistry

- Decrease in cholesterol and triglycerides in males and females at ≥0.01 mg/kg/day, mostly dose-related.
- Decrease in potassium and phosphorus, and increase in albumin and A/G ratio in males at ≥0.025mg/kg/day.

Table 81. Clinical chemistry-percent change vs. control (Applicant's table)

Group/Sex	1M	2M	3M	4M	5M
Dose Level (mg/kg/day)	0	0.0025	0.01	0.025	0.1
Chol	3.84	-12	-21*	-21*	-35**
Trig	1.80	-16	-43**	-47**	-66**
Na	142	+0	+1	+0	+1**
K	5.0	-5	-9	-7	-22*
Cl	102	-1	-1	-1	-1
Ca	2.67	+1	-2	-1	-3**
Phos	1.46	-6	-10	-12*	-9*
Total Prot	70	+0	-2	-1	-2
Alb	25	+5	+5	+12*	+10*
a1	14	+11	-2	-11*	-17**
a2	5	-18*	-1	+16*	0
Beta	20	-2	-2	-9*	-11**
Gamma	7	-14	-18	-4	+7
A/G Ratio	0.55	+7	+11	+20**	+22**

Group/Sex	1F	2F	3F	4F	5F
Dose Level (mg/kg/day)	0	0.0025	0.01	0.025	0.1
Chol	3.55	+0	-21*	-14*	-26**
Trig	2.83	-21*	-64**	-66**	-76**

Urinalysis

During Week 103 of treatment, overnight urine samples were collected from 20 males and 20 females (those suitable with the lowest surviving animal numbers in each group). Animals were placed in an individual metabolism cage without food or water at approximately 16.00 hours; urine was collected until approximately 08.30 hours the following day.

Slight decrease in urinary volume, without statistical significance, and in potassium output was observed in HD males and females.

Gross Pathology

- Dose-related increased incidence of masses in the thyroid gland and deep cervical lymph nodes in all treatment groups.
- Increased incidence of forestomach depressions at ≥0.025mg/kg
- Increased incidences of pale areas in the lungs, without dose relationship
- Thickened uterus at all doses and increased masses at HD.

Table 82. Summary of macroscopic findings (Applicant's table)

Tissue and finding	Group/sex: Number Examined:	1M 70	2M 70	3M 70	4M 70	5M 70	1F 70	2F 70	3F 70	4F 70	5F 70
Thyroids											
Mass(es)		4	8	9	13	20	2	5	6	10	10
Ln deep cervical											
Mass(es)		0	1	0	2	3	0	2	1	0	0
Regional to mass		3	6	6	9	12	2	5	2	5	9
Stomach											
Forestomach depression(s)		4	3	6	12	19	6	5	1	7	13
Lungs + bronchi											
Brown area(s)		0	0	0	0	0	0	1	0	0	0
Pale area(s)		12	27	21	22	22	17	19	28	27	34
Pituitary											
Mass		16	15	23	22	31	41	41	45	44	31
Uterus											
Mass(es)		-	-	-	-	-	9	6	9	11	19
Thickened		-	-	-	-	-	5	15	21	13	15

Histopathology

Histopathology was conducted on all animals sacrificed at scheduled necropsy and all animals killed or dying during the study.

Peer Review: Yes. (b) (4)
Inger Thorup from Novo Nordisk.

Neoplastic
Thyroids

- Increased incidence of C-cell adenoma and combined C-cell adenoma and carcinoma was observed in males and females at >0.01 mg/kg/day, reaching statistical significance by pairwise and trend test.
- Increased incidence of C-cell carcinoma was observed in males at ≥0.025 mg/kg/day (pairwise and trend test). Some of the carcinomas were observed to have metastasized to the deep cervical lymph nodes.

Table 83. Summary of neoplastic findings in the thyroid glands

Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Vehicle Control Group in Rats

Organ name	Tumor name	0 mg Vehicle (C)	0.0025 mg Low (L)	0.01 mg Mid (M)	0.025 mg Mid-High (MH)	0.1 mg High (H)
		P-Trend	P-L vs. C	P-M vs. C	P-C vs. MH	P-C vs. H
Male						
Thyroids	C-Cell Adenoma	7/70 (56)	17/68 (52)	29/69 (59)	42/69 (63)	41/69 (63)
		<0.0001 $	0.0106 @	<0.0001 $	<0.0001 $	<0.0001 $
	C-Cell Carcinoma	3/70 (56)	3/68 (50)	12/69 (55)	13/69 (58)	24/69 (59)
		<0.0001 $	0.6053	0.0108 @	0.0081 $	<0.0001 $
	C-Cell Adenoma/ C-Cell Carcinoma	10/70 (57)	19/68 (53)	32/69 (59)	48/69 (63)	53/69 (66)
		<0.0001 $	0.0246 @	<0.0001 $	<0.0001 $	<0.0001 $
Female						
Thyroids	C-Cell Adenoma	6/70 (53)	17/70 (56)	28/69 (52)	31/70 (53)	45/70 (62)
		<0.0001 $	0.0130 @	<0.0001 $	<0.0001 $	<0.0001 $
	C-Cell Carcinoma	2/70 (53)	4/70 (54)	6/69 (48)	5/70 (47)	8/70 (54)
		0.0598	0.3482	0.1049	0.1715	0.0495 @
	C-Cell Adenoma/ C-Cell Carcinoma	8/70 (53)	19/70 (56)	34/69 (54)	32/70 (53)	50/70 (63)
		<0.0001 $	0.0192 @	<0.0001 $	<0.0001 $	<0.0001 $

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
$ = Statistically significant in common tumor at 0.005 level for test of dose response relationship and at 0.01 level for test of pairwise comparisons, or in rare tumor at 0.025 level for test of dose response relationship and at 0.05 level for test of pairwise comparisons;
@ = Not statistically significant in common tumor at 0.005 level for test of dose response relationship and at 0.01 level for test of pairwise comparisons; or in rare tumor at 0.025 level for test of dose response relationship and at 0.05 level for test of pairwise comparisons;
NC = Not calculable.

Table excerpted from Dr. Chen's biostatistics review

Table 84. Summary of neoplastic findings in the deep cervical LN (Applicant's table)

Tissue and Finding	Group/Sex: Number:	1M 70	2M 70	3M 70	4M 70	5M 70	1F 70	2F 70	3F 70	4F 70	5F 70
LN Deep Cervical	Number Examined:	3	7	8	12	17	2	5	4	7	9
N-Carcinoma		0	1	3	4	8	1	2	2	3	2
N-Histiocytic Sarcoma		0	1	1	0	0	0	0	0	0	0
N-Lymphoma		0	0	0	1	0	0	0	0	0	0

Non Neoplastic

Thyroid

Increase in focal C-cell hyperplasia in males (minimal to moderate) and females (moderate) at all dose levels.

Duodenum
Increased incidence of minimal to slight Brunner's gland hypertrophy at all doses and minimal dilatation at ≥0.01 mg/kg/day were noted in males and females. These changes were not associated with any inflammation or cellular damage, and were considered to be pharmacologically mediated due to increased glandular activity following reduced food intake and to GLP-1 receptor expression (Körner et al, 2007).

Stomach, non-glandular
Increased incidence of minimal to moderate ulceration and epithelial hyperplasia were observed in males at ≥0.01 mg/kg/day and in females at high dose. Historical controls were not submitted. According to the sponsor, the incidence of ulcers was slightly above the historical control range at 0.1 mg/kg/day. Ulceration of the forestomach is a common spontaneous lesion in rodents. In this study, the etiology of the stomach changes is unknown, but recent literature has associated a variety of factors with these lesions, including feeding regimen (e.g. protein restriction and starvation) and stress (Gopinath C, 2014; Greaves P 2012). There is no counterpart to the non-glandular stomach in humans.

Lung
Increased incidence of minimal to moderate alveolar macrophages aggregations was noted in males at all doses and in females at ≥0.01 mg/kg/day. Incidence and severity of this finding was generally not dose-related. The finding correlated with the macroscopic finding of pale areas of the lungs. As this is a commonly observed background change in rats (Greaves P 2012; McInnes EF 2012), and since there was no clear dose-response, the relationship to treatment was considered to be uncertain, and the changes were considered non-adverse.

Adrenals
Increased incidence of minimal to severe cortical cystic/hemorrhagic degeneration was observed in males at ≥0.01 mg/kg/day, without dose-related incidence or severity. As this is a common age related finding in rats (Chandra S, 2013), the relationship to treatment was considered uncertain and the finding of uncertain toxicological significance.

Table 85. Summary of hyperplastic findings in the thyroid glands (Applicant's table)

Group	1	2	3	4	5
Dose (mg/kg/day)	**0**	**0.0025**	**0.01**	**0.025**	**0.1**
Males					
C cell hyperplasia, diffuse					
Minimal	12	16	10	13	5
Slight	1	8	5	6	2
Moderate	1	1	0	3	0
Total	14	25	15	22	7
C cell hyperplasia, focal					
Minimal	8	11	13	11	10
Slight	2	8	12	11	14
Moderate	1	2	5	7	2
Total	11	21	30	29	26
Number of animals examined	70	68	69	69	69
Females					
C cell hyperplasia, diffuse					
Minimal	12	18	5	6	9
Slight	7	13	6	12	1
Moderate	1	2	2	5	3
Total	20	33	13	23	13
C cell hyperplasia, focal					
Minimal	12	6	10	3	5
Slight	14	12	15	16	13
Moderate	1	5	4	5	7
Total	27	23	29	24	25
Number of animals examined	70	70	69	70	70

Table 86. Summary of non-neoplastic changes in the Brunner's glands (Applicant's table)

Group	1	2	3	4	5
Dose (mg/kg/day)	**0**	**0.0025**	**0.01**	**0.025**	**0.1**
Males					
Hypertrophy					
Minimal	1	3	27	29	40
Slight	0	0	1	2	0
Total	1	3	28	29	40
Dilatation					
Minimal	8	1	5	9	9
Slight	0	1	0	1	0
Total	8	2	5	10	9
Number of animals examined	70	70	70	70	70
Females					
Hypertrophy					
Minimal	1	3	9	32	33
Slight	0	0	0	1	3
Total	1	3	9	33	36
Dilatation					
Minimal	0	0	2	5	7
Total	0	0	2	5	7
Number of animals examined	70	69	70	70	70

Table 87. Summary of non-neoplastic changes in the stomach (Applicant's table)

Group	1	2	3	4	5
Dose (mg/kg/day)	**0**	**0.0025**	**0.01**	**0.025**	**0.1**
Males					
Ulceration, non-glandular region					
Minimal	0	0	1	0	2
Slight	1	0	3	4	6
Moderate	1	1	0	1	2
Marked	0	0	1	0	0
Total	2	1	5	5	10
Epithelial hyperplasia, non-glandular region					
Minimal	0	0	0	0	0
Slight	1	1	6	5	9
Moderate	0	0	3	1	2
Marked	1	0	0	0	0
Total	2	1	9	6	11
Epithelial hyperplasia and ulceration, combined, present	2	1	4	4	5
Number of animals examined	70	70	70	70	70
Females					
Ulceration, non-glandular region					
Slight	2	1	0	0	3
Moderate	1	1	1	2	3
Total	3	2	1	2	6
Epithelial hyperplasia, non-glandular region					
Minimal	0	0	1	0	0
Slight	1	2	1	1	3
Moderate	3	3	1	3	4
Total	4	5	3	4	7
Epithelial hyperplasia and ulceration, combined, present	2	0	0	1	5
Dilated glands					
Slight	15	4	3	6	3
Total	15	4	3	6	3
Number of animals examined	70	70	70	70	70

Table 88. Summary of non-neoplastic changes in the lungs (Applicant's table)

Group	1	2	3	4	5
Dose (mg/kg/day)	**0**	**0.0025**	**0.01**	**0.025**	**0.1**
Males					
Aggregations of alveolar macrophages					
Minimal	3	7	5	5	9
Slight	2	7	10	11	6
Moderate	0	0	0	0	1
Marked	1	0	0	0	0
Total	6	14	15	16	16
Number of animals examined	70	70	70	70	70
Females					
Aggregations of alveolar macrophages					
Minimal	5	5	4	5	2
Slight	3	5	9	4	9
Moderate	1	0	3	5	4
Total	9	10	16	14	15
Number of animals examined	70	70	70	70	70

Table 89. Summary of non-neoplastic changes in the adrenal glands (Applicant's table)

Group	1	2	3	4	5
Dose (mg/kg/day)	**0**	**0.0025**	**0.01**	**0.025**	**0.1**
Males					
Cortical cystic/haemorrhagic degeneration					
Minimal	0	1	0	0	3
Slight	9	10	14	13	7
Moderate	3	3	9	8	14
Marked	0	0	1	0	1
Severe	0	1	0	0	0
Total	12	15	24	21	25
Number of animals examined	70	70	70	70	70

Toxicokinetics

AUC_{0-24} and Cmax increased approximately dose proportionally and some accumulation was observed for both sexes. No clear sex differences were observed.
There was no evidence of anti-drug antibody formation at either week 53 or 105.

Table 90. Summary of TK parameters in the rat (Applicant's table)

Group	Period (Week)	Dose (mg/kg)	Gender	C_{max}	t_{max}	AUCtau*
				(nM)	(h)	((h)*(nM))
2	1	0.0025	Female	<LLOQ	NR	NR
			Male	<LLOQ	NR	NR
	52		Female	<LLOQ	NR	NR
			Male	<LLOQ	NR	NR
	104		Female	<LLOQ	NR	NR
			Male	<LLOQ	NR	NR
3	1	0.01	Female#	11.6	6	NR
			Male#	13.4	3	NR
	52		Female	9.21	1.5	247
			Male	7.94	1.5	NR
	104		Female	22.1	3	321
			Male	13.6	3	264
4	1	0.025	Female	20.2	3	244
			Male	26.1	3	NR
	52		Female	25	3	603
			Male	20.7	3	465
	104		Female	24.4	3	609
			Male	30.9	3	673
5	1	0.1	Female	72.3	6	1060
			Male	78.2	6	1270
	52		Female	127	1.5	2970
			Male	112	3	2490
	104		Female	219	3	4220
			Male	148	3	3410

* $AUC_{tau} = AUC_{0\text{-}24\ hr}$. AUC_{tau} not reported if the last measured time-point was detected before 24hr
# Several animals not included in the plasma concentration time profiles, i.e. parameters only rough estimates
NR No results

Dosing Solution Analysis

The results of achieved concentration analyses were within the range -9 to +6% of the nominal concentration at concentrations of 0.04 mg/mL and above, with the exception of 0.04 mg/mL at Week 15 (-11%) and Week 28 (22%). At 0.01 mg/mL (the lowest concentration) lower recoveries were apparent (-22 to -1%), as were expected at this concentration and these were judged to be a result of analytical variation.

Study title: Carcinogenicity Study by Subcutaneous Administration to CD-1 Mice for 104 Weeks

Study no.:	207362
Study report location:	Module 4.2.3.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 6, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, batch # LP217G1T01, LP217G1V04, LP0217G1V04, LP0217G1V06
CAC concurrence:	Yes. The applicant used the ECAC recommended doses. However, a water injected control was not added.

Key Study Findings

Statistically significant neoplastic findings:
Thyroid C-cell adenoma and combined C-cell adenoma and carcinoma were statistically significant increase in all treated male (pairwise comparison) and female (trend and pairwise tests) groups.

Non-Neoplastic findings:

- There was no treatment related effect on survival.
- Reduced feed consumption and decreased body weight gain was evident in all treated groups.
- Increased incidence of minimal to moderate C-cell focal/multifocal hyperplasia in all treated male and female groups, with dose-related total incidence in males.
- Increased incidence of minimal to moderate Brunner's glands dilatation was observed in all treated groups, without dose relationship.

Adequacy of Carcinogenicity Study

The applicant used the doses recommended by the ECAC, but did not include a water-injected control. The Committee concurred that the study was adequate.

Appropriateness of Test Models

The mouse is a commonly used test model for carcinogenesis. The route and frequency of dosing was consistent with the intended clinical use of semaglutide.

Evaluation of Tumor Findings

The administration of semaglutide once daily by subcutaneous injection to CD-1 mice for two years resulted in statistically significant increase in the incidence of thyroid C-

cell adenoma and combined C-cell adenoma and carcinoma in all treated male (pairwise comparison) and female (trend and pairwise tests) groups.
Tumors were observed at and above 2 and 5 fold the clinical exposure in males and females, respectively.

Methods

Doses:	M: 0, 0.3, 1, 3 mg/kg/day F: 0, 0.1, 0.3, 1 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	1 mL/kg
Route of administration:	Subcutaneous (bolus) injection
Formulation/Vehicle:	1.42 mg/mL disodium (b) (4) phosphate, dehydrate, 14.0 mg/mL propylene glycol and 5.50 mg/mL phenol in water for injection, adjusted to pH 7.4
Basis of dose selection:	MTD (decrease in body weight) in 13-wk study
Species/Strain:	Crl:CD1 (ICR) (CD-1) mice
Number/Sex/Group:	60/sex/group
Age:	36-42 days
Animal housing:	The animals were housed three of one sex per cage in polycarbonate cages with a stainless steel mesh lid
Paradigm for dietary restriction:	NA
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	12/sex/group
Deviation from study protocol:	None affecting the integrity of the study

Observations and Results

Mortality

There was no adverse effect on survival in either sex and there were no factors contributing to death attributable to semaglutide treatment. The most common cause of death was lymphoma.

Table 91. Group distribution of mortality (Applicant's table)

Group/sex Dose (mg/kg/day)	1M 0	3M 0.3	4M 1	5M 3	1F 0	2F 0.1	3F 0.3	4F 1
Number of animals/group	60	60	60	60	60	60	60	60
Number of decedents	35[+]	17	26[++]	25	31	37	36	36
Number surviving	25	43	34	35	29	23	24	24
% Survival	42	72	57	58	48	38	40	40

[+] Includes two animals that died during terminal procedures (not included in statistical analysis)
[++] Includes one animal that died during terminal procedures (not included in statistical analysis)

Figure 23. Kaplan-Meier survival functions for male mice

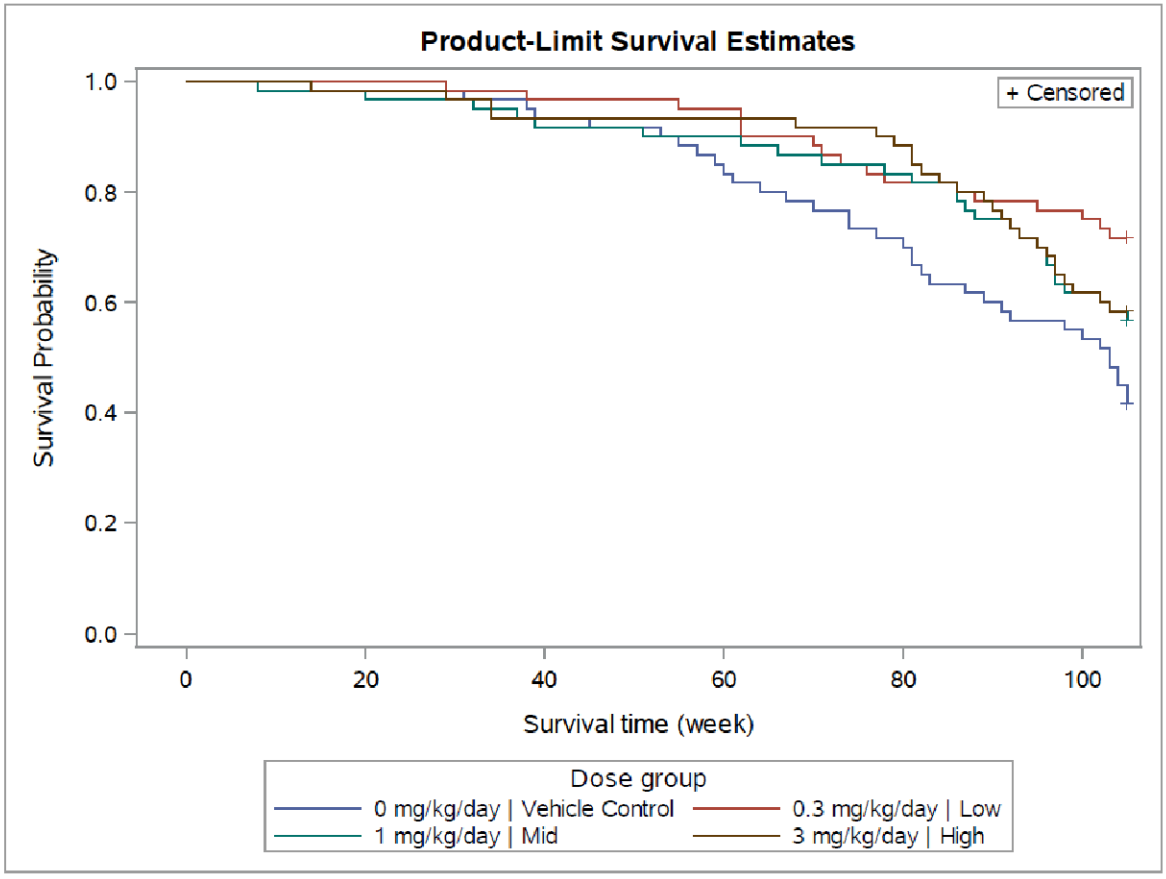
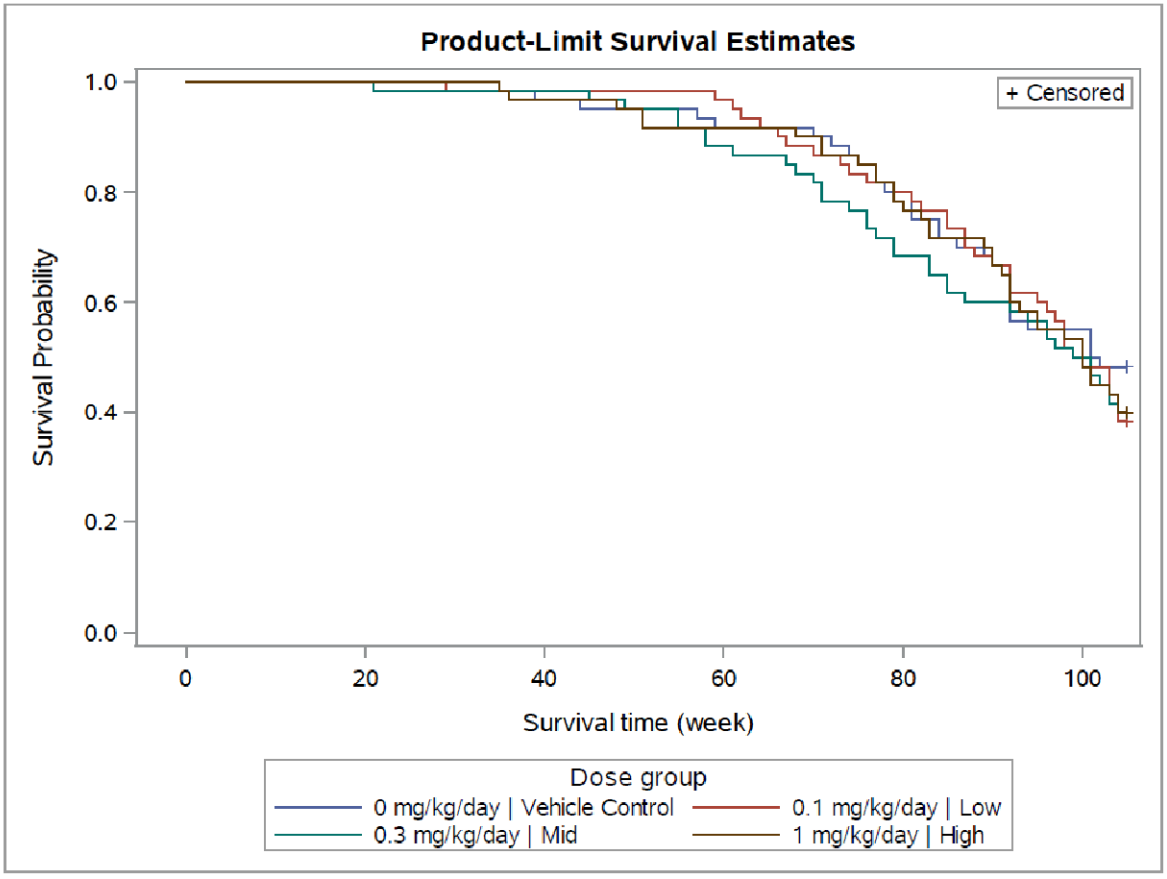


Figure 24. Kaplan-Meier survival functions for female mice



Clinical Signs

Increased incidence of thin appearance, prominent eye, hunched posture, reduced temperature and under-activity was noted in females.

Table 92. Clinical signs (Applicant's table)

Weeks 1 - 106			Number of animals affected							
		Group/sex:	1M	3M	4M	5M	1F	2F	3F	4F
Category	Observation	Number in group:	60	60	60	60	60	60	60	60
Behaviour	Underactive		13	4	8	4	6	13	11	13
Body temperature	Reduced		12	6	8	4	6	10	12	12
Build Conformation	Thin		3	3	4	2	2	7	6	11
Eye	Prominent, Eyes		3	6	6	3	3	13	16	14
Posture	Hunched		13	4	10	7	6	12	14	14

Body Weights

Dose-related decrease in body weight gain was noted during the first year of dosing in all treated male and female groups.

Figure 25. Body weight (Applicant's figure)

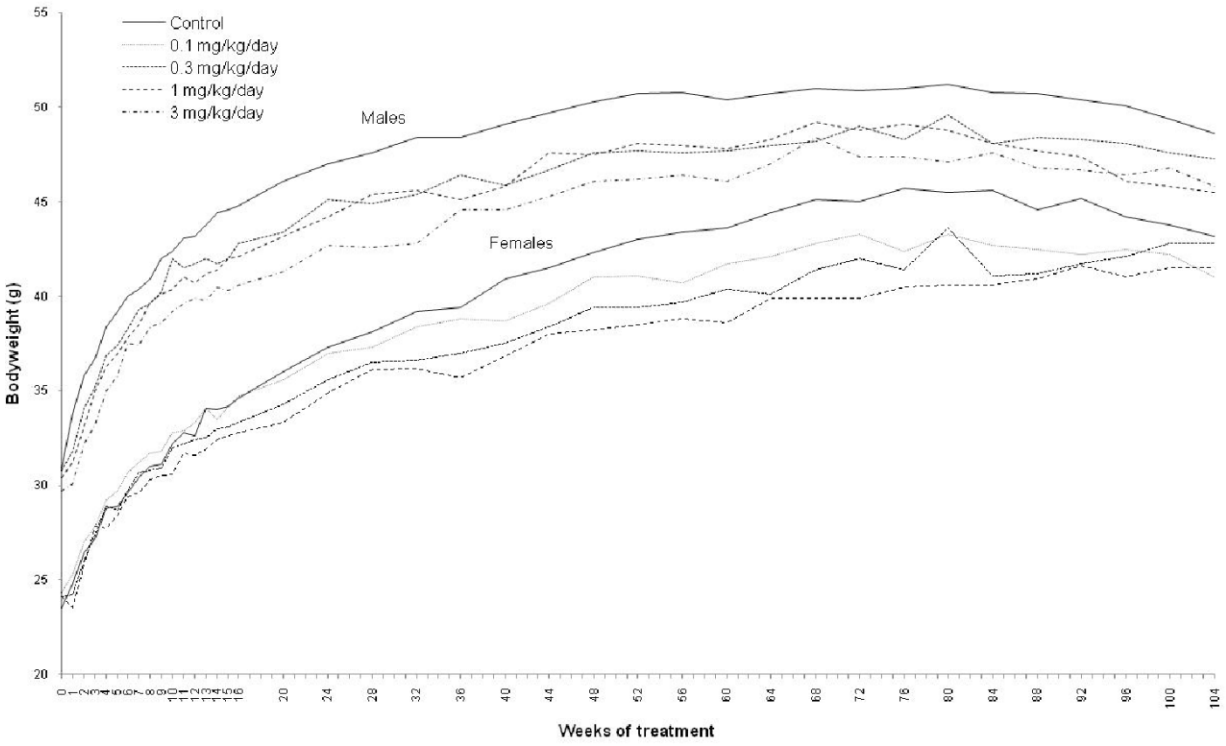


Table 93. Body weight, percent change vs. controls (Applicant's table)

Group/Sex	1M	3M	4M	5M
Dose Level (mg/kg/day)	0	0.3	1	3
Change 0-104	17.6	-7	-13	-10
0-28	16.7	-15**	-10**	-23**
0-52	19.8	-15**	-11**	-18**
28-104	1.1	+106	-64	+203**
52-104+	-	-	-	-

+ Not calculated bodyweight loss for controls

Group/Sex	1F	2F	3F	4F
Dose Level (mg/kg/day)	0	0.1	0.3	1
Change 0-104	19.7	-16	-4	-13
0-28	14.5	-11**	-14**	-18**
0-52	19.5	-14**	-21**	-27**
28-104	6.1	-34	+6	-18
52-104	19.7	-16	-4	-13

Feed Consumption

Decrease in food consumption was observed throughout dosing in all treated groups, and was slightly more pronounced in males.

Table 94. Food consumption-percent change vs. control (Applicant's table)

Group/Sex	1M	3M	4M	5M
Dose Level (mg/kg/day)	0	0.3	1	3
Change 1-104	38	-5*	-7**	-14**
1-28	38	-6**	-9**	-16**
1-52	38	-6*	-8**	-14**
28-104	38	-4	-6*	-11**
52-104	39	-4	-7*	-12**

Group/Sex	1F	2F	3F	4F
Dose Level (mg/kg/day)	0	0.1	0.3	1
Change 1-104	33	-4	-3	-6**
1-28	33	-4*	-4*	-7**
1-52	33	-4*	-3*	-7**
28-104	34	-3	-1	-5*
52-104	34	-2	-1	-4

Hematology

Blood samples were obtained from all surviving animals at the end of the treatment period and, where possible, from mice killed prematurely.

No treatment-related changes were observed.

Gross Pathology

Thyroid
Masses in the thyroid gland were observed in all treated groups with higher incidence in males.

Gall bladder
Increased incidences of distension and/or abnormal content of the gall bladder were noted in all treated groups. There were no associated histopathological changes or any signs of compromised bile efflux. These changes are considered secondary to low food consumption (Cattley RC 2013; Greaves P 2012).

Table 95. Macroscopic findings in the thyroid gland (Applicant's table)

Group/sex	1M	3M	4M	5M	1F	2F	3F	4F
Dose (mg/kg/day)	0	0.3	1	3	0	0.1	0.3	1
Mass(es)	0	4	7	10	0	2	3	3
Enlarged	0	0	1	1	0	0	1	2
Unilaterally enlarged	0	3	1	2	0	0	1	1
Number of animals examined	60	60	60	60	60	60	60	60

Table 96. Macroscopic findings in the gall bladder (Applicant's table)

Group/sex	1M	3M	4M	5M	1F	2F	3F	4F
Dose (mg/kg/day)	0	0.3	1	3	0	0.1	0.3	1
Distended	6	24	24	22	17	29	27	31
Abnormal content	3	20	14	15	1	12	7	14
Number of animals examined	60	60	60	60	60	60	60	60

Histopathology

Histopathology examination was conducted for all animals sacrificed at scheduled necropsy and all animals killed or dying during the study.

Peer Review: Yes. [redacted] (b) (4) Inger Thorup, Novo Nordisk.

Neoplastic
Thyroid
Statistically significant increase in the incidence of thyroid C-cell adenoma and combined C-cell adenoma and carcinoma was observed in males and females at all doses, with dose relationship in females. There was no significant increase in C-cell carcinoma in either males or females.

A few animals had C-cell adenomas in ectopic thyroid tissue in the thymus (one male and one female in the 0.3 mg/kg group) and C-cell carcinoma metastases in the deep cervical lymph nodes (one female at 0.1 and 1 mg/kg/day each).

Table 97. Neoplastic findings in the mouse thyroid gland

Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Vehicle Control Group in Mice

Organ name	Tumor name	Vehicle (C) P - Trend	Low (L) P - C vs. L	Mid (M) P - C vs. M	High (H) P - C vs. H
Male		0 mg	0.3 mg	1 mg	3 mg
Thyroids	C-Cell Adenoma	0/60 (41)	24/60 (51)	23/59 (49)	18/59 (49)
		0.0620	<0.0001 $	<0.0001 $	<0.0001 $
	C-Cell Carcinoma	0/60 (41)	2/60 (50)	2/59 (47)	2/59 (48)
		0.2426	0.2991	0.2824	0.2880
	C-Cell Adenoma/C-Cell Carcinoma	0/60 (41)	24/60 (51)	24/59 (49)	20/59 (49)
		0.0233 @	<0.0001 $	<0.0001 $	<0.0001 $
Female		0 mg	0.1 mg	0.3 mg	1 mg
Thyroids	C-Cell Adenoma	0/58 (43)	16/59 (45)	20/58 (43)	24/60 (47)
		0.0001 $	<0.0001 $	<0.0001 $	<0.0001 $
	C-Cell Carcinoma	0/58 (43)	1/59 (44)	2/58 (42)	2/60 (44)
		0.1434	0.5057	0.2412	0.2529
	C-Cell Adenoma/C-Cell Carcinoma	0/58 (43)	16/59 (45)	21/58 (43)	24/60 (47)
		0.0001 $	<0.0001 $	<0.0001 $	<0.0001 $

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
$ = Statistically significant in common tumor at 0.005 level for test of dose response relationship and at 0.01 level for test of pairwise comparisons, or in rare tumor at 0.025 level for test of dose response relationship and at 0.05 level for test of pairwise comparisons;
@ = Not statistically significant in common tumor at 0.005 level for test of dose response relationship and at 0.01 level for test of pairwise comparisons; or in rare tumor at 0.025 level for test of dose response relationship and at 0.05 level for test of pairwise comparisons;
NC = Not calculable.

Table 98. Summary of findings of ectopic thyroid tumors or metastatic findings (Applicant's table)

Group/sex	**1M**	**3M**	**4M**	**5M**	**1F**	**2F**	**3F**	**4F**
Dose (mg/kg/day)	**0**	**0.3**	**1**	**3**	**0**	**0.1**	**0.3**	**1**
Thymus								
C-cell adenoma (ectopic thyroid tissue)	0	1	0	0	0	0	1	0
Number of tissues examined	52	57	55	55	57	55	57	52
Deep cervical lymph nodes								
C-cell carcinoma	0	0	0	0	0	1	0	1
Number of tissues examined	0	3	5	6	0	2	1	3

Non Neoplastic

- Increased incidence of minimal to moderate C-cell focal/multifocal hyperplasia was observed in all treated groups. Total incidence of this finding was dose-related in males.
- Increased incidence of minimal to moderate Brunner's glands dilatation was noted in all treated male and female groups, without dose relationship.

Table 99. Non neoplastic findings in the thyroid and duodenum (Applicant's table)

Group/sex	1M	3M	4M	5M	1F	2F	3F	4F
Dose (mg/kg/day)	0	0.3	1	3	0	0.1	0.3	1
C-cell hyperplasia, Focal/multifocal								
Minimal	1	8	16	20	2	26	14	17
Slight	0	19	13	11	0	10	19	15
Moderate	0	12	13	13	0	12	12	11
Total	1	39	42	44	2	48	45	43
Number of tissues examined	60	60	59	59	58	59	58	60
Dilated glandular lumens in Brunner`s glands								
Minimal	13	21	23	22	6	19	23	31
Slight	0	10	13	8	0	7	5	5
Moderate	0	0	0	1	0	0	0	0
Total	13	31	36	31	6	26	28	36
Number of tissues examined	59	60	58	60	60	60	59	59

Toxicokinetics

Blood samples were obtained from Satellite study animals on Day 1 and in Week 52 and from Main study animals in Week 105. For immunogenicity analysis at week 53 and 104, blood samples were obtained from animals after a 7 day off dose period.

Exposure increased linearly with the dose. Minor accumulation, up to 2-fold, was observed in both sexes up to 1 mg/kg/day, and a 4-fold accumulation was seen for males receiving 3 mg/kg/day. No clear sex differences were observed in the toxicokinetics.

Anti-drug antibodies were found in one mid-dose female at week 105 and one HD female at week 53. There was no cross-reaction to endogenous GLP-1. Due to a lack of sufficient residual serum sample, the neutralizing effect of these antibodies could not be assessed.

Table 100. Summary of toxicokinetic parameters (Applicant's table)

Dose (mg/kg)	Week	Sex	Cmax (nM)	Tmax (h)	AUC ((h)*(nM))	AUC_{tau} ((h)*(nM))	AUCextra (%)	Rac_{Pred}	Rac_{Obs}
0.1	1	Female	79.5	8	-	750	-	-	
	52	Female	102	4	1430	1190	17.2	1.2	1.6
	105	Female	83.8	4	-	1110	-	-	1.5
0.3	1	Male	185	4	3010	2490	17.3	1.2	
		Female	280	8	3530	3020	14.5	1.1	
	52	Male	291	4	4270	3770	11.6	1.1	1.5
		Female	263	8	4490	3590	19.9	1.2	1.2
	105	Male	245	8	-	3080	-	-	1.2
		Female	225	8	-	3090	-	-	1.0
1	1	Female	771	2	-	6920	-	-	
		Male	717	2	8430	7290	13.6	1.1	
	52	Female	994	4	-	12800	-	-	1.8
		Male	870	2	-	11300	27.2	-	1.6
	105	Female	703	4	11100	8970	19.3	1.2	1.3
		Male	1060	4	-	13900	-	-	1.9
3	1	Male	1720	2	12900	11000	14.8	1.2	
	52	Male	2600	4	-	41900	-	-	3.8
	105	Male	3450	2	-	39500	21.6	-	3.6

AUC_{last} = AUC_{tau} (=$AUC_{0\text{-}24\ hr}$)

Dosing Solution Analysis

Duplicate (5 mL) samples of each concentration were taken immediately after preparation of the formulations prepared for use on Day 1 and Weeks 13, 26, 39, 52, 65, 78, 91 and 104

The results of achieved concentration analyses were generally within the range -10 to +2% of the nominal concentration. The only exception occurred for the 0.1 mg/mL (Group 2 females) in Week 26 where the analyzed result was -13% of the nominal concentration.

Other studies

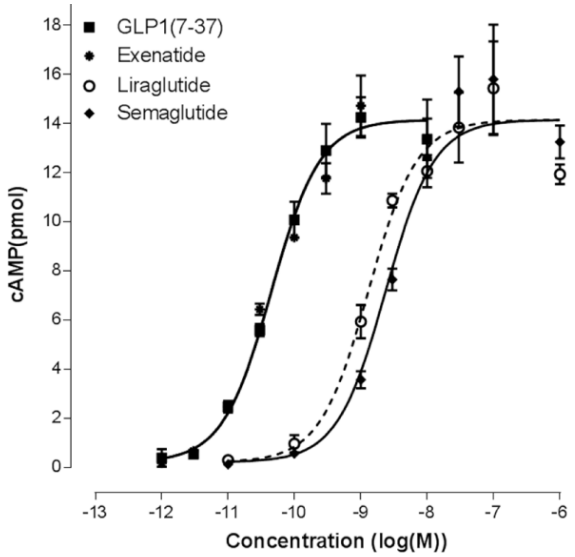
Long-term GLP-1R activation in the thyroid leads to calcitonin secretion, upregulation of calcitonin gene transcription, and subsequently to C-cell proliferation and tumor formation in rats and mice. It has previously been shown that rat thyroid C-cell GLP-1 receptors are activated by GLP-1, exenatide and liraglutide. The ability of semaglutide to activate the rat thyroid C-cell receptor was therefore investigated and compared with the aforementioned compounds. The effect of semaglutide on plasma calcitonin concentrations, biomarker of increased C-cell activity in the thyroid C-cells, was also evaluated in dedicated rodent studies to assess if increased levels of calcitonin occurred prior to the first observations of C-cell hyperplastic or neoplastic changes in rodents.

Activation of rodent thyroid C-cell GLP-1 receptors (Study # LBKN150301)

The objective of this in vitro study was to investigate the activation of thyroid C-cell GLP-1 receptors using the rat thyroid C-cell line MTC 6-23 (established from a rat C-cell carcinoma). The in vitro potency of semaglutide was compared with that of native GLP-1, exenatide and liraglutide.

Semaglutide activated the rat thyroid C-cell GLP-1R with a potency comparable to that of liraglutide. Both liraglutide and semaglutide activate the receptor with an apparent lower potency than native GLP-1 and exenatide.

Figure 26. Activation of rat thyroid GLP-1 receptors by various ligands (Applicant's figure)



	GLP-1	Exenatide	Liraglutide	Semaglutide
EC_{50} (pM) mean±SEM	84±16	69±12	2200±350	3000±400
N	6	6	6	6
Statistical analysis (paired t-test)				P=0.1121 vs. liraglutide P=0.0006 vs. GLP-1 and vs. exenatide

Single Dose Study in Mice to Assess Plasma Calcitonin Levels after Subcutaneous Administration (study # 213448 and 208422)

Since C-cell hyperplasia was observed in mice at ≥0.078 mg/kg/day after 2 weeks of dosing (Study # 206447), single dose studies were conducted to confirm an increase in plasma calcitonin in mice. Initially, the highest dose level used in the 2-week study, 10 mg/kg/day, was explored, but after completion of the carcinogenicity studies, where C-cell tumors were observed at lower doses, the study was repeated at a lower dose level, 1 mg/kg/day.

Design

CD-1 mice (12/sex/group) were given a single subcutaneous injection of semaglutide at doses of 1 mg/kg ((Study # 213448) and 10mg/kg (Study # 208422). Blood samples for measurement of plasma calcitonin and test item exposure were taken pre-dose and 1, 3, 6, 12 and 24 hours after dosing. The animals were terminated after blood sampling without further examination.

Results

Following a single dose of semaglutide, higher plasma calcitonin levels were observed beginning at 12h post-dose at 1 mg/kg, and at 1h post-dose at 10 mg/kg. Calcitonin levels remained elevated for at least 24 hours after dosing.

Figure 27. Plasma calcitonin levels in mice after a single dose of 1 mg/kg

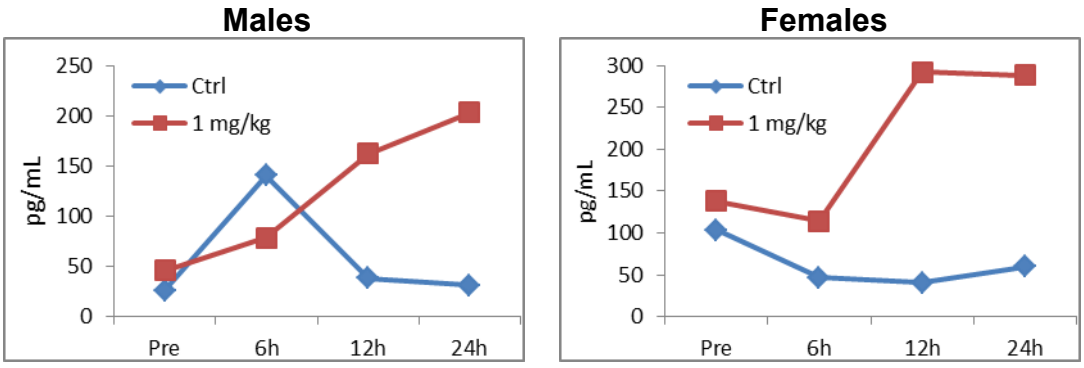


Figure 28. Plasma calcitonin levels in mice after a single dose of 10 mg/kg (Applicant's figure)

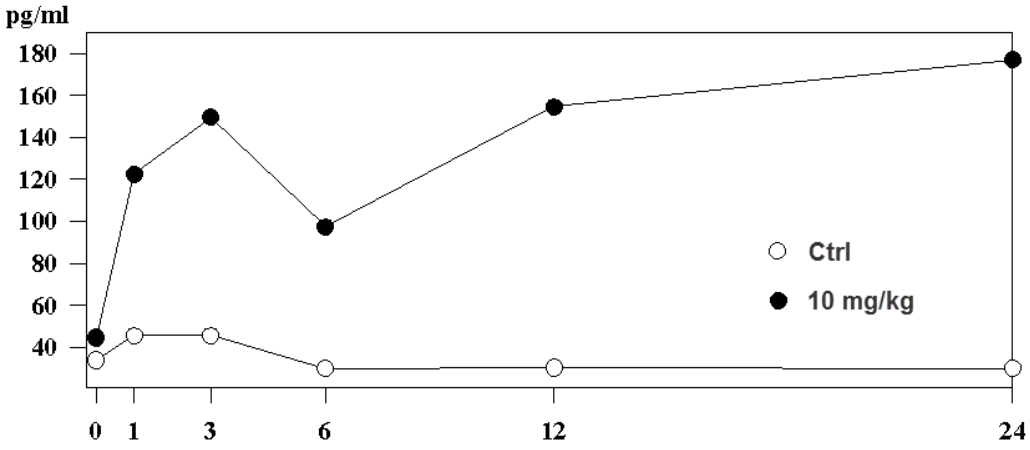
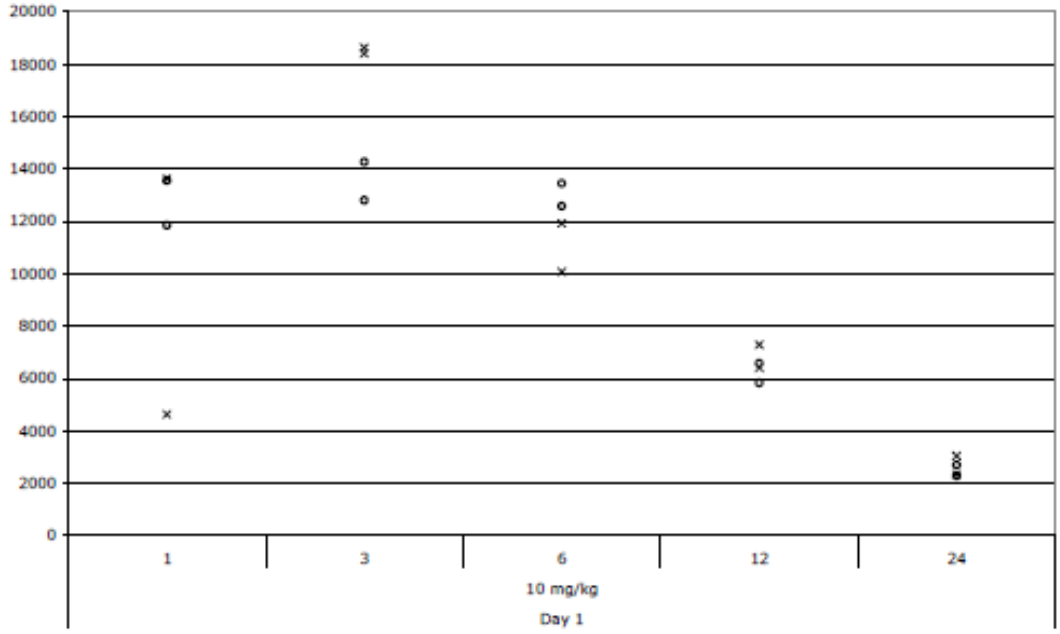


Figure 29. Semaglutide concentration at 10 mg/kg (Applicant's figure)



Six Week Toxicity Study in Rats to Assess Plasma Calcitonin Levels after Subcutaneous Administration (Study # 208456)

In rats, increased incidences of C-cell hyperplasia and neoplasia were only observed in the 2-year carcinogenicity study. The effect of semaglutide on plasma calcitonin in rats was assessed after 6-weeks of dosing, as it had been shown previously that a GLP-1R agonist induced increases in plasma calcitonin levels after one month of dosing in rats (Knudsen LB et al, 2010).

Design
SD rats (12/sex/group) were dosed subcutaneously once daily for six weeks with semaglutide at dose levels of 0, 0.01, 0.1 and 0.86 mg/kg/day. Plasma levels of calcitonin and semaglutide were measured, and the thyroid gland was examined by light microscopy.

Results

- Calcitonin was dose dependently increased in all treated males (up to 2X the control values). No significant increase was noted in females.

- The histopathological examination revealed no treatment related increase in the incidence of focal or diffuse C-cell hyperplasia in any treated animals.

Table 101. Plasma calcitonin in rats at week 6 (Applicant's table)

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Mean conc. (pg/ml)	31.8	53.4*	60.4*	65.6*	83.2	65.9	110.9	85.1
Mean conc. as percent of Group 1		+68%	+90%	+107%		-21%	+33%	+2%

Table 102. Percentage of histological sections with diffuse C-cell hyperplasia (Applicant's table)

Sex	Male				Female			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
% sections with diffuse C-cell hyperplasia	8	18	8	4	4	3	4	1

Table 103. Number of animals with focal C-cell hyperplasia (Applicant's table)

Sex	Male				Female			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Number of animals	12	12	12	12	12	12	12	12
Number of animals with focal C-cell hyperplasia	3	1	0	1	1	1	0	0

9 Reproductive and Developmental Toxicology

Reproductive toxicity studies were initially performed in rats and rabbits. Subsequently, the cynomolgus monkey was used as an embryo-fetal development and pre- and postnatal species, since poor maternal tolerability in the rabbit precluded establishment of a safety ratio between this species and humans. Additionally, observed embryo-fetal toxicity in the rat suggested a need to evaluate the embryo-fetal development in a species with a yolk sac anatomy and function more relevant to humans than the rat.

9.1 Fertility and Early Embryonic Development

Two preliminary studies and one pivotal fertility and embryo-fetal development study were conducted in rats. Major skeletal and visceral malformations were observed in the rat and were considered at least in part to reflect the extent of the effect of treatment on body weight. However, due to the extent and severity of the findings additional causative agents couldn't be excluded. A series of mechanistic studies were therefore conducted with the intent to establish human relevance of the rat embryotoxicity findings.

Study title: Preliminary Study of Effects on Fertility and Development Toxicity in Rats (Study # 206616)

Key findings

- Decrease in maternal body weight gain at ≥0.195 mg/kg (3X MRHD)
- Embryo and fetal toxicity (reduction of live implants, fetal weight, and malformations) at ≥0.025 mg/kg (1X MRHD)
- Death of all embryo at 0.8282 mg/kg (14X MRHD)
- Fetal NOAEL: <0.025mg/kg/day (1X MRHD)

Study design

Sprague-Dawley rats (8/sex/group) were dosed once daily by subcutaneous injection (achieved doses were 0, 0.025, 0.195 and 0.828 mg/kg/day) following up to 2 weeks dose escalation regime. The full dose was administered to animals from 2 weeks prior to mating until Day 19 of gestation. The females were sacrificed on Day 20 of gestation and the reproductive tracts examined. The number of implants and their status recorded, followed by visceral and skeletal examination of the foetuses. Males were sacrificed after completion of 7 weeks of treatment at the full dose and their reproductive organs were weighed and preserved.

Group	Treatment	Nominal Dose (mg/kg/day)*			Number of Animals	
		Week -4	Week -3	Week -2 to Week 5 (males) or to GD19 (females)	Males	Females
1	Control (Vehicle)	0	0	0	8	8
2	Semaglutide	0	0	0.03	8	8
3	Semaglutide	0	0.03	0.21	8	8
4	Semaglutide	0.05	0.21	0.86	8	8

*The start of Week 1 is defined as the start of the mating period

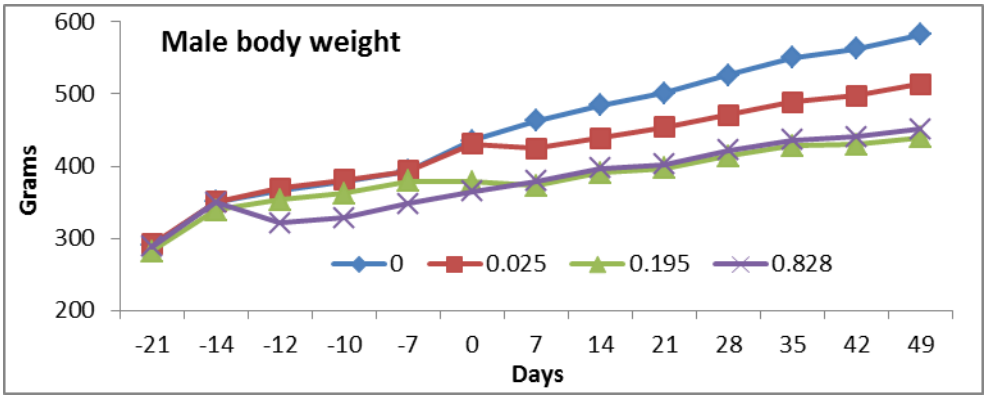
Observations and Results

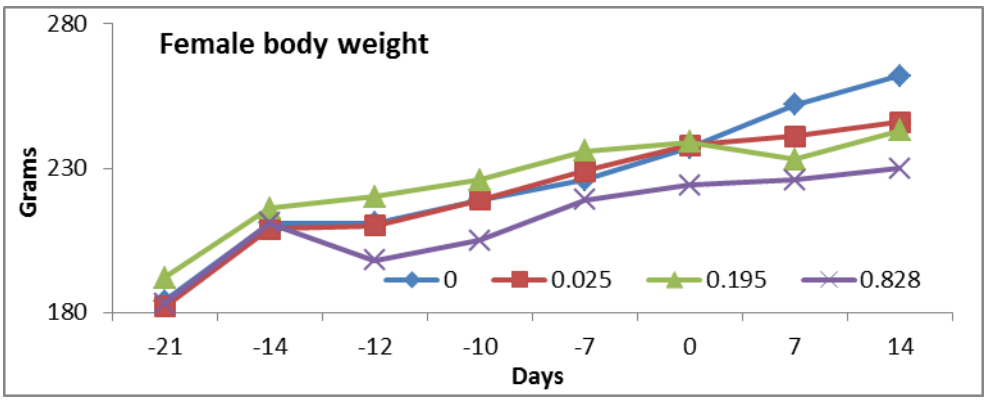
Mortality

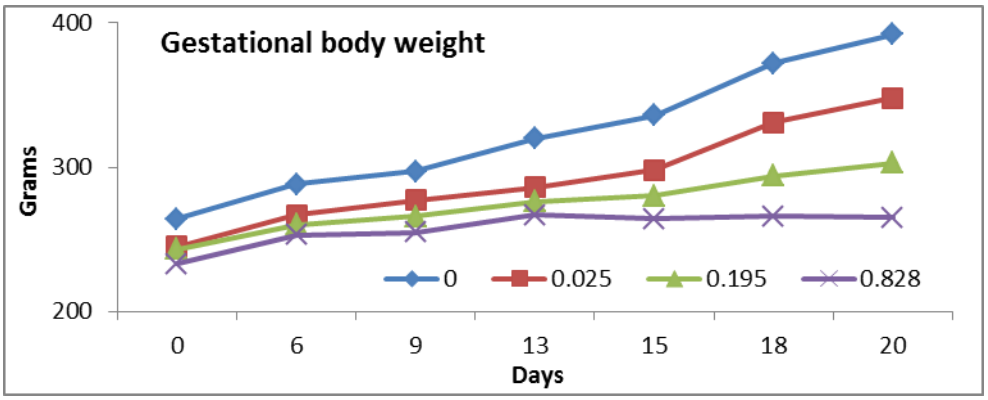
None

Clinical signs
Hunched body was seen at the high dose in most females (7/8) and in one male.

Body weight
Body weight loss occurred in high-dose males and females during the first escalation week. Thereafter, body weight gain remained lower in all treated male groups and in high-dose females. During gestation, lower body weight gain was noted at ≥0.195 mg/kg/day. The weight loss in the HD groups from GD 13 onward most likely reflected the loss of all embryos.







Food consumption
Dose-related decrease in food consumption was observed throughout dosing in males and females.

Mating Performance, Fertility Indices and Estrous Cycles
Early embryonic death at the high dose, marked reduction of live implants, comprising both early and late embryonic deaths, at ≥0.195 mg/kg, and low fetal weight at all doses were noted.

Table 104. Pregnancy Performance (Applicant's table)

	Group/Dose Level (mg/kg/day)			
	1 (0)	2 (0.025)	3 (0.195)	4 (0.828)
Number of animals mated	8	8	8	8
Number pregnant	8	7	8	7
Number of premature decedents	0	0	0	0
Number pregnant at necropsy	8	7	8	7
Pregnancy frequency as %	100	88	100	88
Total corpora lutea graviditatis	115	89	107	91
Total number of implants	113	88	102	89
Pre-implantation loss as %	2	1	5	2
Total live implants (%)	104 (92)	84 (95)	55 (54)	0
Total dead implants (%)	9 (8)	4 (5)	47 (46)	89 (100)
Total early embryonic deaths (%)	8 (7)	4 (5)	30 (29)	89 (100)
Total late embryonic deaths (%)	1 (1)	0	17 (17)	0
Total dead foetuses (%)	0	0	0	0
Mean corpora lutea graviditatis	14.4 ± 1.9	12.7 ± 2.0	13.4 ± 1.6	13.0 ± 1.2
Mean implants	14.1 ± 1.7	12.6 ± 1.7	12.8 ± 1.4	12.7 ± 1.6
Mean live implants	13.0 ± 1.9	12.0 ± 2.2	6.9 ± 3.3	0
Mean dead implants	1.1 ± 1.5	0.6 ± 0.8	5.9 ± 3.0	12.7 ± 1.6
Mean early embryonic deaths	1.0 ± 1.4	0.6 ± 0.8	3.8 ± 2.9	12.7 ± 1.6
Mean late embryonic deaths	0.1 ± 0.4	0	2.1 ± 2.4	0
Mean dead foetuses	0	0	0	0
Total live male foetuses (%)	54 (52%)	40 (48%)	39 (71%)	-
Total live female foetuses (%)	50 (48%)	44 (52%)	16 (29%)	-
Live foetal sex ratio (M:F)	1: 0.93	1: 1.00	1:0.41	-
Mean total uterus weight (g)	79 ± 8	68 ± 12	30 ± 13	-
Mean litter mean foetal weight (g)	3.66 ± 0.13	3.51 ± 0.17	2.14 ± 0.39	-

Means are given ± Standard Deviation

Fetal findings

Various external, visceral, and skeletal abnormalities were observed at 0.195mg/kg/day. The skeletal abnormalities included malrotated, absent, shortened, curved, kinked, misaligned, partially fused, and/or displaced bones (scapula, long bones, ribs, digits, vertebrae and cranial bone(s)) in addition to incompletely ossified bones. The visceral minor abnormalities included subcutaneous edema/hemorrhage, testes not fully descended, left-sided umbilical artery, kinked tail tip and smaller fetuses than normal. At the low dose, incomplete ossification and minimally kinked ribs were observed.

Table 105. Group incidence of major fetal abnormalities (Applicant's table)

Abnormality	Group/Dose Level (mg/kg/day)		
	1 (0)	2 (0.025)	3 (0.195)
	Incidence of Foetuses (Litters)		
Omphalocele (small intestines exposed)	1(1)	0	0
Occipital condyles reduced (in size)	0	0	1(1)
Scapula curved/misshapen/incompletely ossified	0	0	12(6)
Humerus/ulna/radius bent	0	0	1(1)
Hindlimb(s) malrotated	0	0	3(2)
Tibia absent	0	0	10(3)
Tibia markedly shortened/markedly reduced (in size)/reduced (in size)	0	0	4(3)
Femur/tibia/fibula shortened and/or incompletely ossified	0	0	3(3)
Fibula bent/curved	0	0	1(1)
Rib(s) kinked	0	0	24(7)
Forepaw digit(s) absent/reduced (in size)	0	0	10(4)
Hindpaw digit(s) absent/reduced (in size)	0	0	13(5)
Number with major abnormality	1(1)	0	37(8)
Total number examined	104(8)	84(7)	55(8)

Table 106. Incidence of minor abnormalities and variants (Applicant's table)

Abnormality/Variant	Group/Dose Level (mg/kg/day)		
	1 (0)	2 (0.025)	3 (0.195)
	Incidence of Foetuses (Litters)		
Visceral			
Subcutaneous haemorrhage:			
Head	1(1)	0	0
Limbs	0	0	2(2)
Tail	0	0	1(1)
Subcutaneous oedema cervical/thoracic/abdominal region(s)	0	0	3(2)
Cervical remnant of thymus	3(2)	1(1)	0
Intrathoracic haemorrhage	0	1(1)	0
Additional liver lobe within median cleft	10(4)	1(1)	1(1)
Bilateral testis not fully descended to pelvic position	0	0	6(2)
Umbilical artery left-sided	0	1(1)	5(4)
Tail tip kinked	0	0	14(3)
Foetus smaller than normal for day 20	0	0	54(7)
Number with minor visceral abnormality/variant	13(5)	4(3)	55(8)
Skeletal			
Cranial bone(s) small discrete unossified/incompletely ossified area(s)	0	1(1)	0
Cranial bone(s)incompletely ossified	0	0	5(1)
Cranial bone(s) additional ossified area(s)	0	1(1)	0
Spine of scapula/e incompletely ossified	0	0	4(2)
Cervical rib(s)	5(2)	3(3)	0
Additional ossified area arising from sternebra 6	1(1)	0	0
Rib(s) minimally kinked	0	6(3)	25(7)
Rib(s) incompletely ossified	0	0	17(5)

Abnormality/Variant	Group/Dose Level (mg/kg/day)		
	1 (0)	2 (0.025)	3 (0.195)
	Incidence of Foetuses (Litters)		
Skeletal (cont)			
Rib(s) partially fused with/without costal cartilage(s) fused	0	0	14(6)
Rib(s) costal cartilage(s) fused distally/at point of attachment to sternum	0	0	21(7)
Rib(s) costal cartilage(s) cranially displaced	0	0	1(1)
Rib(s) costal cartilage(s) asymmetrically aligned at point of attachment to sternum	0	1(1)	0
Unilateral/bilateral rib 7 costal cartilages not attached to sternum	4(1)	0	5(4)
Proximal and/or distal caudal vertebrae, cartilaginous precursor(s) misaligned	0	0	18(5)
Number of ribs			
12^{th} reduced rib(s)	0	0	1(1)
12 complete rib(s)	0	0	5(3)
13th vestigial rib(s)	0	0	6(5)
13th reduced rib(s)	3(2)	0	6(3)
13 complete ribs	99(8)	81(7)	37(8)
Vestigial supernumerary rib(s) on 1st lumbar vertebra	2(2)	3(3)	0
Number with minor skeletal abnormality/variant	7(3)	12(5)	50(8)
Total number examined	104(8)	84(7)	55(8)

Table 107. Incidence of skeletal ossification parameters (Applicant's table)

Abnormality	Group/Dose Level (mg/kg/day)		
	1 (0)	2 (0.025)	3 (0.195)
	Incidence of Foetuses (Litters)		
Incomplete ossification affecting			
≥4 skull bones	5(3)	12(3)	35(8)
≤3 skull bones	25(4)	27(7)	12(5)
Cervical vertebral arch(es)	1(1)	5(2)	26(7)
Thoracic centrum(a)	1(1)	1(1)	3(2)
Pubis(es)	1(1)	7(3)	19(7)
Ischium(a)	0	2(1)	24(7)
Sacral vertebral arch(es)	12(3)	20(5)	24(8)
Sacral centrum/a	0	0	27(6)
Lumbar centrum(a)	0	0	1(1)
Lumbar vertebral arch(es)	1(1)	2(1)	12(5)
2nd to 4th metacarpal(s)	1(1)	0	13(5)
2nd to 5th metatarsal(s)	1(1)	1(1)	19(6)
Unossified			
2nd to 5th metacarpal(s)	42(8)	43(6)	51(8)
2nd to 5th metatarsal(s)	0	0	33(7)
Ossified			
Anterior arch of atlas ossified	19(6)	18(4)	3(3)
>2 cervical vertebral centra ossified	5(3)	7(2)	0
One or more sacrocaudal vertebra with connection between centrum and arch(es)	23(5)	8(1)	1(1)
Phalangeal elements ossified	3(2)	3(2)	0
Mean number of caudal vertebral centra	4.0	3.8	1.2
Number of sternebrae retarded:			
0	39(6)	23(6)	0
1	39(6)	30(7)	0
2	26(6)	27(5)	7(2)
>2	0	4(4)	48(8)
Total number examined skeletally	104(8)	84(7)	55(8)

Toxicokinetics

Exposure on GD 6 increased proportionally with the dose. No appreciable difference in exposure was noted between GD 6 and GD 16.

Table 108. Toxicokinetic parameters, GD 6 and GD16 (Applicant's table)

Group	Day	Achieved Mean Dose (mg/kg) [Ratio]	Achieved Mean Dose (nmol/kg) [Ratio]	AUC(0-∞) (nmol.h/L) [Ratio]	AUC(0-24) (nmol.h/L) [Ratio]	AUC(0-t) (nmol.h/L) [Ratio]	Cmax (nmol/L) [Ratio]
2	6	0.025 [1]	6.08 [1]	296 [1]	242 [1]	242 [1]	16.8 [1]
	16			250 [1]	205 [1]	205 [1]	16.3 [1]
3	6	0.195 [7.8]	47.4 [7.8]	NR [-]	2360 [9.8]	2360 [9.8]	131 [7.8]
	16			NR [-]	2050 [10.0]	2050 [10.0]	107 [6.6]
4	6	0.828 [33.1]	201 [33.1]	NR [-]	8100 [33.5]	8100 [33.5]	574 [34.2]
	16			NR [-]	9510 [46.4]	9510 [46.4]	562 [34.5]

Group	Day	Achieved Mean Dose (mg/kg)	Achieved Mean Dose (nmol/kg)	Tmax(obs) (h)	T½el (h)	Kel (h^{-1})	CL/F (L/h/kg)	Vd/F (L/kg)	Rac(predicted)
2	6	0.025	6.08	3.00	9.20	0.0754	0.0251	0.333	1.20
	16			6.00	8.60	0.0806	0.0297	0.369	1.17
3	6	0.195	47.4	3.00	14.13	0.0491	0.0201	NR	1.45
	16			6.00	20.58	0.0337	0.0231	NR	1.80
4	6	0.828	201	3.00	27.03	0.0256	0.0248	NR	2.18
	16			6.00	17.34	0.0400	0.0212	NR	1.62

Study title: Preliminary combined fertility and embryo-fetal toxicity study by subctaneous administration to female CD rats (Study # 207359)

Key findings

- Reduced maternal body weight gain and reduced food/water consumption at all doses (1X MRHD).
- Decreased number of corpora lutea, increased embryo-fetal mortality and reduced fetal weight at all doses.
- Fetal findings of tail kinked/caudal elements misaligned and cardiovascular vessel abnormalities at all doses, with dose-related trends.

Study design

Group	Treatment	Nominal Dose (mg/kg/day)* Week -4	Week -3	Week -2 to GD17 or GD20	Number of Animals Females treated until GD 17	Females treated until GD20
1	Control (Vehicle)	0	0	0	10	3
2	Semaglutide	0	0	0.05	10	3
3	Semaglutide	0	0.05	0.10	10	3
4	Semaglutide	0.05	0.10	0.15	10	3

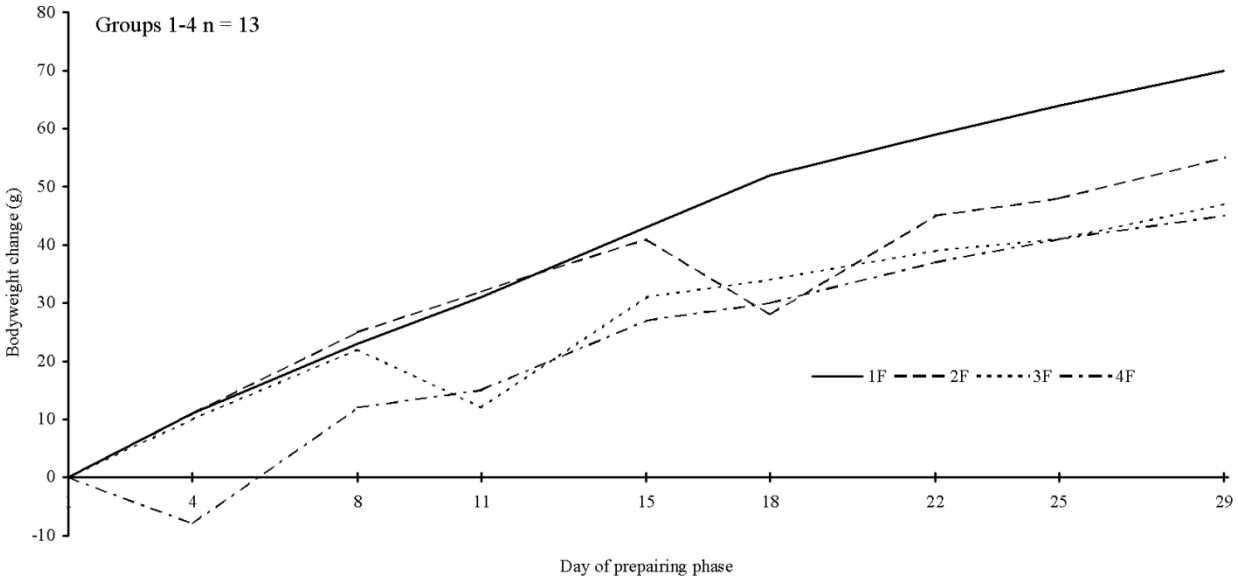
*The start of Week 1 is defined as the start of the mating period

Observation and results
Mortality
None

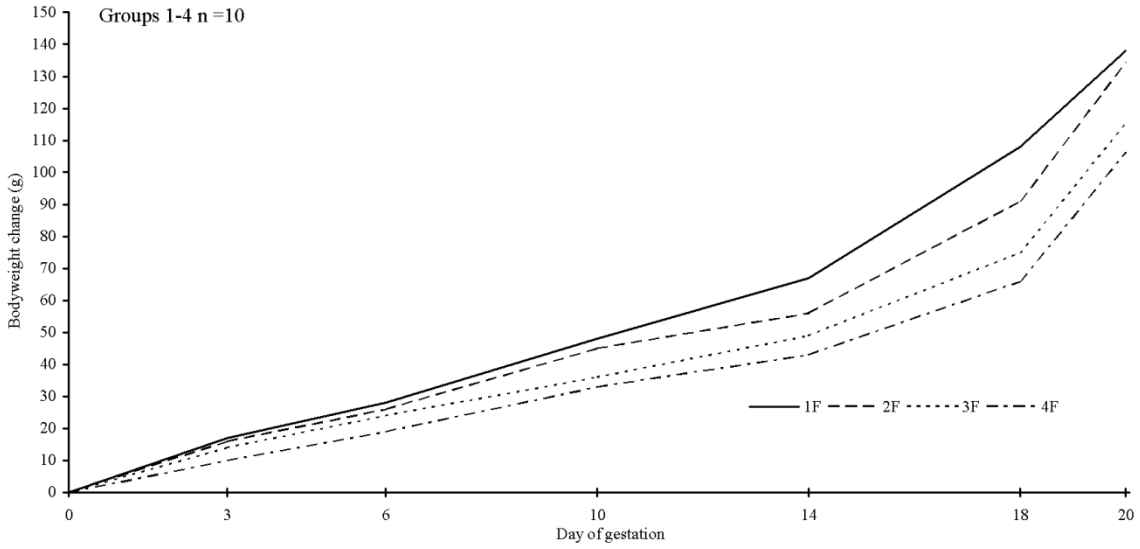
Clinical signs
None treatment-related

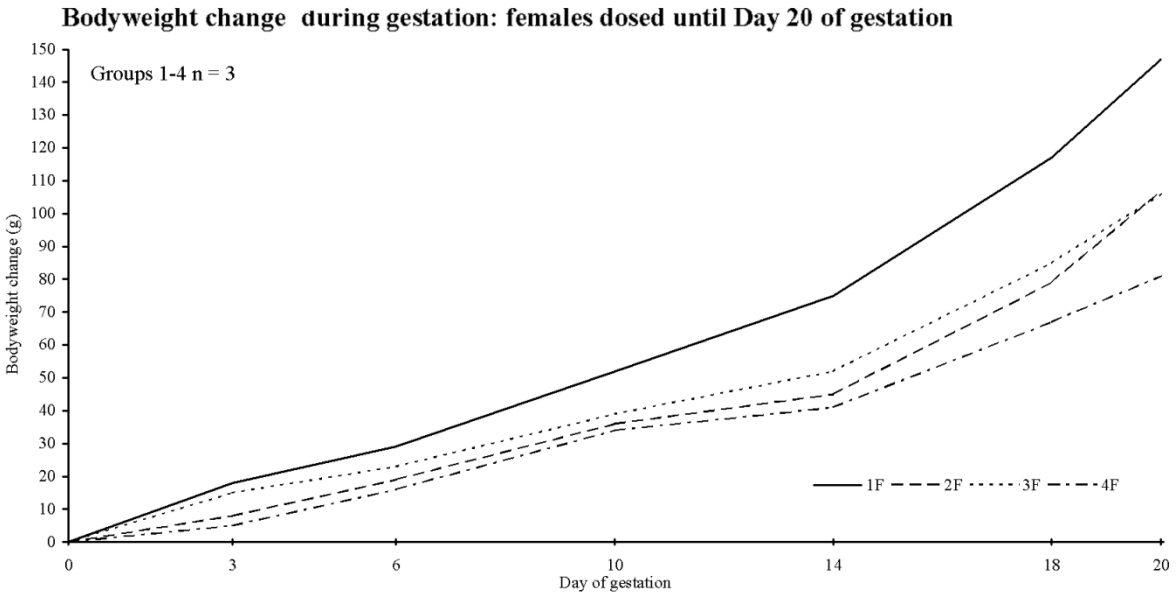
Body weight
Initial and transient body weight loss and reduced body weight gain were seen in all treated groups. During gestation, lower body weight gain was observed in all treated groups, starting at GD 6 in the mid- and high-dose groups and at GD 10 in the low-dose group.

Bodyweight change - group mean values (g) before pairing



Bodyweight change during gestation: females dosed until Day 17 of gestation





Mating and pregnancy performance

Estrous cycles, pre-coital interval, mating performance and fertility were unaffected by treatment.

Toxicokinetics

C_{max} and AUC_{tau} (tau=24hr) increased dose proportionally. There was an indication of a slightly lower exposure on Day 17 compared to Day 6 in all dose groups. Fetal exposure was approximately 3-4% of the maternal exposure.

Table 109. Maternal and fetal toxicokinetic parameters (Applicant's table)

Period	Dose (mg/kg)	Subject	C_{max} (nMolar)	T_{max} (h)	AUC_{tau} ((h)*(nMolar))	AUC_{last} ((h)*(nMolar))
6	0.05	Maternal	54.3	6	811	811
	0.1	Maternal	87.5	6	1660	1660
	0.15	Maternal	82.5	10	1530	1530
17	0.05	Maternal	39	6	555	555
	0.1	Maternal	78.4	6	1370	1370
	0.15	Maternal	81	10	1440	1440
20	0.05	Fetus	1.81	0	NC	NC
	0.1	Fetus	2.9	0	NC	NC
	0.15	Fetus	3.37	0	NC	NC

NC; not calculated

Litter data

Slight reduction in the mean number of corpora lutea and implantations was noted in all treated groups, without apparent dose response. Increase number of embryo-fetal deaths (early or late resorptions), post-implantation loss and live fetuses in all treated groups compared to controls. Placental, litter and fetal weights at ≥0.10 mg/kg were markedly lower than for controls.

Table 110. Litter data, group mean values on GD 20 (Applicant's table)

Group /Sex		Corpora Lutea	Implantations	Resorptions Early	Resorptions Late	Resorptions Total	Live Young Male	Live Young Female	Live Young Total	Sex ratio (%M)	Implantation Loss (%) Pre-	Implantation Loss (%) Post-
1F*	Mean	17.2	16.0	0.6	0.0	0.6	7.4	8.0	15.4	48.5	6.5	3.8
	SD	1.93	1.25				1.51	2.05	1.43			
	N	10	10	10	10	10	10	10	10	10	10	10
#	Mean	16.3	15.7	0.7	0.0	0.7	6.3	8.7	15.0	41.5	3.5	4.3
	SD	2.52	1.53				2.52	1.53	1.73			
	N	3	3	3	3	3	3	3	3	3	3	3
2F*	Mean	14.8	14.1	0.9	0.0	0.9	7.2	6.0	13.2	55.0	5.1	6.5
	SD	1.69	2.18				1.40	1.63	2.44			
	N	10	10	10	10	10	10	10	10	10	10	10
#	Mean	13.3	13.0	1.0	0.0	1.0	7.0	5.0	12.0	58.4	3.3	9.5
	SD	3.06	3.61				3.00	2.65	4.36			
	N	3	3	3	3	3	3	3	3	3	3	3
3F*	Mean	15.2	14.3	1.5	0.3	1.8	5.9	6.6	12.5	46.7	4.9	12.5
	SD	3.46	2.54				2.13	1.84	2.55			
	N	10	10	10	10	10	10	10	10	10	10	10
#	Mean	15.7	15.3	1.0	0.3	1.3	7.0	7.0	14.0	51.9	4.3	8.8
	SD	0.58	1.53				2.65	4.36	1.73			
	N	3	3	3	3	3	3	3	3	3	3	3
4F*	Mean	14.6	14.1	1.9	0.8	2.7	5.6	5.8	11.4	49.2	5.8	19.0
	SD	1.90	2.42				2.32	2.66	3.20			
	N	10	10	10	10	10	10	10	10	10	10	10
#	Mean	13.3	13.0	1.3	0.3	1.7	7.7	3.7	11.3	67.8	2.4	12.8
	SD	0.58	0.00				0.58	0.58	1.15			
	N	3	3	3	3	3	3	3	3	3	3	3

* Animals treated until Day 17 after mating # Animals treated until Day 20 after mating

Table 111. Placental, litter and fetal weights, group mean values on GD 20 (Applicant's table)

Group /Sex		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
1F*	Mean	0.52	53.85	15.40	3.61	3.39	3.50
	SD	0.053	6.242	1.430	0.309	0.271	0.293
	N	10	10	10	10	10	10
#	Mean	0.49	53.44	15.00	3.68	3.51	3.56
	SD	0.061	6.150	1.732	0.190	0.018	0.064
	N	3	3	3	3	3	3
2F*	Mean	0.53	46.45	13.20	3.63	3.41	3.53
	SD	0.040	7.849	2.440	0.185	0.197	0.176
	N	10	10	10	10	10	10
#	Mean	0.51	38.83	12.00	3.30	3.05	3.20
	SD	0.057	15.394	4.359	0.276	0.188	0.272
	N	3	3	3	3	3	3
3F*	Mean	0.44	34.69	12.50	2.86	2.67	2.76
	SD	0.059	8.169	2.550	0.220	0.276	0.231
	N	10	10	10	10	10	10
#	Mean	0.47	42.18	14.00	3.12	2.97	3.04
	SD	0.065	0.964	1.732	0.284	0.338	0.353
	N	3	3	3	3	3	3
4F*	Mean	0.38	27.37	11.40	2.54	2.26	2.38
	SD	0.043	8.810	3.204	0.341	0.279	0.271
	N	10	10	10	10	10	10
#	Mean	0.37	27.85	11.33	2.57	2.27	2.47
	SD	0.016	3.541	1.155	0.423	0.175	0.345
	N	3	3	3	3	3	3

* Animals treated until Day 17 after mating # Animals treated until Day 20 after mating

Fetal findings

Dose-related increased incidence of various skeletal and visceral abnormalities was observed mostly at the high-dose. Skeletal abnormalities included malrotated, absent, shortened, curved, kinked, misaligned, partially fused, and/or displaced bones (scapula, long bones, ribs, digits, vertebrae and cranial bones) in addition to incompletely ossified bones. The visceral abnormalities included folded retina, hydrocephaly and dilated brain ventricles, and abnormalities of the major blood vessels such as retro-oesophageal aortic arch and right subclavian artery, subclavian artery arising from the aortic arch, left umbilical and short innominate artery.

Table 112. Fetal examinations-major abnormalities (Applicant's table)

	Fetuses				Litters			
Group	1	2	3	4	1	2	3	4
Number examined	199	168	167	148	13	13	13	13
Number affected	3	0	3	25	2	0	2	8
Folded retina	-	-	1	1[j]	-	-	1	1
Hydrocephaly	-	-	-	5[jlogh]	-	-	-	2
Retroesophageal right subclavian artery	-	-	-	2	-	-	-	1
Retroesophageal aortic arch	-	-	-	1	-	-	-	1
Membranous ventricular septal defect	-	-	-	1[h]	-	-	-	1
Diaphragmatic hernia	-	-	1	-	-	-	1	-
Bent scapula/clavicle	-	-	1	10[abdgkn]	-	-	1	7
Short/bent/thickened long bones	2	-	-	8[abdfgkmn]	1	-	-	6
Absent tibia	-	-	-	5[ekmnp]	-	-	-	2
Absent deltoid tuberosity	-	-	-	2[np]	-	-	-	1
Ectrodactyly	-	-	-	12[cefhiklmnopq]	-	-	-	4
Brachydactyly	-	-	-	1[o]	-	-	-	1
Syndactyly	-	-	-	3[op]	-	-	-	3
Malrotated hindlimbs	-	-	-	9[efhiklnpq]	-	-	-	3
Complete situs inversus	1	-	-	-	1	-	-	-

Superscript denotes fetuses with more than one abnormality

Group	:	1	2	3	4
Dose (mg/kg/day)	:	0	0.05	0.10	0.15

Table 113. Fetal examinations-skeletal and visceral abnormalities (Applicant's table)

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined		199	168	167	148	13	13	13	13
Vertebral	tail kinked/caudal elements misaligned	-	1	11	38	-	1	6	12
Appendicular	bent clavicle/scapula	-	-	1	10	-	-	1	7
Fore limbs/paws	bent/thickened/short/incompletely ossified long bones*/absent deltoid tuberosity	2	-	-	7	1	-	-	5
	ectrodactyly/syndactyly /brachydactly	-	-	-	9	-	-	-	3
Hind limbs/paws	bent/absent/short/incompletely ossified long bones*	-	-	-	7	-	-	-	3
	ectrodactyly/syndactyly	-	-	-	12	-	-	-	4
	malrotated hindlimb(s)	-	-	-	9	-	-	-	3
Additional observations at necropsy									
	shiny skin	2	-	14	6	2	-	4	4
Total affected by one or more of the above		4	1	20	45	3	1	6	12

Note: Individual fetuses/litters may occur in more than one category.
*Fore limb long bones include radius, ulna, humerus
*Hind limb long bones include femur, tibia, fibula

Group	:	1	2	3	4
Dose (mg/kg/day)	:	0	0.05	0.10	0.15

Table 114. Fetal examinations-minor skeletal abnormalities/variants (Applicant's table)

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined		100	84	83	75	13	13	13	13
Skeletal abnormalities									
Cranial	sutural bone	-	2	-	-	-	2	-	-
	bridge of ossification maxilla to jugal	-	-	6	6	-	-	4	4
Vertebral element abnormality									
	sacrocaudal	-	-	-	1	-	-	-	1
Ribs	medially thickened/kinked	2	3	3	20	1	2	3	10
	partially fused	-	-	3	11	-	-	3	6
	interrupted 13th	-	-	-	2	-	-	-	2
Sternebrae	bipartite ossified	1	-	-	-	1	-	-	-
	partially fused	-	-	-	1	-	-	-	1
	wide	1	-	-	-	1	-	-	-
Costal cartilage	partially fused/fused	-	1	5	19	-	1	4	8
	interrupted	-	-	-	1	-	-	-	1
	7th not connected to sternum	-	1	3	3	-	1	2	2
Appendicular	misshapen scapula cranial margin	-	1	1	1	-	1	1	1
Total affected by one or more of the above		3	7	18	38	2	5	9	11

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined		100	84	83	75	13	13	13	13
Incomplete ossification/unossified									
Cranial centres		19	15	27	54	6	8	11	12
Hyoid		7	5	10	5	6	4	5	4
Vertebrae	cervical	3	2	1	11	2	2	1	6
	thoracic	4	-	7	24	4	-	5	11
	lumbar	-	-	2	9	-	-	2	4
	sacral centra/sacrocaudal	7	6	7	13	4	4	3	7
	caudal	-	-	2	8	-	-	2	5
Appendicular	clavicle/scapula	-	-	1	3	-	-	1	3
Rib		-	1	4	10	-	1	3	5
Sternebrae	5th and/or 6th	71	62	71	71	13	13	13	13
	other	2	10	44	58	2	8	13	13
	total	71	63	74	71	13	13	13	13
Pelvic bones		4	1	3	10	3	1	2	8
Metacarpals		1	-	-	2	1	-	-	2
Metatarsals		1	-	5	18	1	-	5	10

Note: Individual fetuses/litters may occur in more than one category.

Group :	1	2	3	4
Dose (mg/kg/day) :	0	0.05	0.10	0.15

Table 115. Fetal examinations-minor visceral abnormalities (Applicant's table)

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined		99	84	84	73	13	13	13	13
Visceral abnormalities									
Brain	dilated ventricles	-	-	1	3	-	-	1	2
	hydrocephaly	-	-	-	5	-	-	-	2
Eye(s)	folded retina	-	-	1	1	-	-	1	1
Thymus lobe	partially undescended	1	2	1	-	1	2	1	-
Innominate artery	short	2	2	1	6	2	1	1	3
Subclavian artery	arises from aortic arch	-	3	5	11	-	2	3	5
	retroesophageal	-	-	-	2	-	-	-	1
Aortic arch	retroesophageal	-	-	-	1	-	-	-	1
Azygos vein	additional	-	-	-	1	-	-	-	1
Ventricular septum	membranous defect	-	-	-	1	-	-	-	1
Lungs	unexpanded	-	-	1	-	-	-	1	-
Caudal vena cava	anomalous confluence left hepatic vein	-	2	-	-	-	2	-	-
Diaphragmatic	hernia	-	-	1	-	-	-	1	-

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined		99	84	84	73	13	13	13	13
Liver	posterior caudate lobe fissure	1	1	-	1	1	1	-	1
Testis(es)	displaced	-	3	3	5	-	3	3	4
Umbilical artery	left	-	2	7	9	-	1	6	9
Whole body	subcutaneous oedema	-	-	2	1	-	-	2	1
Haemorrhages	brain/spinal cord	-	1	2	-	-	1	2	-
	thoracic cavity	-	1	-	-	-	1	-	-
	abdominal cavity	4	3	2	-	3	2	1	-
	liver lobe(s)	3	-	-	1	3	-	-	1
	subcutaneous	-	2	2	-	-	1	2	-

Note: Individual fetuses/litters may occur in more than one category.

Group : 1 2 3 4

Dose (mg/kg/day) : 0 0.05 0.10 0.15

Study title: Combined fertility and embryo-fetal toxicity study by subcutaneous administration to CD rats

Study no.:	(b) (4) #JLY0145 (Novo Nordisk #NN207361)
Study report location:	Module 4.2.3.5.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 6, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, Batch #LP0217G1T01, 93.64% pure

Key Study Findings

- Dose-related decrease in body weight gain was observed at all dose levels in males and females (1X MRHD); food consumption generally reflected the body weight performance.
- Longer estrous cycle, decrease in the number of corpora lutea, implantations, live litter sizes at ≥0.03 mg/kg (0.4X MRHD)
- Decreased litter weights, placental and fetal weights at HD (1X MRHD)
- Major malformations were observed ≥0.03 mg/kg (0.4X MRHD), including cardiovascular abnormalities (retro-esophageal aortic arch, double aortic arch, and membranous ventricular septal defect) and short tibia (malrotated hindlimb).
- The NOAEL for female fertility was 0.01 mg/kg/d (0.4X MRHD) based on an abnormal estrus cycle and decreased mean number of corpora lutea. The NOAEL for embryonic development was 0.01 mg/kg/d based on a slightly increased number of major malformations. The NOAEL for male fertility was the high dose of 0.09 mg/kg/d (1X MRHD).

Methods

Doses: Adaptive phase, week 1 and 2

Group	Adaptive period (escalating dose phase) - mg/kg/day	
	Week 1	Week 2
1	Control (vehicle)	Control (vehicle)
2	Vehicle	Vehicle
3	Vehicle	0.01
4	0.01	0.03

Treatment phase (target dose levels) - Week 3 onwards

Group	Treatment	Dose (mg/kg/day)
1	Control	0
2	NNC 0113-0217	0.01
3	NNC 0113-0217	0.03
4	NNC 0113-0217	0.09

Frequency of dosing: Once daily

Dose volume: 1 mL/kg

Route of administration: Subcutaneous

Formulation/Vehicle: 1.42 mg/mL disodium (b) (4) phosphate, dihydrate; 14.0 mg/mL propylene glycol, and 5.50 mg/mL phenol in water for injection

Species/Strain: Rat/Sprague Dawley

Number/Sex/Group: 22/sex/group

Satellite groups: 5 females/group

Study design: To avoid significant effects on maternal body weight during gestation, dams were treated prior to mating using a dose escalation scheme. After the 2-week escalating dose phase, males and females received the target doses by once daily subcutaneous injection for 4 weeks (males) and 2 weeks (females) before pairing and until Day 17 after mating (or Day 20 for satellite females). Adult females were examined macroscopically at necropsy on GD20. All fetuses from all litters were examined macroscopically at necropsy and subsequently by detailed internal visceral examination or skeletal examination. Adult males were examined macroscopically at necropsy and organ weights were taken.

Deviation from study protocol: There were no protocol deviations that impacted the validity or interpretability of the study

Observations and Results
Mortality
None treatment related.

Clinical Signs
There were no treatment-related adverse effects on behavior or appearance.

Body Weight
Body weight was statistically significantly decreased for males at all dose levels, beginning on Day 15 for the mid- and high-dose groups and Day 32 for the low-dose group. Before mating, female weights became statistically significantly less than controls at Day 15 for high-dose females and at Day 18 for mid-dose females. After mating a dose-related decrease in body weight gain was observed for all dose groups.

Table 116. Body weights (Applicant's table)

Males

Dose (mg/kg/d)	0	0.01	0.03	0.09
Weight (g) - Day 1	345	342	341	342
Weight (g) - Day 71	541	510*	489**	480**
% diff from control (final weight)		↓6%	↓10%	↓11%
Weight gain (g)	196	168	148	138
Diff from control (g)		-28	-48	-58
% diff from control (weight gain)		↓14%	↓24%	↓30%

*p<0.05; **p<0.01.

Females (before mating)

Dose (mg/kg/d)	0	0.01	0.03	0.09
Weight (g) - Day 1	218	219	216	219
Weight (g) - Day 29	258	251	244**	241**
Weight gain (g)	40	32	28	22
Diff from control (g)		-8	-12	-18
% diff from control		↓20%	↓30%	↓45%

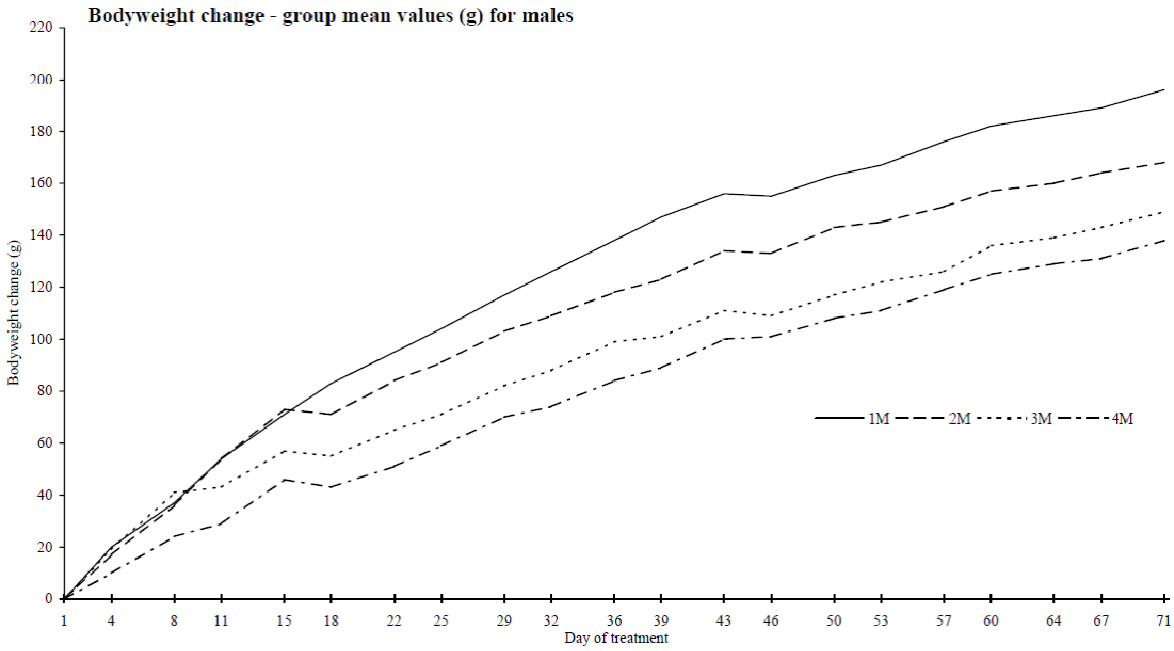
**p<0.01.

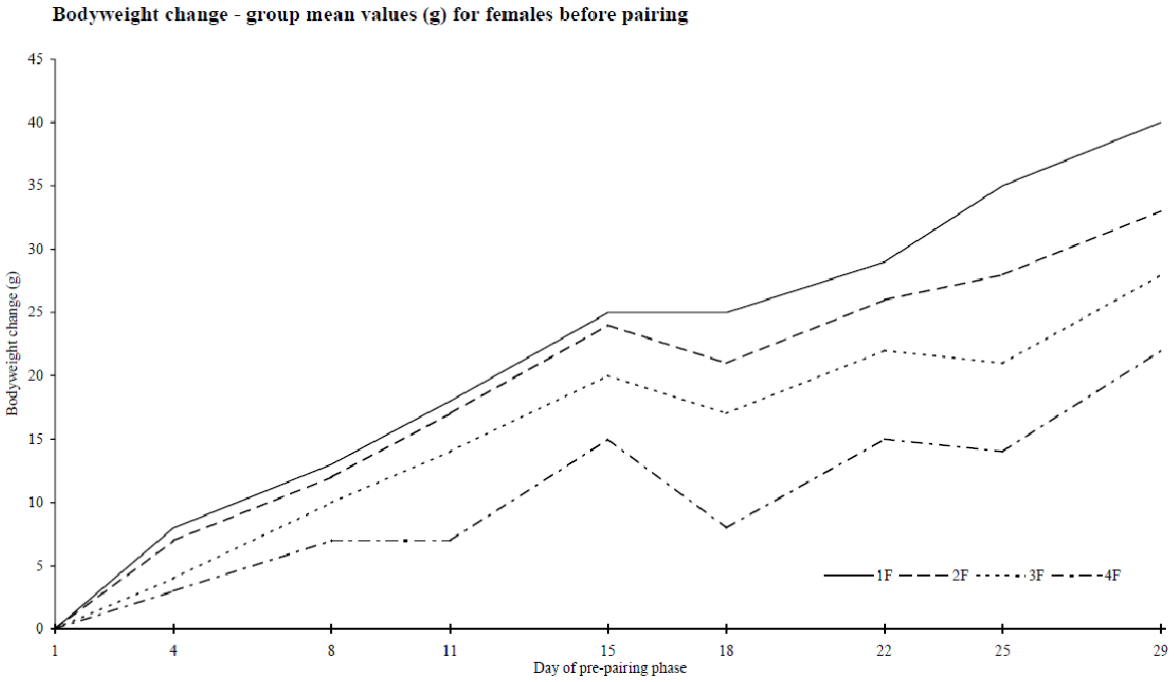
Females (after mating)

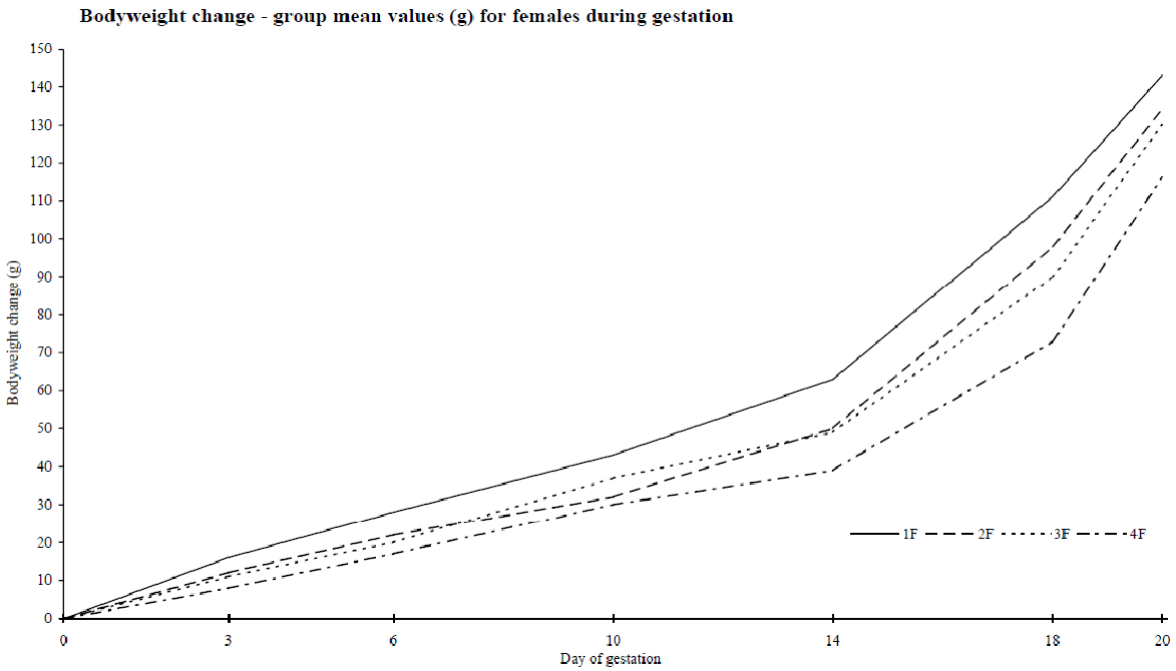
Dose (mg/kg/d)	0	0.01	0.03	0.09
Weight (g) - Gestation Day 0	263	257	249**	243**
Weight (g) - Gestation Day 18	374	356**	338**	316**
% diff from control (final weight)		↓5%	↓10%	↓16%
Weight gain (g)	111	99	89	73
Diff from control (g)		-12	-22	-38
% diff from control (weight gain)		↓11%	↓20%	↓34%

**p<0.01.

Figure 30. Body weights (Applicant's figure)

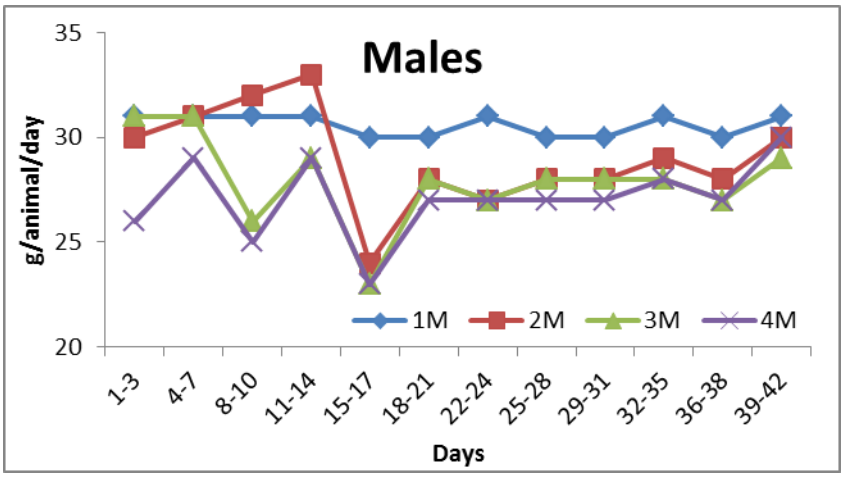


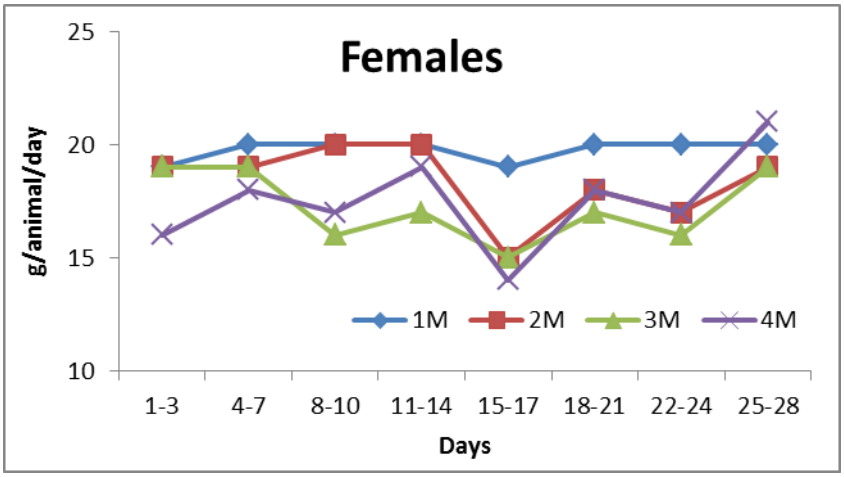


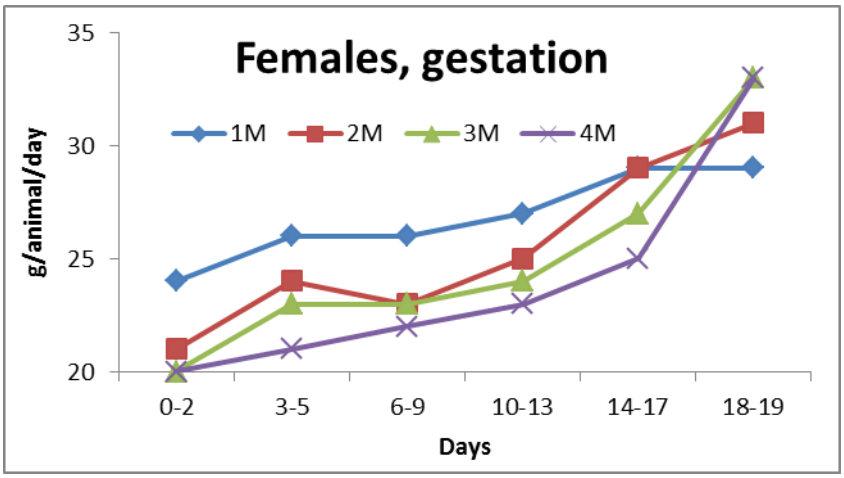


Feed Consumption

Food consumption was decreased in all male and female treated groups before mating. During gestation, incraese in food consumption was noted in all treated females.







Water Consumption
There were no definitive effects on water consumption for treated males or for pregnant females during the gestation period. During the pre-mating period, high-dose females appeared to show a marginal increase in mean water consumption compared with controls.

Calcitonin
There was no apparent treatment-related effect on maternal or fetal calcitonin levels.

Necropsy
Mean gravid uterine weight was statistically significantly decreased for the high-dose females (↓24%). No other treatment related changes were observed.

Toxicokinetics
Exposure to semaglutide increased linearly with the dose on both GD6 and GD17. Slightly lower exposure (0.7-fold) was seen on Day 17 compared to Day 6 in all dose groups.

Table 117. Toxicokinetic parameters on GD6 and GD17 (Applicant's table)

Day	Gender	Dose (mg/kg)	Cmax (nMolar)	Tmax (h)	AUC ((h)*(nM))	AUCtau ((h)*(nM))	AUCextrap (%)
6	Female	0.01	7.70	6.00	NC	94.1	NC
		0.03	17.3	3.00	NR	286	29.0
		0.09	58.6	3.00	NR	880	39.6
17	Female	0.01	5.45	10.0	NC	71.6	NC
		0.03	13.4	3.00	NR	208	32.4
		0.09	38.4	3.00	NR	590	43.3

NC; not calculated NR; not reported

Dosing Solution Analysis

Dosing formulations were assessed for concentration during Week 3 pre-mating, and on Gestation Days 6 and 17.

Recovery for the 0.01 mg/mL samples were between 72% and 77%.
Recovery for the 0.03 mg/mL samples were between 87% and 89%.
Recovery for the 0.09 mg/mL samples were between 90% and 92%.
Recovery for the 1 mg/mL stock solution was between 93% and 95%.

The concentration of the low dose formulation was slightly lower than the acceptance criteria. The applicant was unable to find an obvious reason for the apparent low recovery. This does not impact the interpretability of the study and the resulting exposure from a lower than intended dose will be reflected in the TK data.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

- There was no difference in the number of animals mating, conception rate, or fertility index.

- There was an increase in females with estrous cycles of 5 days or other irregular durations at the mid-dose (14%) and high-dose (23%) compared with controls (9%). A statistically significant decrease in the mean number of corpora lutea was observed for the MD and HD groups, resulting in a decreased number of implantation sites and slightly fewer live young on GD 20 in the high-dose group. There was not a definitive drug effect on pre- or post-implantation loss. The effect on estrus cycle and the number of corpora lutea may be considered adaptive responses secondary to the pharmacological effect of semaglutide on food consumption and body weight, as feed restriction and body weight loss have been demonstrated to cause increased estrus cycle length and reduced numbers of corpora lutea in Sprague Dawley rats (Terry KK et al, 2005).

- Mean placental, litter, and individual fetal weights were statistically significantly decreased in the HD group compared with control.

Table 118. Estrous cycles (Applicant's table)

Group	Number of animals		4 day	Regular cycles 4/5 day	5 day	Irregular cycle λ	Extended oestrus ϕ	Acyclic ψ
Statistical test: Lt								
1	22	n	20	1	0	0	0	1
		(%)	(91)	(5)				(5)
2	22	n	16	5	0	1	0	0
		(%)	(73)	(23)		(5)		
3	22	n	19	0	2	1	0	0
		(%)	(86)		(9)	(5)		
4	22	n	15	2	2	2	1	0
		(%)	(68)	(9)	(9)	(9)	(5)	

λ At least one cycle of two, three or six to ten days
ϕ At least four consecutive days of oestrus
ψ At least ten days without oestrus

Table 119. Historical control data for Crl:CD (SD) female rats approximately 11 to 13 weeks old at pairing (Applicant's table)

Pairing date	Number of animals		4 day	Regular cycles 4/5 day	5 day	Irregular cycle λ	Extended oestrus ϕ	Acyclic ψ
Mar-05	22	%	91	5	5	0	0	0
Apr-05	22	%	82	5	0	9	0	5
Sep-06	22	%	100	0	0	0	0	0
Oct-06	22	%	91	9	0	0	0	0
Jun-07	22	%	95	5	0	0	0	0
Apr-08	22	%	86	9	0	5	0	0
Jun-08	21	%	90	5	0	0	0	5
Total of 7 studies								
Mean (%)			90.7	5.4	0.7	2.0	0.0	1.4
Minimum (%)			82	0	0	0	0	0
Maximum (%)			100	9	5	9	0	5

λ At least one cycle of two, three or six to ten days
ϕ At least four consecutive days of oestrus
ψ At least ten days without oestrus

Table 120. Litter data - group mean values on GD20 (Applicant's table)

Group /Sex		Corpora Lutea	Implantations	Resorptions Early	Resorptions Late	Resorptions Total	Live Young Male	Live Young Female	Live Young Total	Sex ratio (%M)	Implantation Loss (%) Pre-	Implantation Loss (%) Post-
Statistical test:		Wi	Wi	Wc	Wc	Wc	Wi	Wi	Wi	Wa	Wa	Wa
1F	Mean	17.3	16.0	0.8	0.0	0.9	8.0	7.1	15.1	52.5	7.3	5.5
	SD	2.16	2.27				2.21	1.38	2.47			
	N	22	22	22	22	22	22	22	22	22	22	22
2F	Mean	16.5	15.3	0.4	0.0	0.4	7.6	7.4	15.0	50.7	7.2	2.4
	SD	1.63	1.35				1.91	1.99	1.32			
	N	21	21	21	21	21	21	21	21	21	21	21
3F	Mean	15.9*	14.8	0.9	0.0	1.0	7.5	6.4	13.9	53.3	5.9	6.9
	SD	2.51	1.87				2.04	1.44	2.57			
	N	22	22	22	22	22	22	22	22	22	22	22
4F	Mean	14.2**	13.6**	1.0	0.1	1.0	6.3*	6.2	12.5**	49.8	4.7	7.8
	SD	2.22	2.22				2.59	2.27	2.44			
	N	22	22	22	22	22	22	22	22	22	22	22

* p<0.05, ** p<0.01, *** p<0.001 vs. control (pairwise test)

Table 121. Placental, litter and fetal weights - group mean values (g) GD 20 (Applicant's table)

Group /Sex		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
Statistical test:		Sh	Wi	Wi	Sh	Wi	Wi
1F	Mean	0.55	53.50	15.14	3.64	3.41	3.53
	SD	0.065	9.556	2.475	0.261	0.205	0.214
	N	22	22	22	22	22	22
2F	Mean	0.55	54.81	14.95	3.78	3.55	3.67
	SD	0.042	5.031	1.322	0.161	0.157	0.152
	N	21	21	21	21	21	21
3F	Mean	0.56	50.52	13.86	3.77	3.51	3.64
	SD	0.109	9.828	2.569	0.252	0.286	0.258
	N	22	22	22	22	22	22
4F	Mean	0.50*	41.22**	12.55**	3.41*	3.16**	3.28**
	SD	0.052	9.191	2.444	0.357	0.301	0.310
	N	22	22	22	22	22	22

* p<0.05, ** p<0.01, *** p<0.001 vs. control (pairwise test)

Offspring (Malformations, Variations, etc.)

Major malformations were identified in 3 (2), 1 (1), 2 (2), and 4 (4) fetuses (litters) from the control, LD, MD, and HD groups. Major malformations included retroesophageal aortic arch, double aortic arch, membranous ventricular septal defect, and short tibia (malrotated hindlimb) in the HD group; absent cervical vertebral arch and retroesophageal aortic arch in the MD group; diaphragmatic hernia in the LD group; and bent scapula, short/bent/thickened humerus, and bent radius in the control group.

A slight increase in the incidence of some minor skeletal and visceral abnormalities/variants was observed in the MD and HD groups, including the cranium (sutural bone or bridge of ossification/partially fused), ribs (medially thickened/kinked or partially fused), costal cartilage (partially fused), misaligned caudal vertebral elements/kinked tail, misshapen scapula cranial margin, and shiny skin. A slight

increase in observations of incomplete ossification/unossified cranial centers and sternebrae (other) was noted for the HD group. Potential minor visceral abnormalities were observed in the MD and HD group, including brain (dilated ventricles - HD only) and subclavian artery (arising from aortic arch). The incidence for most of these findings was generally small; for example, 1 to 4 out of 139 fetuses in the HD group.

Table 122. Offspring data (Applicant's table)

Nominal Dose Levels (mg/kg/day)	0 (Control)	0.01	0.03	0.09
Initial number of animals	**22 M & 22 F**	**22 M & 22 F**	**22 M & 22 F**	**22 M & 22 F**
Foetal pathology (No. of foetuses (No. of litters))				
Major abnormalities				
Number examined	333 (22)	314 (21)	305 (22)	276 (22)
Retro-oesophageal aortic arch	0	0	1 (1)	1 (1)
Double aortic arch	0	0	0	1 (1)
Membranous ventricular septal defect	0	0	0	1(1)
Short tibia: malrotated hindlimb	0	0	0	1 (1)
Minor visceral abnormalities				
Number examined	166 (22)	155 (21)	153 (22)	137 (22)
Dilated brain ventricles	0	0	0	2 (1)
Dilated foramen interventriculare	0	0	1 (1)	0
Subclavian artery arises from aortic arch	0	0	1 (1)	5 (4)
Tail kinked	0	0	0	1 (1)
Minor skeletal abnormalities and variants				
Number examined	167 (22)	159 (21)	152 (22)	139 (22)
Cranial, bridge of ossification/partially fused maxilla to jugal	0	0	0	4 (4)
Caudal vertebral elements misaligned	0	0	0	4 (3)
Ribs partially fused	0	0	0	2 (2)
Costal cartilage partially fused/fused	0	0	0	3 (2)
Skeletal variants				
Cervical rib	1 (1)	1 (1)	5 (5)	5 (5)
Incomplete ossification/unossified				
Cranial centres	11 (8)	4 (4)	16 (9)	24 (14)
Cervical vertebrae	0	0	1 (1)	2 (2)
Lumbar vertebrae	0	0	3 (1)	2 (2)
Ribs	0	0	1(1)	4 (4)

9.2 Embryonic Fetal Development

Study title: Embryo-Fetal Toxicity Study in the Rabbit by Subcutaneous Administration

Study no.:	(b) (4) #JLY0143, NN207360
Study report location:	Module 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Marc 31, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, Batch #LP0217G1T01, 93.64% pure

Key Study Findings

- Body weight loss was observed throughout GD 11-14 at ≥0.0025 mg/kg/day (0.3X MRHD). Effects on body weight correlated with decreases in food consumption.
- There was a trend for a slight increase in post-implantation loss due to early resorptions at ≥0.0025 mg/kg/day (0.3X MRHD), which resulted in slightly lower mean litter sizes and weights.
- There was not a definitive treatment-related effect on the incidence of major abnormalities. Minor skeletal abnormality (additional sternebral centers, bridge of ossification/partially fused/fused sternebra, unossified/incompletely ossified metacarpals/phalanges) at ≥0.0025 mg/kg/day (0.3X MRHD) and minor visceral abnormalities (dilated renal pelvis, additional liver lobe, and forepaw flexure) at 0.0075 mg/kg/day (2X MRHD) were observed.
- Early pregnancy losses and increased incidences of minor fetal abnormalities were possibly secondary to the marked maternal effects, but a direct effect of semaglutide could not be excluded. Consequently, the NOAEL was considered to be the lowest dose tested, 0.001 mg/kg/day.

Methods

Doses:	0, 0.001, 0.0025, 0.0075 mg/kg/day
Frequency of dosing:	Once daily from GD 6 through GD 19
Dose volume:	0.1 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	1.42 mg/mL disodium (b) (4) phosphate, dihydrate; 14.0 mg/mL propylene glycol and 5.50 mg/mL phenol in water for injection
Species/Strain:	Rabbit / New Zealand white
Number/Sex/Group:	22
Satellite groups:	TK group: 3 main dose animals from each treatment group were used for TK sampling pre-dose and 4, 8, 12, and 24 hours after dosing on GD 6 and GD 19. Developmental data from these animals were combined with the data from the other animals in the group.
Study design:	Three groups of 22 female rabbits received NNC 0113-0217 at doses of 0.001, 0.0025 or 0.0075 mg/kg/day from Days 6 to 19 after mating. A similarly constituted Control group received the vehicle, at the same volume-dose throughout the same period. Animals were killed on Day 29 after mating for reproductive assessment and fetal examination.
Deviation from study protocol:	There were no protocol deviations that impacted the validity or interpretability of the study

Observations and Results

Mortality

There were no unscheduled mortalities.

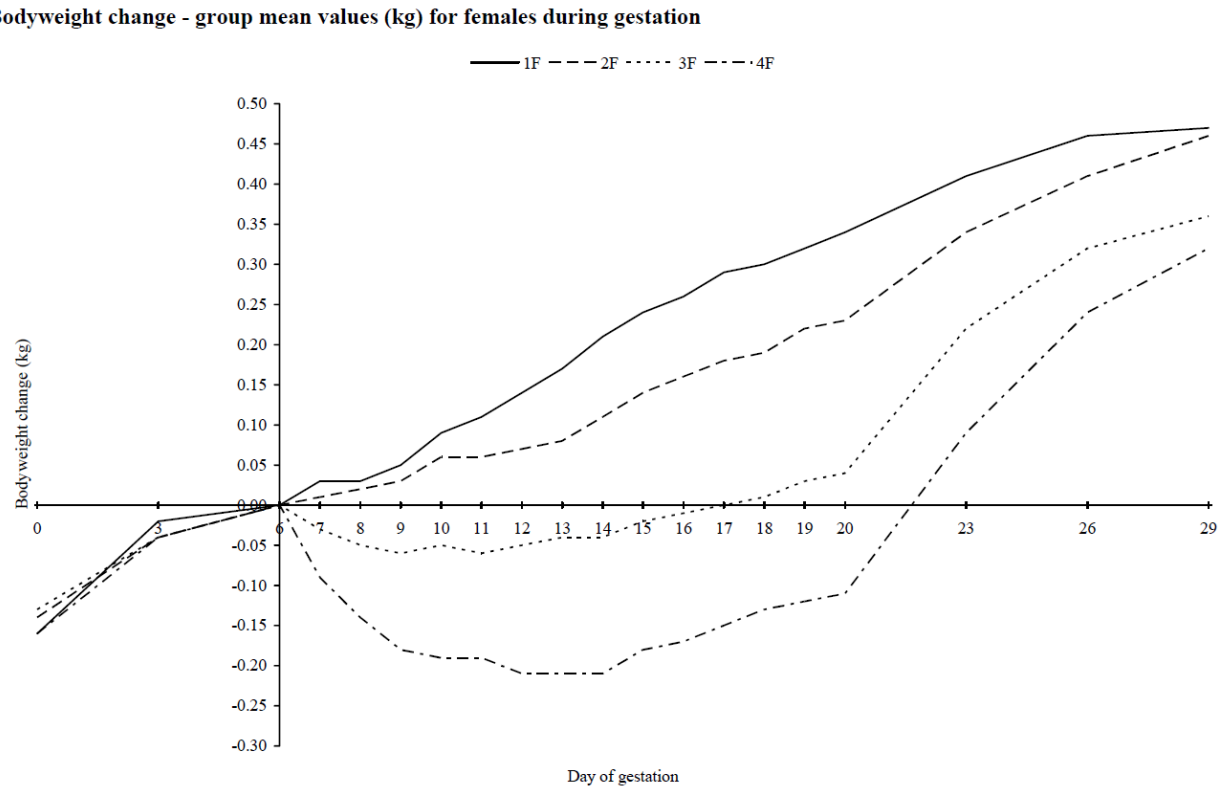
Clinical Signs

There was an increase in the number of animals having few feces in the MD (2) and HD (7) groups compared with zero in the control and LD groups. One to 2 animals in the MD and HD groups were noted as having yellow forepaws, head, hindpaws, or pinna compared with zero in the control and LD groups. The HD group had 2 animals noted as having an abnormal whole body (brown) and 2 to 3 animals from each of the three treatment groups were noted as having abnormal whole body color (yellow).

Body Weight

After initiation of dosing, the MD and HD groups lost weight and the LD group showed decreased mean body weight gain. On the day after the final dose (GD20), the mean body weight for the HD does was still below their weight on GD6. Body weight gain between GD6 and GD20 was 25%, 87%, and 132% less for the LD, MD, and HD groups, respectively, compared with the control group. The treatment-related effects on body weight are shown in the applicant-generated figure below.

Figure 31. Body weight, female rabbit, gestational period (Applicant's figure)



Feed Consumption
Effects on body weight correlated with decreased food consumption, with profound reductions in food consumption occurring in the HD group. Once dosing ended, food consumption increased for all treatment groups, surpassing the value for the control group; food consumption for the MD and HD groups was statistically significantly higher than the control value from GD 22 until the end of the study.

Serum glucose
Samples were collected pre-dose and 4, 8, 12, and 24 hours after dosing on GD 6 and 19. A slight decrease in mean serum glucose was noted for HD animals at 4, 8, and 24 hours after dosing on GD 6 (range 6.78 to 6.98 mmol/L) compared with pre-dose values or control values (range 7.30 to 7.82 mmol/L). MD values at 4 and 24 hours post-dose on GD 6 were slightly lower than pre-dose values but were similar to control values. There was no apparent effect serum glucose at the LD on GD 6 or at any dose level on GD 19.

Toxicokinetics
Blood samples were taken from three animals per dose group on Day 6 (on the first day of dosing) and Day 19 at the following nominal time points: predose, 4, 8, 12 and 24 hours after dosing.

Overall, the exposure to NNC 0113-0217, as evaluated by Cmax, increased with dose on both Day 6 and Day 19. Cmax generally increased in a more than proportional manner with dose level. There was a higher exposure to NNC 0113-0217 on Day 19 compared to Day 6 in all dose groups for both Cmax and AUClast (11-fold in the highest dose group).

Table 123. Toxicokinetic parameters, female rabbits, GD6 and GD19 (Applicant's table).

Period	Dose (mg/kg)	Subject	C_{max} (nMolar)	T_{max} (h)	T_{last} (h)	AUC_{last} ((h)*(nMolar))	Rac_{Obs}
6	0.001	42	0	NC	NC	NC	NC
		43	0	NC	NC	NC	NC
		44	0	NC	NC	NC	NC
		Mean					
		Stdev					
		%CV					
		Median					
	0.0025	64	1.5	12	12	11.8	NC
		65	1.24	8	8	4.7	NC
		66	1.44	8	12	11.2	NC
		Mean	1.39	9.3		9.22	
		Stdev	0.136	2.3		3.93	
		%CV	9.77	25		42.6	
		Median	1.44	8		11.2	
	0.0075	86	11.3	8	24	173	NC
		87	6.74	8	24	126	NC
		88	7.78	8	24	136	NC
		Mean	8.61	8		145	
		Stdev	2.39	0		24.5	
		%CV	27.8	0		16.9	
		Median	7.78	8		136	
19	0.001	42	2.02	0	24	46.8	NC
		43	1.18	4	8	4.68	NC
		44	1.33	12	12	9.08	NC
		Mean	1.51	5.3		20.2	
		Stdev	0.448	6.1		23.1	
		%CV	29.7	110		115	
		Median	1.33	4		9.08	
	0.0025	64	12.3	24	24	279	NC
		65	11.5	4	24	225	NC
		66	5.92	4	24	120	NC
		Mean	9.91	11		208	
		Stdev	3.48	12		81	
		%CV	35.1	110		39	
		Median	11.5	4		225	

Dosing Solution Analysis

Formulations were 77% to 82% of nominal for the LD, possibly due to test material adhesion to the sampling container; all other dose levels were 85% to 95% of nominal.

Necropsy

There were no gross pathology findings attributed to the test article.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There was a slight decrease in mean litter weight for the MD and HD groups, which was likely due to the slightly smaller litter size, as there was no difference in overall fetal weight.

There were no effects on the number of corpora lutea, implantation sites, or the male to female ratio. There was a trend for a slight increase in post-implantation loss due to early resorptions in the MD and HD groups.

Table 124. Cesarean section data (Applicant's table)

Litter data - group mean values on Day 29 of gestation

Group /Sex		Resorptions Early	Resorptions Late	Resorptions Total	Implantation Loss (%) Pre-	Implantation Loss (%) Post-
Statistical test:		Wc	Wc	Wc	Wa	Wa
1F	Mean	0.6	0.5	1.1	6.8	9.5
	SD					
	N	22	22	22	22	22
2F	Mean	0.6	0.5	1.1	9.5	9.3
	SD					
	N	20	20	20	20	20
3F	Mean	0.8	0.7	1.5	10.0	13.5
	SD					
	N	21	21	21	21	21
4F	Mean	1.1	0.5	1.6	9.9	15.3
	SD					
	N	21	21	21	21	21

Placental, litter and fetal weights - group mean values (g) on Day 29 of gestation

Group /Sex		Placental Weight	Litter Weight	Litter Size	Overall Fetal Weight
Statistical test:		Wi	Wi	Wi	Wi
1F	Mean	5.1	410.1	10.5	39.5
	SD	0.69	78.56	2.52	4.17
	N	22	22	22	22
2F	Mean	5.2	402.4	9.8	41.5
	SD	0.71	81.11	2.31	3.07
	N	20	20	20	20
3F	Mean	5.4	384.9	9.4	41.6
	SD	0.66	91.68	2.40	4.75
	N	21	21	21	21
4F	Mean	5.3	373.8	9.4	40.7
	SD	0.90	82.72	2.46	5.76
	N	21	21	21	21

Offspring (Malformations, Variations, etc.)

There was not a definitive treatment-related effect on the incidence of major abnormalities. The major abnormalities occurred at a low incidence and often did not occur in a dose-related manner.

In the 0.0075 mg/kg/day group, there were marginally higher than expected incidences of fetuses/litters with the minor visceral abnormalities of dilated renal pelvis, additional liver lobe, and forepaw flexure and minor skeletal abnormalities/variants, including

additional sternebral centers, bridge of ossification/partially fused/fused sternebrae, unossified/incompletely ossified metacarpals/phalanges compared with the concurrent controls and background control range (see applicant-generated tables below, which were modified to remove insignificant findings). In the 0.0025 mg/kg/day group, there was a marginally higher than expected incidence of fetuses/litters with the minor skeletal abnormality of additional sternebral centers compared with the concurrent controls and background control range. Although a relationship to treatment cannot be completely ruled out, the study director concluded that the findings were not considered to represent an adverse effect on fetal development because they are not detrimental to normal subsequent development.

Table 125. Fetal examination (Applicant's table)

Fetal examinations - major abnormalities - group incidences

	Fetuses				Litters			
Group	1	2	3	4	1	2	3	4
Number examined	232	196	198	197	22	20	21	21
Number affected	3	3	6	2	2	1	4	2
Open/partially open/punctate open eyelid(s)	3	3	1	-	2	1	1	-
Multiple folded retina: absent vitreous humour	-	-	2	-	-	-	2	-
Misshapen heart: dilated pulmonary trunk	-	-	1	-	-	-	1	-
Absent kidney/ureter	-	-	1	1	-	-	1	1
Absent adrenals	-	-	1	-	-	-	1	-
Bent scapula: hyperextension forelimb	-	-	-	1	-	-	-	1

Fetal examinations - minor visceral abnormalities - group incidences

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined at necropsy		232	196	198	197	22	20	21	21
Number heads examined at detailed visceral		122	103	104	105	22	20	21	21
Head	folded retina	1	2	1	-	1	2	1	-
	subdural haemorrhage	11	-	3	3	8	-	3	1
Number of heads affected by one or more of the above		**12**	**2**	**4**	**3**	**8**	**2**	**4**	**1**
Additional observations at necropsy									
Liver	additional lobe	2	4	1	10	2	2	1	3
Kidneys	dilated renal pelvis	2	2	1	5	2	2	1	4
	misshapen	-	-	-	1	-	-	-	1
Forelimb/paw flexure		-	-	2	3	-	-	2	3
Total number affected by one or more of the above		27	12	28	33	14	9	14	16

Note: Individual fetuses/litters may occur in more than one category.

Fetal examinations - minor skeletal abnormalities - group incidences

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined		232	196	198	197	22	20	21	21
Number intact		110	93	94	92	22	20	21	21
Skeletal abnormalities									
Cranial	sutural bone	-	2	1	3	-	2	1	3
	fissures/extra sutures	-	2	5	7	-	2	4	5
Sternebrae	additional centre(s)	3	4	21	20	3	3	10	9
	bridge of ossification/partially fused/fused	-	2	1	6	-	2	1	6
	bipartite ossified/offset alignment	-	2	3	1	-	2	3	1
	misshapen	1	1	2	-	1	1	2	-
	bifurcated 6th	-	-	-	2	-	-	-	2
Ribs	medially thickened	1	-	-	1	1	-	-	1
	partially fused	1	-	1	1	1	-	1	1
	interrupted 13th	1	-	3	1	1	-	3	1
Xyphoid cartilage	bifurcated/perforated	-	-	1	3	-	-	1	2
Costal cartilage	offset alignment	-	1	1	-	-	1	1	-
	partially fused/branched	-	1	3	-	-	1	2	-
	7th not connected to sternum	-	-	3	-	-	-	1	-
	8th connected to sternum	-	1	1	1	-	1	1	1
Appendicular	elongated acromion process	-	4	4	2	-	1	2	2
Total number of fetuses affected by one or more of the above		9	16	41	41	8	11	18	19
Rib and vertebral configuration									
Cervical rib		-	-	1	1	-	-	1	1
Short 1st rib with/without costal cartilage		-	-	4	-	-	-	2	-
Incomplete ossification/unossified									
20 thoracolumbar vertebrae		106	82	99	114	20	17	19	19
Offset alignment pelvic girdle		10	7	3	7	6	6	3	6
Sternebrae	5th	2	1	-	-	2	1	-	-
	other	-	1	2	6	-	1	1	5
	total	2	2	2	6	2	2	1	5
Epiphyses		8	4	3	4	6	1	3	3
Metacarpals/phalanges		13	8	18	36	5	4	8	15
Metatarsals/phalanges		-	-	1	-	-	-	1	-
Precocious ossification									
Ossified olecranon processes		-	1	3	8	-	1	2	4

Note: Individual fetuses/litters may occur in more than one category.

Study title: Injection Bolus (Subcutaneously) Embryo-Fetal Development Study in the Cynomolgus Monkey

Study no.:	NN208486
Study report location:	Module 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 27, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0113-0217, Batch LP0217G1V04, 92.42% pure

Key Study Findings

- Marked maternal body weight loss and reduced food consumption were observed during the dosing phase in all animals.
- There were no apparent treatment-related effects on fetal parameters (survival, body weights, organ weights, or placental weights).

- Few sporadic abnormalities (focal reddening of the skin, kinked and stiff wrist, blood accumulation under the skull causing misshapen right brain hemisphere, fused kidneys, liver cysts and shift in alignment of the vertebrae, ribs, and first sternebra, at the cervico-thoracic border) were observed at the mid and high dose (5 and 15X MRHD). Because these findings exceeded the concurrent control values and the historical control range, a relationship to treatment could not be excluded. NOAEL was established at 0.015 mg/kg, 1 X MRHD.

Methods

Doses:	0.015, 0.075, 0.15 mg/kg
Frequency of dosing:	GD 16, 18, 20, and every 3rd day thereafter until GD 50
Dose volume:	0.1 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	1.42 mg/mL disodium (b) (4) phosphate, dihydrate; 14.0 mg/mL propylene glycol; 5.50 mg/mL phenol in water for injection (WFI), adjusted to pH 7.4.
Species/Strain:	Monkey/Cynomolgus (*Macaca fascicularis*)
Number/Sex/Group:	16
Satellite groups:	None
Study design:	Three groups of 16 pregnant females were dosed during their pregnancy starting with two escalation dosing steps on gestation day 16 and 18 and then every third gestation day between day 20 and 50 at final target doses of 0.015 mg/kg/3 days, 0.075 mg/kg/3 days, and 0.15 mg/kg/3 days. The pregnancies were ultrasonographically monitored between gestation day 15 and 86. The pregnancies were terminated on gestation day 100±1 with a cesarean section to examine the fetuses. The females were necropsied and the fetus underwent a detailed external, visceral and skeletal examination.
Deviation from study protocol:	Several protocol deviations occurred during the course of the study. Of note, one control animal was injected with 0.075 mg/kg on GD 50 instead of control vehicle. The study deviations did not affect the validity or interpretation of the study.

Observations and Results

Mortality
No maternal deaths occurred.

Clinical Signs

Signs including dehydration, hypoactivity, or vomiting was occasionally noted for MD or HD animals, which was considered to be related to the pharmacological activity of the test article. On one occasion on GD20, animal 15039 from the 0.15 mg/kg group appeared to be pale, cool, and weak. It was discovered that this animal had received a dose of 0.15 mg/kg on the first day (GD16), thereby omitting the escalation phase in error.

Body Weight

Decrease in body weight was observed in all treatment groups throughout dosing, followed by body weight gain at dose cessation.

Figure 32. Maternal body weight (Applicant's figure)

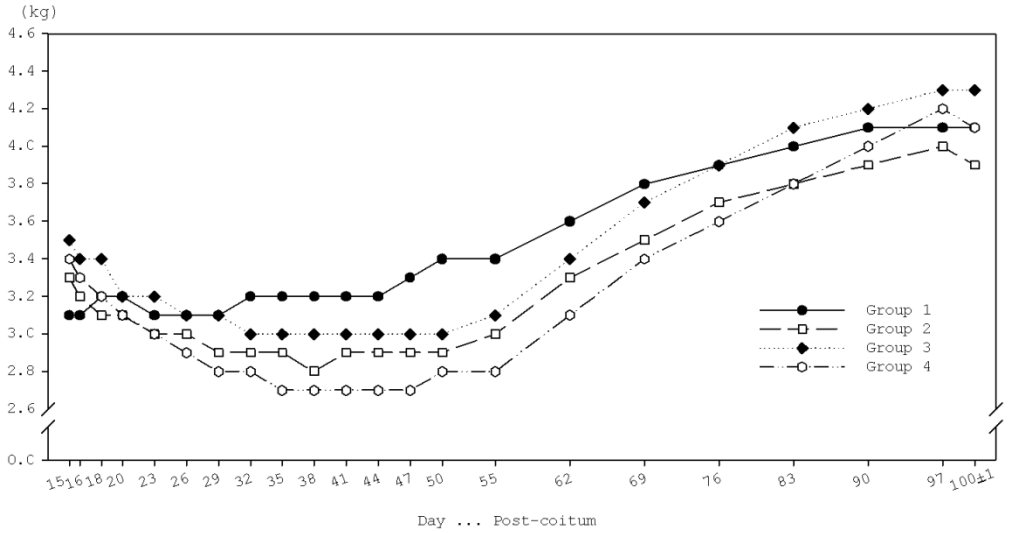


Table 126. Gestational body weight, monkey (Applicant's table)

Body weight change in %	Group 1: 0.0 mg/kg	Group 2: 0.015 mg/kg	Group 3: 0.075 mg/kg	Group 4: 0.15 mg/kg
GD 15 to GD 50	+ 9.7	- 12.1	-14.3	-17.6
GD 50 to GD 100	+20.6	+34.5	+43.3	+46.4
GD 15 to GD 100	+32.3	+18.2	+22.9	+20.6
% different from control GD 15	3.1 kg	+6.5	+12.9	+9.2
% different from control GD 50	3.4 kg	-14.7	-11.7	-17.6
% different from control GD 100	4.1 kg	-4.9	+4.9	0.0

Feed Consumption

Reduced food consumption was observed in all treated groups.

Toxicokinetics

For Group 2 blood samples (full profiles) were taken from each animal on the first day (on target dose level) and on GD 50 (last dosing day) at the following nominal time points: 0, 4, 8, 12, 24, 48 and 72 hours after dosing. During the up titration for Group 3 and 4, compliance samples were taken at 24hr post dose, as well predose on the very first day of dosing.

The C_{max} and AUC_{0-48h} increased with dose level, and the increase in exposure was proportional or somewhat more than proportional to dose level after single target dose and at steady state. Minor accumulation (less than 2-fold) was observed in all animals.

Table 127. Toxicokinetic parameters, gestational monkey (Applicant's table)

Day	Dose (mg/kg)	Group	C_{max} (nM)	T_{max} (h)	AUC_{tau} (nM*h)	$AUC_{(0-24h)}$ (nM*h)	$AUC_{(0-48h)}$ (nM*h)
16	0.015	2	32.4	21	1270	581	1270
18	0.075	3	182	17	7310	3580	7310
20	0.15	4	443	11	24000	8970	17400
50	0.015	2	42.1	12	2000	856	1530
	0.075	3	224	8	10400	4410	7910
	0.15	4	625	9	30000	12000	22700

AUC_{tau}=AUC_{0-72hr}, with the exception of Groups 2 and 3 on GD 16 and GD18 where AUC_{tau}=AUC_{0-48hr}, due to the dosing schedule.

Dosing Solution Analysis
Except for the HD formulation on GD 20, which was 80% of nominal, all other formulations were between 94% and 106% of the nominal concentrations.

Necropsy
There were no treatment related findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Fetal Mortality
There was no treatment-related increase in fetal mortality.

Fetal Growth
There were no apparent treatment-related effects on mean placental weight, fetal weight, or other external fetal growth measurements. Mean fetal organ weights were similar to control values except for testicular weights, which were slightly higher for the high-dose group. This difference appears to be within the expected range based on the standard error values, but considering increased testicular weights were observed in pre-pubescent or early pubescent monkeys with other GLP-1 receptor agonists, the data are presented below.

Mean Fetal Testicular Weights (Gestational Day 100)

Dose (mg/kg)	Control	0.015	0.075	0.15
N	8	7	11	7
Testis - left	0.0137	0.0150	0.0137	0.0167 (↑22%)
Testis - right	0.0142	0.0149	0.0136	0.0173 (↑22%)

Offspring (Malformations, Variations, etc.)
There were no malformations or variations that could be definitively attributed to treatment. Overall, the fetal pathology findings in this study were without commonality in nature, but the incidences exceeded the incidences in the control group and in the limited historical control data, thus a relationship to treatment could not be excluded.

Fetal macroscopic and stereomicroscopic visual inspections revealed no effects on the morphology of the fetal heart or on major blood vessel organization. The majority of the fetal variations observed were within the historical control range. However, incidences of sporadic malformations and variations at the mid and high dose levels exceeded the historical control ranges. These included generalized or focal reddening of the skin, kinked and stiff wrist, blood accumulation under the skull causing misshapen right brain hemisphere, fused kidneys, liver cysts and shift in alignment of the vertebrae, ribs, and first sternebrae, at the cervico-thoracic border. Only one fetus had more than one abnormality.

One low dose fetus had marked skin reddening affecting a large area of the left side of the body, appearing autolytic at the histopathological examination. This animal had a kinked and stiff wrist, but with no skeletal abnormalities. An area on the left side of the chest, which corresponded with the size and shape of the left arm, was not reddened. Together with the stiff wrist, this indicates that the position of the fetus in uterus might be the cause of the findings. Less widespread skin reddening was observed in the other treated groups, as well as in the control group. In some cases, the skin appeared normal when the fetus was removed from the mother, but reddening appeared when the fetus was injected with barbiturate. Histopathological examination of the reddened skin generally revealed correlating histological changes of minimal to slight multifocal congested vessels and/or minimal to slight multifocal hemorrhages in the dermis and subcutis. Based on findings from the pre- and postnatal study in cynomolgus monkeys, where no skin reddening was observed, the skin reddening was considered to be incidental or possibly related to the terminal procedures.

A misshapen right brain hemisphere in a HD fetus, categorized as a malformation, was due to accumulation of blood between the dura mater and the brain. This was initially observed on ultrasound on GD71, but the fetus was alive and in otherwise normal condition at the caesarean section on GD100. This finding had not been recorded previously in control monkeys.

Fused kidneys were observed in a mid-dose fetus. The kidneys were fused in the hilus area with a continuous cortical and partly medullar layer on the cranial and caudal side of the joined hilus. Each kidney had a pelvis and its own ureter and there were no signs of dysplasia or prematurity in the different structural elements of the kidneys. The overall development of the fetus did not indicate any impaired functionality of the kidneys. Fused kidneys had not been observed previously in control monkeys.

Skeletal malformations were observed in one high dose and one mid dose fetus, both with slight shifts in the alignment of the vertebrae, ribs and sternebrae at the cervico-

thoracic border. Such findings had not been recorded previously in control monkeys. The shift was considered unlikely to be of major functional significance in post-natal life.

An expert panel consulted by the applicant noted that with the exception of the two fetuses with a skeletal alteration, none of the findings was morphologically similar to other findings. The fact that not all of the isolated findings had been seen previously in control fetuses did not necessarily imply that they were treatment related. According to one panelist, the skeletal alteration observed in one mid-dose and one high-dose fetus was considered to be due to a slight shift in alignment of the vertebra, ribs, and first sternebra at the cervico-thoracic border. Although classified in the report as malformations because of its rare occurrence, the general opinion of the panel was that it would be unlikely to be of major functional significance in post-natal life. No similar finding was observed in the X-ray of any treated infant from the PPND study, assessed on PND 120. No morphological abnormalities were observed in infants from females in the PPND study. Also, it was agreed that the fetal findings in the primate fetuses were very different from those seen in the rat fetuses (limb, tail and aortic arches abnormalities). In conclusion, the expert panel agreed unanimously that the findings in primates were not indicative of a teratogenic response (expert panel meeting minutes are in the appendix).

Table 128. Summary of fetal malformations, monkeys (Applicant's table)

Parameter	Group 1 ##	Group 2 ##	Group 3 ##	Group 4 ##
Number of fetuses examined	15	14	14	14
External malformations				
Number of fetuses with malformations	1	1	0	0
Thorax - two additional nipples	1	0	0	0
Extremity/ies - wrist kinked and stiff	0	1	0	0
Visceral malformations				
Number of fetuses with malformations	0	0	1	1
Kidney(s) - fused	0	0	1	0
Brain - misshapen, right hemisphere	0	0	0	1
Inspection of fixed heart				
Number of fetuses with malformations	0	0	0	0
Skeletal malformations				
Number of fetuses with malformations	0	0	1	1
Vertebral column				
Vertebra(e), between 1 and 7, additional vertebra	0	0	1	1
Total number of fetuses with malformations	1	1	2	2

Parameter	Group 1 ##	Group 2 ##	Group 3 ##	Group 4 ##
Number of fetuses examined	15	14	14	14
Total number of fetuses with				
- no external abnormalities	4	3	2	4
- no visceral abnormalities	2	3	1	3
- no skeletal abnormalities	0	0	0	0
Total number of fetuses with variations	15	14	14	14
External variations				
Number of fetuses with variations	10	11	12	10
Skin - reddened	0	3	2	2
Skin - red focus/i	1	0	1	0
Skin - red spotted	1	0	0	1
Skeletal variations				
Number of fetuses with variations	15	14	14	14
Greater wing of sphenoid bone, incompletely ossified	3	3	6	11
Rib(s)				
Shortened	0	0	1	1
Thickened	0	0	0	1
Bent	0	0	0	1
Supernumerary, last cervical vertebra	0	0	1	1
Insertion on first and second sacral vertebra	0	0	1	1

Skin reddening was observed in several fetuses including controls. Overall, the most severe incidence of skin reddening occurred in Group 2. Samples of reddened and control fetal skin were examined microscopically by the study pathologist and digital images with special emphasis on bleedings and the number of capillaries were taken. Blood vessel congestion and/or hemorrhages in the dermis and subcutis, partly associated with an increased number of cutaneous blood vessels, were present in all fetuses affected by reddening of the skin. These findings could be observed throughout all dosing groups and the control group and correlated well with the macroscopic finding of skin reddening. The cause of the vessel congestion and hemorrhages could not be determined. As Group 1 fetuses were also affected, the pathologist concluded that a relation to the treatment of the mother animals seemed unlikely.

The expert panel noted that in a small number of fetuses skin reddening developed after terminal injection of pentobarbitone, and skin reddening has subsequently been recorded in control fetuses in the same laboratory. Dosing of the females was completed 50 days before termination of pregnancy on GD 100. At this time the skin of the fetus is immature. It is possible that terminal necropsy procedures may have played

a part in the development of skin reddening. No skin reddening was seen in the infants that were delivered naturally in the pre- and post-natal study, where dosing continued until day 140 of gestation. Therefore, the expert panel concluded skin reddening to be of no toxicological importance.

Table 129. Summary of external findings (Applicant's table)

	Group 1	Group 2	Group 3	Group 4
General reddening	15029**	149130	14790 *	14312
Focal reddening	14702 14803	14975 14604*	15036 149190 15047	149580 15154
Genital swelling				149440
Kinked/stiff wrist		149130		
Additional nipples	15000			

* Fetus 14604 and 14790 were normal in skin color when removed from the mother. The reddening appeared after euthanizing the fetus with barbiturates.
** Skin reddening of 15029 is apparent from the images (see Annex 1: Individual Fetal Photographs Group 1, Female no. 15029), the reddening was not recorded at necropsy The reddening appeared after euthanizing the fetus with barbiturates.

Table 130. Histopathology findings (Applicant's table)

Number of fetuses	Group 1	Group 2	Group 3	Group 4
- evaluated	6	3	4	3
- with blood vessel congestion	3	2	4	2
- with hemorrhages	3	2	4	2

9.3 Prenatal and Postnatal Development

Study title: Study for Effects on Embryo-Fetal and Pre- and Postnatal Development in Cynomolgus Monkeys

Study no.: NN210061
Study report location: Module 4.2.3.5.2
Conducting laboratory and location: (b) (4)
Date of study initiation: April 9, 2010
GLP compliance: Yes, except for X-ray image evaluation and mCG measurements
QA statement: Yes
Drug, lot #, and % purity: NNC 0113-0217 (semaglutide)
Batch LP0217K2X09, 96.82% pure
Batch LP0217K2X07, 95.92% pure

Key Study Findings

- Maternal weight loss was observed in all treated groups until GD 50.
- Increased incidence of early pregnancy loss (GD 16 to GD 50) at ≥0.075 mg/kg (3X MRHD).
- Lower infant body weights at birth in the HD group (7X MRHD). By Day 91 body weight was similar across all groups.
- Maternal toxicity during the first trimester may have directly contributed to the increased fetal mortality. However, a direct drug-related effect on the fetus cannot be ruled out. Therefore, the NOAEL was set at 0.015 mg/kg (1X MRHD).

Methods

Doses:	0.015, 0.075, and 0.15 mg/kg/ every 3 days
Frequency of dosing:	On GD 16, 18, 20, and every 3rd day thereafter until GD 140, inclusive, for a total of 43 doses. Mothers were not dosed during lactation.
Dose volume:	0.1 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	1.42 mg/mL disodium (b) (4) phosphate, dihydrate; 14.0 mg/mL propylene glycol; 5.50 mg/mL phenol in water for injection (WFI), the vehicle was adjusted to pH 7.4
Species/Strain:	Monkey/Cynomolgus (*Macaca fascicularis*)
Number/Sex/Group:	24
Satellite groups:	None
Study design:	Pregnant females were dosed by subcutaneous administration of NNC 0113-0000-0217 at target dose levels of 0.015, 0.075, and 0.15 mg/kg/ every 3 days from Gestation Day (GD) 20 to 140 after a dose escalation phase on Gestation Day 16 and 18 to ensure tolerability.
Deviation from study protocol:	None affecting the validity of the study

Observations and Results

F_0 Dams

Mortality/Clinical signs

There was no treatments related mortality or clinical signs.

Body weight

A dose dependent initial maternal body weight loss was observed in all treated groups between GD 16-50. Body weight gain was observed thereafter, although at lower rate in the mid- and high-dose groups compared to controls.

Table 131. Gestational body weight (Applicant's table)

Body weight change	Group 1: 0.0 mg/kg	Group 2: 0.015 mg/kg	Group 3: 0.075 mg/kg	Group 4: 0.15 mg/kg
GD 16 to GD 50	3%	-6%	-15%	-16%
GD 50 to GD 98	22%	27%	24%	19%
GD 16 to GD 98	25%	20%	6%	0%
GD 16 to GD 140	42%	37%	21%	14%

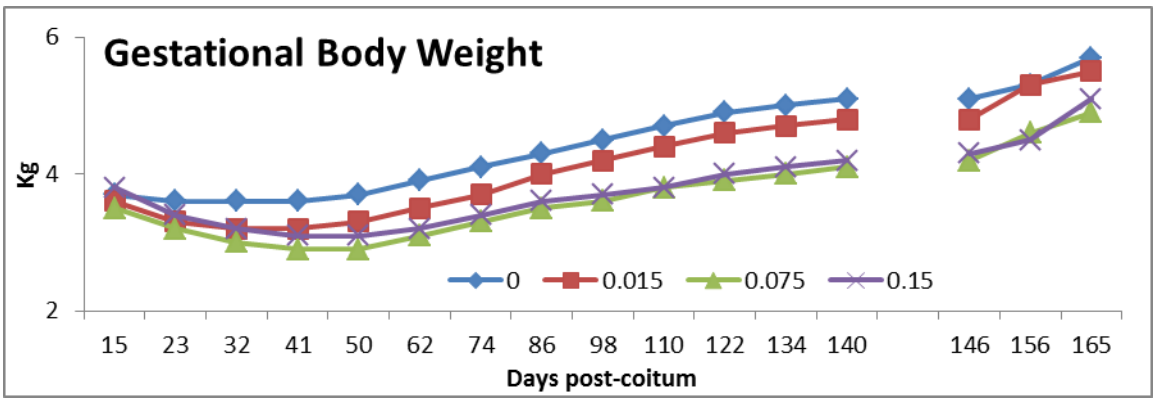
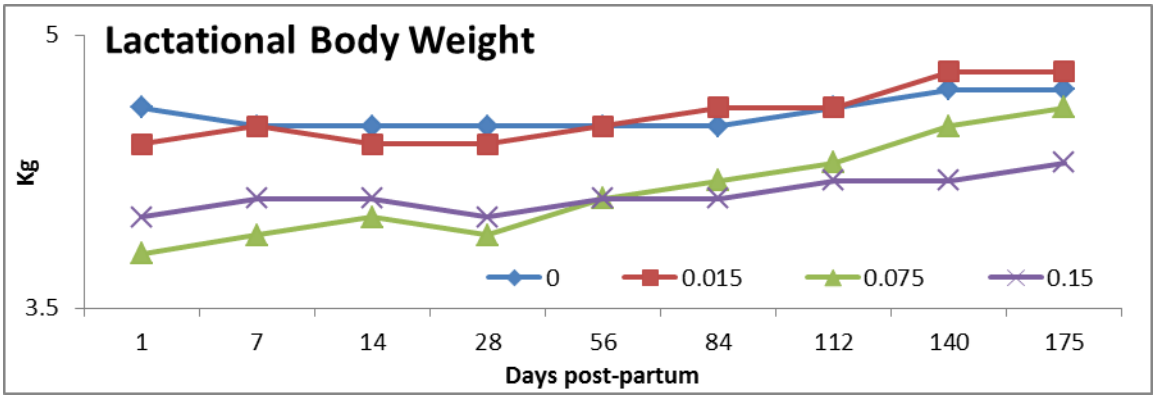


Table 132. Maternal body weight during lactation (Applicant's table)

Body weight change in %	Group 1: 0.0 mg/kg	Group 2: 0.015 mg/kg	Group 3: 0.075 mg/kg	Group 4: 0.15 mg/kg
Lactation Day 1 to Day 175	2%	9%	21%	8%
GD 16 to Lactation Day 175	31%	37%	35%	16%



Food consumption
Food consumption was qualitatively assessed as part of the evaluation of clinical signs.

Necropsy observations
There were no treatment related findings.

Toxicokinetics

Blood samples for toxicokinetics were taken from animals in selected dosing groups on GD 16 and 18 at predose (0), 4, 12, 24, and up to 48 hours after dosing, and on GD 20, 50, and 140 (last dosing day) at predose (0), 4, 12, 24, 48 and up to 72 hours after dosing.

Cmax and $AUC_{0\text{-}72h}$ increased with the dose. The increase in exposure was proportional to dose after repeated dosing both at GD 50 and at GD 140. Following repeated subcutaneous administration, exposure to NNC 0113-0217 in plasma (AUC0- 72h) generally tended to increase slightly from GD 20 to GD 50 with individual accumulation ratios in the range of 0.8 to 1.5. When comparing GD 20 with GD 140, exposure to NNC 0113-0217 tended to decrease and the individual accumulation ratios were in the range 0.4 to 0.8.

Table 133. Toxicokinetic parameters on GD 16, 18, 20, 50, and 140 (Applicant's table)

Period	Group	Dose (mg/kg)		C_{max} (nmol/L)	t_{max} (h)	$AUC_{0\text{-}48h}$ (nmol/L*h)	$AUC_{0\text{-}72h}$ (nmol/L*h)
16	2	0.015	Mean	33.1	24.0	1290	NA
			Stdev	5.27	10.6	206	NA
			%CV	15.9	44.2	15.9	NA
18	3	0.075	Mean	201	20.9	8140	NA
			Stdev	31.8	8.81	1240	NA
			%CV	15.8	42.1	15.2	NA
20	4	0.15	Mean	433	14.5	17700	24500
			Stdev	60.1	6.36	2520	3300
			%CV	13.9	43.7	14.3	13.4
50	2	0.015	Mean	54.0	17.6	2170	2900
			Stdev	9.34	9.57	343	463
			%CV	17.3	54.4	15.8	16.0
	3	0.075	Mean	266	12.5	10500	14000
			Stdev	42.0	5.08	1750	2500
			%CV	15.8	40.7	16.6	17.9
	4	0.15	Mean	512	13.8	20800	27900
			Stdev	76.1	5.46	3110	4530
			%CV	14.9	39.7	14.9	16.2
140	2	0.015	Mean	29.0	15.8	1070	1320
			Stdev	5.06	7.19	201	323
			%CV	17.5	45.6	18.8	24.6
	3	0.075	Mean	166	9.41	5530	6720
			Stdev	25.5	5.47	1270	1810
			%CV	15.4	58.1	23.0	26.9
	4	0.15	Mean	349	7.38	11900	14400
			Stdev	75.7	6.08	2420	3320
			%CV	21.7	82.3	20.3	23.0

NA = not applicable. For Day 16, Group 3 and 4, and for Day 18, Group 4, only 1 post-dose sample (24h) was available as according to the protocol, therefore no PK parameters are reported.

Dosing solution analysis
Dosing formulations were sampled on GDs 16, 18, 20, 50, 100, and 140. The results indicate that the dosing formulations were within the acceptance range of ±10% of the nominal concentration.

F1 generation
Increased early pregnancy losses were observed between GD16-50 at the mid and high dose levels and were considered likely related to the maternal body weight loss. However, due to the limited historical control data available and the lack of experience with cynomolgus monkey studies with such marked effects on maternal body weight, it cannot be excluded that the early pregnancy losses were treatment related, adverse effects.

Post-natal mortality was also increased at all dose levels compared with the control group. However, these values were within the normal range of expected infant mortalities of the reference data. Additionally, all infant deaths appeared to have different etiologies and/or clinical signs. Therefore, the infant deaths were not considered treatment related.

Dose (mg/kg)	Number of Confirmed Pregnant by mCG	Number of Live Infants at Birth	Pregnancy Loss				Post Natal Mortality†	Number of Live Infants at Study End
			GD 20-50	GD 50-150	Still-births‡	Total		
0	24	19	2	2	1	5 (21%)	0 (0%)	19 (79%)
0.015	22	17	1	3	1	5 (23%)	5 (29%)	12 (55%)
0.075	22	15	5	1	1	7 (32%)	3 (20%)*	12 (55%)
0.15	24	14	8	2	0	10 (42%)	3 (21%)	11 (46%)

GD = gestational day; mCG = monkey chorionic gonadotropin.
*In error, no lung swim test was performed on an infant that was found dead on post-partum Day 0. It is therefore possibly a stillbirth.
†Found dead or sacrificed moribund.
‡Stillbirth is defined as a born infant with a lung that has not been inflated with air (determined by a lung swim test).

Early pregnacy losses were not observed in the embryofetal development study. The panel of experts consulted by the applicant noted the following:

- Plasma levels of mCG from females which were reported pregnant and aborted between GD 16 and 50 showed that the early early pregnancy loses (prior to GD 25) were not due to false positive pregnancies.
- Females in the PPND study were housed three to a cage (two per cage in the EFD study). This may have lead to psycological stress which can interfere with the HPA axis and can lead to hormonal imbalance that may affect the mainteinance of pregnancy (Nakamura et al. 2008; Douglas 2010, Parker and Douglas, 2010). However, there were no records in the clinical signs of the PPND females to suggest any obvious adverse interaction between the females in any cage. Also, pregnancy outcome was not affected in other studies in whici animals were housed three per cage, but it was noted that none of these studies showed severe weight loss during early pregnancy.

- A wide range of prenatal loss rates In the cynomolgus monkeys has previously been reported in various facilities (Jarvis et al. 2010 and references therein).
- Stress of pregnancy alone, combined with the poor nutritional state of the semaglutide treated monkeys, was sufficient to result in pregnancy failure in some animals.

Clinical signs and external observations
There were no treatment-related clinical signs for infants. The type, pattern, and frequency of clinical signs, such as lesions/swellings, skin reddening/spots/wheals, and scratches/bites, were similar between all groups. There were no treatment-related external abnormalities observed. The skin reddening that was observed in earlier studies was not observed in this study.

Body weight
Mean infant body weights were lower at the MD and HD levels, with the effect being statistically significantly lower for HD infants on Day 1 (↓14%). The differences in mean body weight between controls and MD and HD groups diminished over time, with a 4% difference at Day 91 and only 1% at Day 181.

Table 134. Infant body weight after birth (Applicant's table)

Body weight	Group 1: 0.0 mg/kg	Group 2: 0.015 mg/kg	Group 3: 0.075 mg/kg	Group 4: 0.15 mg/kg
Mean ± SD Day 1 p.p. [g]	338 ± 41	334 ± 57	299 ± 38	290 ± 39*
Day 1 p.p. [n]	19	17	13	13§
Mean ± SD Day 91 p.p. [g]	694 ± 72	684 ± 101	665 ± 104	666 ± 59
Day 91 p.p. [n]	19	12	12	11
Mean ± SD Day 181 ± 1 p.p. (necropsy) [g]	1053 ± 160	1076 ± 193	1039 ± 127	1040 ± 113
Day 181 ± 1 p.p. (necropsy) [n]	19	12	12	11

§ One infant not weighed on Day 1 p.p. due to poor physical condition
* The mean of Dose Group 4 are significantly lower than the Dose Group 1 (vehicle control) (t-test; $p \geq 0.05$)

Physical development
Skeletal development:
Skeletal development was assessed around post-partum day 120 by conducting a whole-body X-ray. Dual-energy X-ray absorptiometry (DEXA) was conducted to evaluate bone mineral density and content, body fat, and body muscle mass.

No treatment-related effects on skeletal development were observed.

Morphological examinations:
Measurements of head circumference, distance between the eyes, crown-rump length, crown-heel length, tail length, chest circumference, length of upper and lower extremities, and ano-genital distance were conducted on postpartum days 1, 21, 56, 84, and 180.

Mean crown-heel length and chest circumference values were statistically significantly shorter for MD and HD infants on PPD 1, although in a non-dose-related manner. This

was not observed at other time points. The mean length of extremities was slightly shorter for HD infants on PPD 1 and PPD 21. Overall, differences observed at early time points were not observed on PPD 180 and were not considered to be toxicologically meaningful.

Clinical pathology:
Hematology, coagulation, and clinical chemistry evaluations were conducted on post-partum days 28, 98, and 178.

No toxicologically meaningful differences in clinical pathology endpoints were observed for semaglutide-exposed groups.

Neurological assessment
There was no treatment-related neurobehavioral impairment in neurobehavioral test battery conducted on post-partum day 1 and 7.

Necropsy:
Organ weights:
Spleen, adrenal glands, kidneys, liver, gallbladder, ovaries, uterus, testes, epididymides, thyroid (with parathyroid), thymus, heart, and brain were weighed.

No biologically meaningful differences in mean organ weights were observed across the treatment groups.

Gross pathology:
Findings at necropsy were reported to be generally consistent with the expected spectrum of background pathology in Cynomolgus monkeys and concurrent controls. There were no unusual macroscopic findings suggestive of target organ toxicity.

Histopathology:
There were no treatment-related microscopic findings in infants that died prematurely or in infants evaluated after terminal sacrifice.

11-Week Subcutaneous Toxicity Study in Juvenile Rats Followed by a 4-Week Recovery Period

Study no.:	214479
Study report location:	Module 4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 27, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC0113-0000-0217 and NNC0113-0217, Batch # 412_N14309 and 415-N15050, 96.3% and 95.8% pure

Key Study Findings

- Reduced food consumption and body weight gain was observed for all treated groups (1X MRHD) compared with the pair-fed control group.
- Sexual maturation was delayed in males and females at all dose levels (1X MRHD); body weight at attainment of sexual maturation was higher than in pair-fed controls.
- There were no treatment related effects on estrous cyclicity, mating performance or fertility.
- Minimal to slight Brunner's gland hypertrophy was observed in all treated female group and in males at $\geq$0.13 mg/kg.
- NOAEL: 0.6 mg/kg/day, 22X MRHD

Methods

Doses:	0 (ad libitum, reference controls), 0 (pair fed to the HD, pair-fed controls), and 0.02, 0.13, 0.6 mg/kg/day
Frequency of dosing:	Once daily from PND 21 to 97
Route of administration:	Subcutaneous
Dose volume:	1 mL/kg
Formulation/Vehicle:	Aqueous solution containing 1.42 mg di-sodium [redacted] (b) (4) phosphate, dihydrate, 14.0 mg propylene glycol, 5.5 mg phenol and water for injection. The vehicle was adjusted to pH 7.4.
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	10/sex/group
Age:	21 days old
Weight:	34 to 58 g (males), 34 to 55 g (females)
Satellite groups:	20/sex/group for reproductive assessment 4 weeks after dose cessation
Unique study design:	A pair-fed control group and an ad libitum fed control group were included. The pair-fed control, and the low and mid dose semaglutide treated groups, were pair-fed to the high dose semaglutide treated group, which was fed ad libitum, but expected to have reduced food consumption. Treatment commenced for the ad libitum control group and the high dose group three days earlier than the other study groups to permit pair-feeding based on the actual consumption of the high dose animals.
Deviation from study protocol:	None affecting the integrity of the study

Group	Treatment	Feeding Regimen[a]	Dose (mg/kg/day)			Number of animals			
			Day 21-24 of age	Day 25-27 of age	Day 28-97 of age	Main phase		Recovery/ Reproductive phase[ϕ]	
						Males	Females	Males	Females
1	Control	*Ad libitum* fed	0	0	0	10	10	20	20
2	Control	Pair-fed	0	0	0	10	10	20	20
3	Semaglutide	Pair-fed	0.02	0.02	0.02	10	10	20	20
4	Semaglutide	Pair-fed	0.02	0.13	0.13	10	10	20	20
5	Semaglutide	*Ad libitum* fed	0.02	0.13	0.6	10	10	20	20

[ϕ] Mating trial conducted after a minimum of 4 weeks recovery

[a] Groups 2, 3 and 4 were pair-fed to the food consumption of the high dose semaglutide treated group (Gr. 5).

Observations

The following parameters were assessed during and/or at the end of the dosing and recovery periods: viability, clinical condition, body weight, food consumption, hematology, clinical chemistry, urinalysis, toxicokinetics, sexual maturation, neurobehavior, estrous cycle, limb measurements, organ weights, macropathology and histopathology. Furthermore, mating performance and fertility were assessed after the recovery period.

Results

Mortality

None treatment related.

Clinical Signs

No treatment related clinical signs were observed.

Body Weights

Body weight gain in all treated male groups was generally lower than reference controls throughout the dosing period (17 to 21% lower); during the recovery period overall mean body weight gains of these animals were greater than in reference controls.

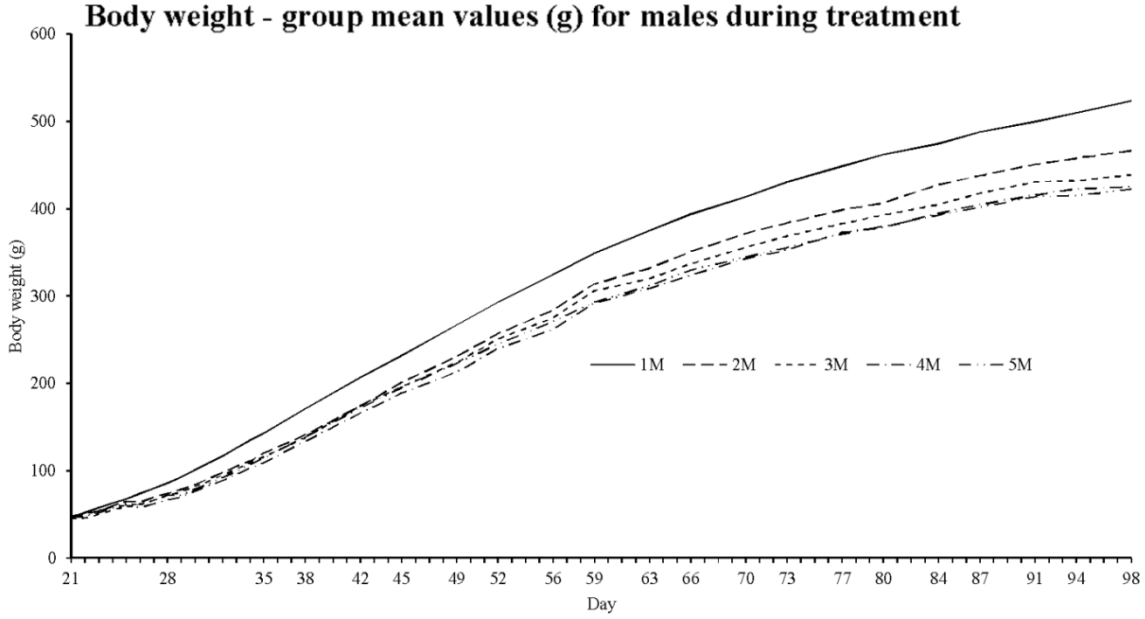
Body weight gain in treated females was lower than reference control from Day 21 to Day 31 of age but thereafter body weight gain was generally similar to or slightly greater than in controls until the end of the recovery period.

The mean absolute body weight for the pair-fed control males and females on Day 98 of age was 11% and 7% lower than reference control, respectively. Despite the pair-feeding, the body weight gain was lower in the semaglutide treated groups than in the pair-fed controls.

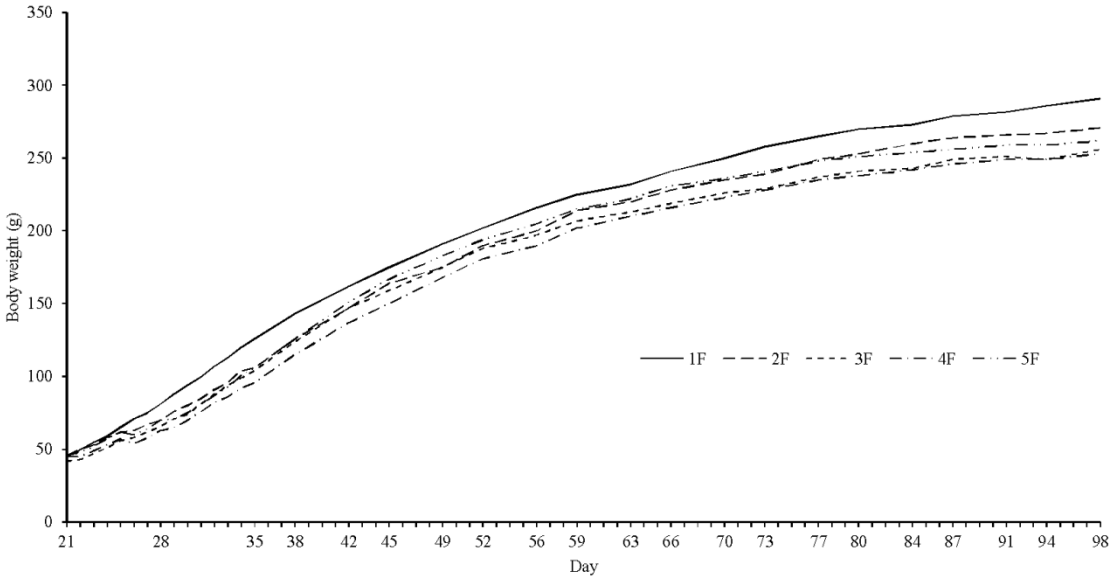
There was no effect of previous treatment on body weight gain during gestation.

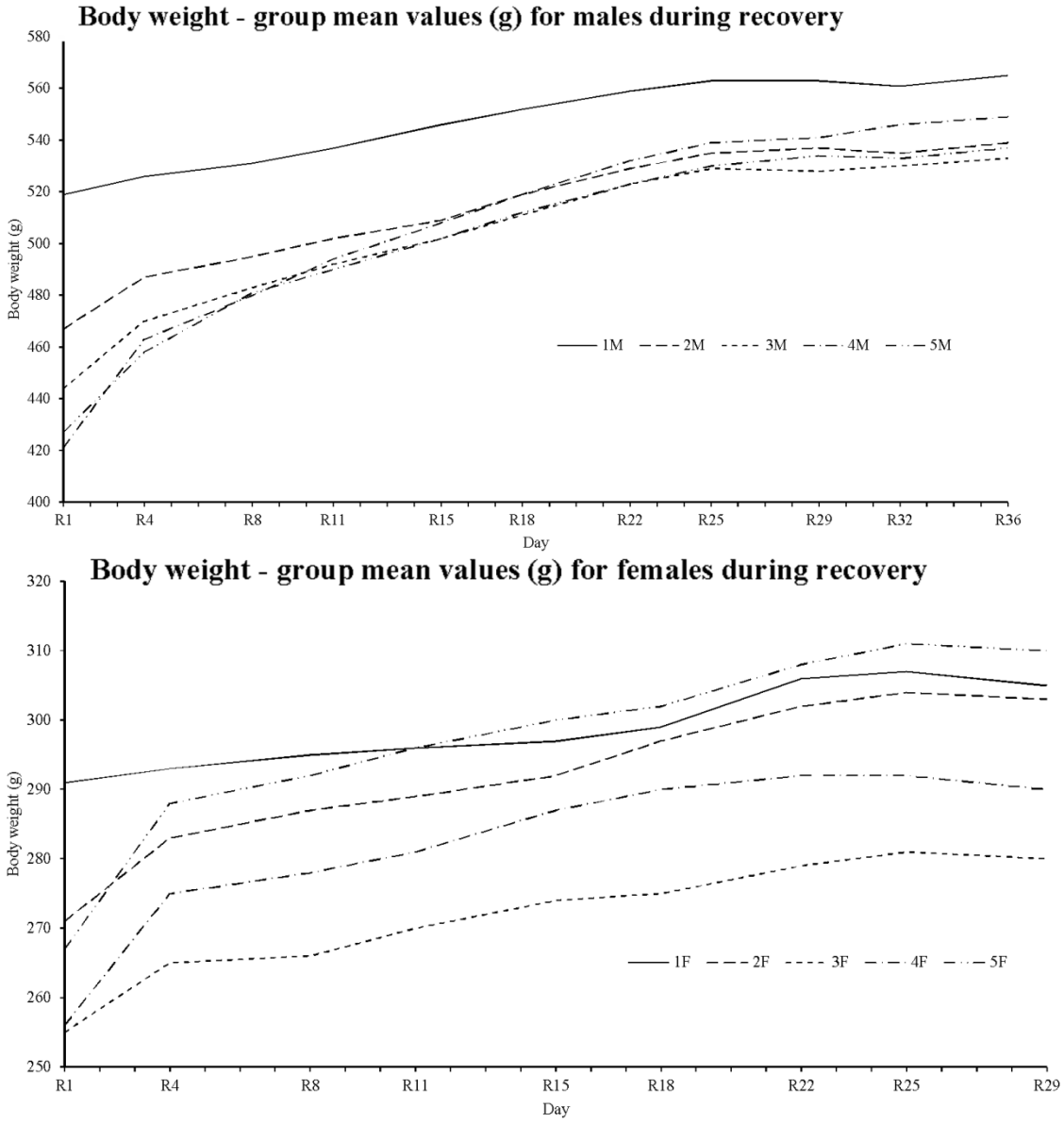
BW gain PND 21-98								
Males					Females			
	g	% vs. 1M	% vs. 2M			g	% vs. 1F	% vs. 2F
1M	477				1F	246		
2M	420	-12			2F	225	-9	
3M	394	-17	-6		3F	214	-13	-5
4M	377	-21	-10		4F	208	-15	-8
5M	377	-21	-10		5F	216	-12	-4

Body weight - group mean values (g) for males during treatment



Body weight - group mean values (g) for females during treatment





Feed Consumption

Food consumption was decreased during the first two weeks of treatment in high-dose males and females when compared with the reference control group (28-30% lower over the period of Day 21-34 of age). Thereafter, food intake was similar to reference controls in females, and steadily increased in males, although it remained slightly lower than reference control. During the first two weeks of recovery (Days 1-14), food intake of all semaglutide-treated males and females was markedly higher than reference control, and was thereafter similar to marginally higher than reference control until scheduled termination.

Nominal Dose (mg/kg/day)	0 (Control)	0 (Control)	0.02	0.13	0.6	0 (Control)	0 (Control)	0.02	0.13	0.6
Food consumption (all animals)										
Day 21-34 of age (g/animal/day)	15	10	10	9	10	14	10	10	9	10
Day 35-97 of age (g/animal/day)	29	26	25	25	25	20	18	17	17	19
Day 21-97 of age (g/animal/day)	26	23	22	22	23	19	17	16	16	17

Physical development

Bone length

Overall, there was no effect on ulna growth. Ulna length was slightly decreased between Day 21 and Day 49 of age in all treated males and females compared to pair-fed controls.

Sexual maturation

In control females, the lower body weight of the pair-fed control group, compared with the ad libitum fed control group, was reflected in a minimal delay of sexual maturation and minimally lower body weight at the time of vaginal opening, although the changes were not statistically significant. Delay in vaginal opening was observed in all treated female groups when compared to pair-fed controls despite higher body weight.

In males, the reduction of body weight in the pair-fed control group compared with the ad libitum fed control group did not cause a delay of sexual maturation, measured as the age at onset and completion of preputial separation. Despite similar body weights in the semaglutide treated groups and the pair-fed control group, at the age at onset of preputial separation in the pair-fed control group, a delay in onset of preputial separation occurred in the high dose semaglutide treated males, and body weight at the onset of preputial separation was higher in the high dose males than in the pair-fed controls. Completion of preputial separation was delayed in all semaglutide treated groups compared with the pair-fed controls and the body weight at completion of preputial separation was higher than in the pair-fed control group.

Nominal Dose (mg/kg/day)	0 (Control)	0 (Control)	0.02	0.13	0.6	0 (Control)	0 (Control)	0.02	0.13	0.6
Ulna growth (all animals)										
Day 21 - Day 35 of age (mm)[c]	6.1	5.4^^	5.3	5.2**	5.5**	5.9	5.5	5.2	4.5**	5.2
Day 21 - Day 91 of age (mm)[c]	16.9	16.9	16.2	16.2	16.6	13.8	13.4	13.4	13.2	13.8
Sexual maturation (Recovery/Reproductive phase animals)										
Vaginal opening [c]										
Age at attainment (days)	N/A	N/A	N/A	N/A	N/A	33.1	34.4	37.3**	41.8**	38.5**
Body weight at attainment (g)	N/A	N/A	N/A	N/A	N/A	114	108	122*	137**	130**
Preputial separation [c]										
Age at onset (days)	41.6	41.2	40.9	40.4	45.2**	N/A	N/A	N/A	N/A	N/A
Body weight at onset (g)	204	178^^	164**	151**	197**	N/A	N/A	N/A	N/A	N/A
Age at completion (days)	46.4	45.7	48.5**	50.9**	50.6**	N/A	N/A	N/A	N/A	N/A
Body weight at completion (g)	239	213^^	225	229*	234**	N/A	N/A	N/A	N/A	N/A

* - p<0.05 ** - p<0.01 (pairwise test, semaglutide treated groups versus pair-fed control)

^ - p<0.01 ^^ - p<0.01 (pairwise test, pair-fed control group versus *ad libitum* fed control group)

ΔΔ -p<0.01 (for the 0.6 mg/kg/day groups compared to the vehicle group)

[c] Pair-fed control versus *ad libitum* fed control and semaglutide treated groups versus pair-fed control.

Neurobehavioral examination

During week 8 and 9 of the dosing period, motor activity, functional observations battery, and learning test(morris maze) were evaluated in all recovery/reproductive phase animals.

No treatment related cganges were observed.

Clinical pathology

Slight increase in hematocrit and hemoglobin in high dose males and a trend towards an increase in hemoglobin and red blood cell count in high dose females were noted compared to both control groups. These findings were accompanied by a trend towards a minimal increase in mean cell hemoglobin concentration in semaglutide treated groups compared with the ad libitum fed control group. No changes were observed after the 4-week recovery period. In the absence of any correlating pathology findings, the above changes were considered non-adverse.

Dose-related decrease in triglycerides was noted in all treated animals

Table 135. Clinical pathology (Applicant's table)

Nominal Dose (mg/kg/day)	0 (Control)	0 (Control)	0.02	0.13	0.6	0 (Control)	0 (Control)	0.02	0.13	0.6
Haematology[c]										
Haematocrit (L/L)										
Week 11	0.443	0.445	0.440	0.440	0.461*	0.436	0.419^^	0.426	0.435*	0.442**
Week R4	0.476	0.458^	0.471	0.462	0.471	0.456	0.413^	0.450	0.461**	0.463**
Haemoglobin (g/dL)										
Week 11	15.0	15.2	15.0	15.2	15.8**	15.0	14.7	14.7	15.2*	15.6**
Week R4	15.4	14.9	15.1	14.8	15.3	15.2	13.9	14.7	15.1	15.2
Red blood cells (*10^{12}/L)										
Week 11	8.48	8.37	8.41	8.08	8.68	8.11	7.93	8.10	8.36**	8.43**
Week R4	8.79	8.45^	8.47	8.45	8.45	8.36	7.40^	7.99	8.24*	8.14*
Mean cell haemoglobin concentration (g/dL)										
Week 11	33.8	34.0	34.1	34.6**	34.2	34.3	35.0^^	34.6	35.0	35.3
Week R4	32.3	32.5	32.1	32.2	32.6	33.2	33.7^	32.7**	32.7**	33.0**

Nominal Dose (mg/kg/day)	0 (Control)	0 (Control)	0.02	0.13	0.6	0 (Control)	0 (Control)	0.02	0.13	0.6
Clinical chemistry[c]										
Glucose (mmol/L)										
Week 11	8.16	7.40	7.69	8.34	6.75	7.34	7.77	7.30	6.08**	5.96**
Week R4	6.87	6.79	7.08	7.35	6.91	6.71	7.79^	7.76	8.37	6.78*
Triglycerides (mmol/L)										
Week 11	0.91	0.66	0.36**	0.28**	0.20**	0.31	0.36	0.30	0.22*	0.18**
Week R4	0.72	0.74	0.77	0.49	0.64	0.36	0.41	0.39	0.47	0.34
Total protein (g/L)										
Week 11	60	60	60	60	61	65	63	62	61	60*
Week R4	65	67	64	64	63	66	65	66	64	67
Albumin (g/L)										
Week 11	34	34	34	33	34	39	38	36*	36**	35**
Week R4	35	35	34	35	35	39	38	39	37	39

* p<0.05, ** p<0.01 (pairwise test, semaglutide treated groups versus pair-fed control)

Urinalysis

When compared with pair-fed controls, a statistically significant slight decrease in urinary pH in HD males and in all treated females and lower sodium/chloride output in males at ≥0.02 mg/kg/day and in all treated females were observed. All differences were, however, within the historical control range and were considered to be of no toxicological significance.

Table 136. Urinalysis, percent change vs. control (Applicant's table)

Urinalysis - Week 11 of treatment

Group/Sex	2M	3M	4M	5M	1M	2M
Vol	4.9	+29	+16	-6	6.1	-20
pH	7.1	-4	+1	-7**	6.9	+3
SG	1047	-1	-1	0	1041	+1
Prot	0.82	+6	+18	+55*	1.19	-31^
U-Na	187.6	-43**	-11	-43**	107.3	+75^^
U-K	306.2	-32	-19	-15	237.0	+29
U-Cl	212.1	-52**	-16	-49**	108.7	+95^^

Urinalysis - Week 4 of recovery

Group/Sex	2M	3M	4M	5M	1M	2M
Vol	5.1	+8	+2	+14	6.3	-19
pH	7.0	+4	+3	+3	7.0	0
SG	1049	0	0	0	1044	0
Prot	1.03	-12	+13	+24	1.34	-23
U-Na	93.8	-16	-25	+12	84.3	+11
U-K	252.9	-7	0	+1	226.6	+12
U-Cl	79.7	-16	-17	+34	81.4	-2

Urinalysis - Week 11 of treatment

Group/Sex	2F	3F	4F	5F	1F	2F
Vol	4.3	-7	-12	-16	4.0	+8
pH	6.7	-6**	-7**	-7**	6.3	+6^^
SG	1040	0	0	0	1044	0
Prot	0.18	+22	+11	+50**	0.25	-28^
U-Na	144.3	-37*	-25*	-24*	123.7	+17
U-K	207.5	-27	-12	-18	207.0	0
U-Cl	156.7	-53**	-31**	-33**	120.0	+31

Urinalysis - Week 4 of recovery

Group/Sex	2F	3F	4F	5F	1F	2F
Vol	4.3	-21	-23	0	3.9	+10
pH	6.3	+2	+2	+5	6.5	-3
SG	1040	0	+1	0	1042	0
Prot	0.19	+26	+42*	+26*	0.21	-10
U-Na	78.4	+8	+17	+52**	102.5	-24
U-K	181.4	+18	+26	0	186.4	-3
U-Cl	98.4	-13	0	+16	113.0	-13

* p<0.05, ** p<0.01 (pairwise test, semaglutide treated groups versus pair-fed control)
^ p<0.01, ^^ p<0.01 (pairwise test, pair-fed versus ad lib fed control group

Reproductive performance

For the reproductive assessment, control males were paired with control females, previously treated males were paired with untreated females, and previously treated females were paired with stock males. All females were terminated on Day 14 after mating (mid-gestation) for a uterine assessment.

No effects were oberved on estrous cycle regularity, pre-coital interval, mating performance and fertility, or litter data, including mean number of corpora lutea, implantation sites, resorptions, live embryos and percentages of pre- and post-implantation losses.

Gross Pathology

There were no treatment related changes.

Organ Weights

As a consequence of the lower terminal body weight, when compared to pair-fed controls, a decrease in absolute organ weights and a corresponding increase in relative organ weights were seen for various organs, predominantly at ≥0.13 mg/kg/day. However, there were no macroscopic or microscopic correlates for any of the organ weight differences and the differences were all considered non-adverse.

Histopathology

Adequate Battery: Yes

Peer Review: Yes, by Inger Thorup, Novo Nordisk

Histological Findings
Treatment-related histopathological changes were limited to the duodenum. Minimal or slight hypertrophy of the Brunner's glands were seen in all treated female group and in males at ≥0.13 mg/kg, with dose-related increase in severity. This finding persisted into recovery for one LD and MD female each (minimal in severity).

Table 137. Histopathology (Applicant's table)

Tissue and Finding	Group/Sex: / Number:	1M 10	2M 10	3M 10	4M 10	5M 10	1F 10	2F 10	3F 10	4F 10	5F 10
Duodenum	Number Examined:	10	10	10	10	10	10	10	10	10	10
Brunner's Glands - Hypertrophy	Minimal:	0	0	6	5	3	0	0	4	4	3
	Slight:	0	0	0	5	7	0	0	0	5	7
	Total:	0	0	6	10	10	0	0	4	9	10

Toxicokinetics

The Cmax and AUCtau increased linearly with the dose and were slightly higher on Week 11. No apparent differences in the exposure to semaglutide were observed between female and male rats.

Exposure in juvenile rats following 11 weeks of daily subcutaneous administration was similar to the exposure in adult rats following 26 weeks of daily subcutaneous. For comparison, the AUCtau after repeated dosing of 0.6 mg/kg was 15000 and 18100 nmol.h/L in juvenile and adult rats, respectively.

Table 138. Toxicokinetic parameters at week 5 and 11 (Applicant's table)

Dose (mg/kg)	Week	Sex	t_{max} (hr)	C_{max} (nmol/L)	AUC_{tau} (hr*nmol/L)
0.02	5	Male	4	17.6	313
		Female	4	22.6	344
		Mean	**4.00**	**20.1**	**329**
0.02	11	Male	4	25.6	439
		Female	8	27.6	472
		Mean	**6.00**	**26.6**	**456**
0.13	5	Male	8	151	2290
		Female	2	168	2400
		Mean	**5.00**	**160**	**2340**
0.13	11	Male	8	187	3560
		Female	8	196	3660
		Mean	**8.00**	**191**	**3610**
0.60	5	Male	4	646	11100
		Female	4	848	11200
		Mean	**4.00**	**747**	**11100**
0.60	11	Male	8	696	13900
		Female	4	845	16100
		Mean	**6.00**	**770**	**15000**

AUC_{tau} = $AUC_{0\text{-}24hr}$

Dosing Solution Analysis

Analysis of the formulations prepared for use on Day 1 of dosing revealed that the achieved concentrations for Groups 3 and 4 (0.02 and 0.13 mg/mL) were outside the acceptance criteria and found to be 129.8% and 112.6% of nominal concentrations, respectively. Re-analysis of the samples confirmed the original result.

10 Special Toxicology Studies

Mechanistic studies to explore effects on embryo-fetal development in rats

Background

In the rat developmental toxicity studies, increased frequency of embryo-fetal loss, fetal growth retardation and major fetal malformations were observed at approximately the clinical dose. Increase in minor skeletal variations in the rabbit EFD and few sporadic fetal malformations in the monkeys EFD were observed, but these were considered mostly related to the marked maternal body weight loss. In order to understand the mechanism and to assess the potential human relevance of the embryotoxicity findings in rats, a series of mechanistic studies was performed. The majority of rat abnormalities involved anatomical structures (tail, digits and major blood vessels) for which development is generally initiated between gestational day 10 and 13, which coincides with the period when the rat embryo is highly dependent on nutrition supplied via the inverted yolk sac (Fig. 31) (DeSesso JM 2012). It was therefore hypothesized if impairment of the inverted yolk sac placenta could cause the observed embryotoxicity. Embryotoxicity caused by functional impairment of the inverted yolk sac placenta function in rats has been reported in the scientific literature for other compounds, e.g. trypan blue (Roger JM 1985) and a Haemoglobin Based Oxygen Carrier (HBOC-201) (Holson JF 2005).

Table 139. Summary of fetal findings in rats (Applicant's table)

	Maternal response		Embryo-foetal effects				
	Dose level (mg/kg)	GD 18 BW diff vs ctrl	Embryo-foetal loss[1]	Foetal weight vs control	Skeletal findings	Visceral findings	Exposure rati to humans[3]
Pre-liminary studies	0.83	32% ↓	100 %	N/A	N/A	N/A[2]	15
	0.20	23% ↓	46 %	42% ↓	+++	N/A[2]	3.1
	0.15	19% ↓	19 %	32%↓	++	++	2.2
	0.10	17% ↓	13 %	21%↓	++	++	2.1
	0.05	10% ↓	6.5 %	-	-	-	0.8
Main study	0.09	16 % ↓	7.8 %	7%↓	+	+	0.9
	0.03	10 % ↓	6.9 %	-	-	(+)	0.3
	0.01	5 % ↓	2.4 %	-	-	-	0.1
	0 (vehicle)	N/A	5.5 %	N/A	-	-	N/A

[1] Post implantation loss; [2]Visceral examination not included; [3] Calculated from arithmetic mean AUC_{0-24h} on GD 16/17 (corrected for dosing frequency) in pregnant rats (Table 2.6.7.3, study no 206616, 207359, 207361) and geometric mean for AUC_{0-168h} (4,602 nM×h) in female postmenopausal subjects with type 2 diabetes (Trial NN9535-3819). - no effect, (+) minimal effect, + moderate effect, ++ marked effect, +++ severe effect

Table 140. Summary of fetal findings in rabbits (Applicant's table)

	Maternal response		Embryo-foetal effects					
	Dose level (mg/kg)	GD 18 BW diff vs ctrl	Embryo-foetal loss[1]	Foetal weight vs control	Skeletal findings		Visceral findings	
					Minor	Major	Minor	Major
Main study	0.0075	11 % ↓**	15.3 %	3% ↑	41/197	1/197	33/197	1/197
	0.0025	6 % ↓**	13.5 %	5%↑	41/198	0/198	28/198	6/198
	0.001	4 % ↓*	9.3 %	5%↑	16/196	0/196	12/196	3/196
	0 (vehicle)	-	9.5 %	N/A	9/232	0/232	27/232	3/232

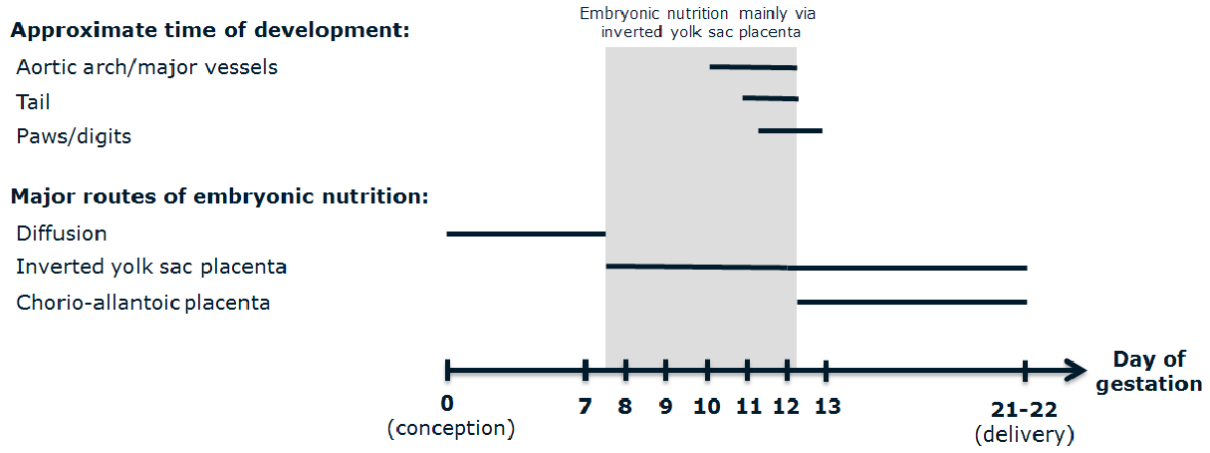
[1] post implantation loss

Table 141. Summary of fetal findings in monkeys (Applicant's table)

	Dose (mg/kg/3d)	Dose duration	BW change GD 15-50	Pregnancy loss		Foetal/infant weight vs control	Major findings [a]
				GD 16-50	GD 50→		
Pilot EFD II (n=6 per dose)	0.15	GD 16-50	18 % ↓	0	0	-	No foetal findings
	0.075	GD 16-50	12 % ↓	1	0	-	Skin reddening
Main EFD II (n=16 per dose)	0.15	GD 16-50	18 % ↓	1	1	0 %	Blood accumulation under skull, fused kidneys, Skeletal mis-association[b]
	0.075	GD 16-50	14 % ↓	2	0	0 %	Liver cysts; skeletal mis-association[b]
	0.015	GD 16-50	12 % ↓	2	0	2 % ↓	Skin reddening*, stiff wrist*
	0 (vehicle)	GD 16-50	10 % ↑	1	0	-	One extra nipple
Main PPND (n=24 per dose)	0.15	GD 16-140	16 % ↓	8	2	14 % ↓	No infant findings
	0.075	GD 16-140	15 % ↓	5	1	12 % ↓	No infant findings
	0.015	GD 16-140	6 % ↓	1	3	1 % ↓	No infant findings
	0 (vehicle)	GD 16-140	3 % ↑	2	2	-	No infant findings

[a] Single events, except for findings marked with *, which occurred in the same foetus. [b] An incorrect association between vertebrae, ribs and sternebrae at the cervico-thoracic border.

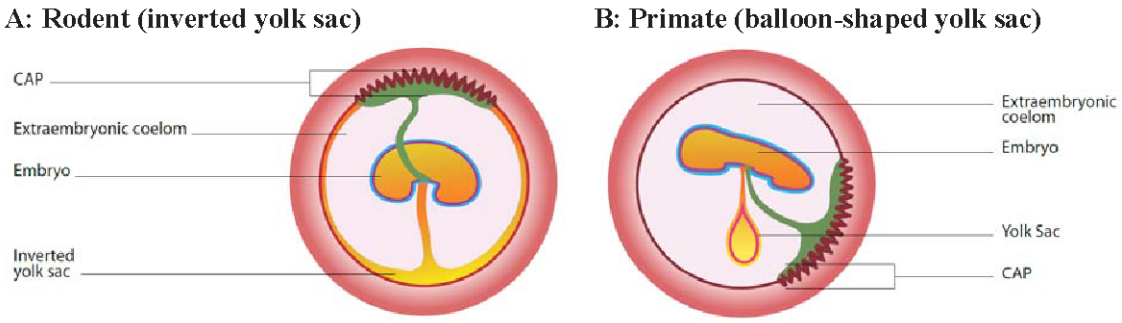
Figure 33. Timing of organogenesis for affected anatomical structures in the rat EFD studies and major routes of embryonic nutritional support (Applicant's figure)



Embryofetal nutrition in rodents versus primates

There are marked differences between the anatomical appearance of the yolk sac and its involvement in early nutrition between rodents and primates. In rodents, the yolk sac inverts and forms an early placenta-like structure, which ensures sufficient early nutritional flow to the rapidly growing embryo, initially alone and at later stages of gestation in parallel with the chorio-allantoic placenta (CAP). Due to the anatomical structure, the rat embryo becomes highly dependent on the inverted yolk sac for nutritional support. In primates, the yolk sac has a less prominent role in early nutrition and it does not form a placenta-like structure as in the rat. It may be involved in early nutritional support to the embryo, but as it retains its balloon-shape with no inversion of the absorptive membranes, thus the nutritional contribution is considered to be limited. When the need for nutrition increases in the primate embryo, the CAP appears to be capable of supporting the embryo alone. As the rodent embryo is more dependent on the nutritional support from the yolk sac than the primate embryo, it is reasonable to expect that the rodent embryo will be more sensitive towards interference with the yolk sac nutritional function compared to the primate embryo. Even though the rabbit embryo develops an inverted yolk sac placenta, no major abnormalities were seen when semaglutide was administered to dams during organogenesis. According to the applicant, this could be because sufficiently high dose levels were not achieved due to maternal tolerability, or because of two major differences between the rat and rabbit inverted yolk sac: 1) the yolk sac in the rabbit takes much longer to completely enclose the embryo than that of the rat, and 2) the size of the extra-coelomic cavity in the rabbit is much larger, meaning that the rabbit embryo has greater reserves within the yolk sac cavity than does the rat embryo and so is better able to withstand adverse effects on histiotrophic nutrition.

Figure 34. Yolk sac appearance in rodent and primate (Applicant's figure)



Schematic presentation of the yolk sac appearance in the rodent (A) and primate (B) embryos. In rodents, the yolk sac membranes increase in size to fully surround and enclose the embryo. After degeneration of the trophoblast membrane and the outer layer of the yolk sac membrane, the absorptive visceral layer (red line) is exposed directly to the maternal uterine epithelium (hence the term "inverted") allowing direct absorption of the nutritious uterine secretions. In primates, the yolk sac remains in the shape of a balloon with the absorptive visceral layer (red line) lining the internal cavity. In this presentation, direct absorption from the uterine secretions cannot take place.
CAP: Chorio-allantoic-placenta.

Mechanistic studies key findings

- GLP-1R is expressed in the inverted yolk sac membrane in the rat, but not in the monkey.
- Embryonic exposure to native GLP-1 and semaglutide caused a dose dependent reduction in yolk sac diameter and embryonic growth. These effects could be prevented by a specific GLP-1R antagonist, demonstrating that the effects were mediated by the GLP-1R.
- Semaglutide reduced the number of pinocytotic vesicles in the rat yolk sac membrane and the rate of pinocytotic transport.
- Continuous intravenous infusion of unacetylated semaglutide (short half-life, ~2-4h) for ten days before mating up to GD 8, GD13, and GD17 caused no malformations on GD 8, when the yolk sac has not begun to invert to optimise supply of nutrients for the embryo. Skeletal malformations (short, bent, thickened long bones, bent scapula and bent clavicola) were observed starting at ≥GD 13 when the rat embryo is dependent on the inverted yolk sac placenta for nutrition. There was a clear correlation between the size of the semaglutide treated embryos and the degree of limb abnormalities, i.e. the smaller embryos had more severe malformations.

Conclusion

Semaglutide activates the GLP-1R on the inverted yolk sac membrane of the rat embryo, leading to decreased pinocytotic transport, embryonic malnutrition, growth retardation and major skeletal and visceral malformations. The applicant concluded that due to anatomical and functional differences between the rat and primate yolk sac, and similarity between the monkey and human structure, embryotoxicity in rat is unlikely to be of relevance in primates; however, involvement of additional mechanisms leading to embryotoxicity in rats cannot be completely excluded.

Mechanistic studies

The following key elements were investigated:

#	Key element	Objective of investigations

1	**Time dependency of embryonic effects**	Investigate when, during the gestational period, semaglutide induced embryotoxicity in rats:
		• Time of initiation of the adverse effects in the embryos was assessed in a modified EFD study in rats using different dosing periods
		• Whole embryo culture (WEC) studies were performed to evaluate
		  ○ if *in vivo* malformations could be replicated in *in vitro* culture
		  ○ during which period of development cultured embryos were susceptible to semaglutide exposure
		• Time of occurrence of digit malformations was investigated by imaging technology

2	**Presence of functional GLP-1R in yolk sac and embryo**	Investigate if GLP-1Rs were expressed and functional in inverted yolk sac placenta tissue and embryonic tissue in the rat: • Presence of GLP-1Rs on yolk sacs and embryonic tissue was examined both at the protein (ISLB) and mRNA (ISH, qPCR) level • As an indicator of GLP-1R functionality, GLP-1R agonist stimulated cAMP accumulation was measured in cells from rat inverted yolk sac placenta (WEC and *in vivo*) • The effects of GLP-1R activation/blocking on yolk sac diameter and crown-rump lengths of embryos were measured in WEC studies using different GLP-1R agonists and an antagonist
3	**Distribution of semaglutide to the inverted yolk sac placenta**	Investigate if maternally administered semaglutide distributed to the inverted yolk sac placenta of the rat: • Presence of labelled semaglutide at inverted yolk sac placenta and in embryonic tissue was investigated by MARG in pregnant rats
4	**Functional effects of semaglutide on the inverted yolk sac placenta**	Investigate if semaglutide affected the pinocytotic transport of nutrients across the yolk sac membrane: • Effects of semaglutide on vesicular transport were evaluated in isolated yolk sac tissue in a diffusion chamber. • Effects of semaglutide on the number of pinocytotic vesicles in inverted yolk sac placenta tissue were evaluated by semi-quantitative TEM

WEC: whole embryo culture; ISH: *in situ* hybridisation; ISLB: *in situ* ligand binding; qPCR: quantitative polymerase chain reaction; MARG: micro-autoradiography; TEM: transmission electron microscopy

Time dependency of embryonic effects

Assessment of potential effects on inverted yolk sac and embryonic development of the rat in vitro and in vivo (Study #209213)

Purpose

a) To compare effects on the inverted yolk sac and embryonic development and survival after in vitro and in vivo exposure over the same developmental period.
b) To investigate the time course and possible etiology of adverse effects on embryonic and fetal development after maternal exposure of pregnant rats to NNC 0113-0217.

Key findings

Maternal treatment with semaglutide affected growth and development of embryo in vitro and in vivo as early as Day 11 of gestation. Malformations and evidence of delayed morphological development were detectable in the early *stage* in vivo and in vitro embryos on GD 11 and became more apparent in the in vivo embryos at GD 13 and 15.

Study Design

Semaglutide or vehicle was administered to two subsets of adult female CD rats for five weeks (three weeks dose escalation, two weeks target dose). The first subset of animals was mated and treatment continued throughout gestation (groups 1-3), while the second subset consisting of unmated females (groups 4-6) was terminated 10 hours after the final treatment and serum collected for subsequent use during in vitro embryo culture. GD9.5 is the earliest time when embryos can be explanted and cultured in vitro. GD11.5 is the latest time the embryo and embryonic membranes can remain viable. The influence of maternal treatment upon embryonic growth and development in vivo was assessed at Days 11, 13 and 15 of gestation. Additionally growth and development in vitro was assessed in Day 9 embryos obtained from treated females and cultured in medium containing serum from treated non-pregnant females. All embryos were evaluated after approximately 48 hours in culture.

Escalating dose phase

Group	Adaptive Period (escalating dose phase) – mg/kg/day		
	Week 1	Week 2	Week 3
1	Control (vehicle)	Control (vehicle)	Control (vehicle)
2	0.03	0.03	0.15
3	0.03	0.15	0.3
4	Control (vehicle)	Control (vehicle)	Control (vehicle)
5	0.03	0.15	0.3
6	0.03	0.15	0.3

Two weeks target dose

Group	Treatment	Dose (target dose phase)# (mg/kg/day)	No. of females	Animal numbers
1	Control	0	15[a]	1-15
2	NNC 0113-0217	0.2	15[a]	16-30
3	NNC 0113-0217	0.4	15[a]	31-45
4	Control	0	4[b]	46-49
5	NNC 0113-0217	0.4	4[b]	50-53
6	NNC 0113-0217	0.8	4[b]	54-57

Expressed in terms of test material as supplied.

[a] 15 mated females per group were dosed with the aim of achieving 12 pregnant females in each group for provision of embryos for direct evaluation.

[b] non-mated females for provision of serum

Mated females in each of Groups 1-3 were allocated to subgroups as follows:
Subgroup A (3 females) Embryos harvested Day 9.5 of pregnancy for culture
Subgroup B (3 females) Embryos harvested Day 11 of pregnancy for evaluation
Subgroup C (3 females) Embryos harvested Day 13 of pregnancy for evaluation
Subgroup D (3 females) Embryos harvested Day 15 of pregnancy for evaluation

Cultured embryos (subgroup A)
For in vitro culture, embryos were harvested from 3 pregnant animals per group (groups 1-3) on Day 9.5 of gestation. Five embryos per female were selected and allocated to the following groups:

Embryonic Group	In vitro Treatment*	Adult target dose with NNC 0113-217 mg/kg/day #		Number of embryos†
		in unmated females providing serum for culture	in mated females providing embryos for culture	
1	CM + serum 0 (Control)	0	0	15
2	CM + serum 0.4	0.4	0.2	15
3	CM + serum 0.8	0.8	0.4	15

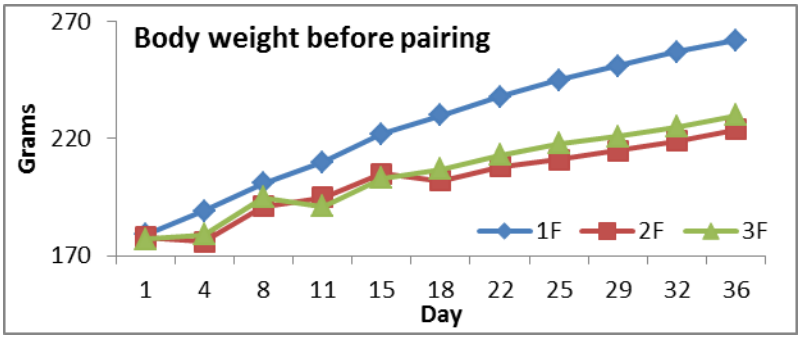
Expressed in terms of the test substance as supplied.
* Standard culture medium (CM) containing 50 % serum from unmated female rats treated at adult target doses.
† 5 embryos per female

Results

Maternal toxicity

- There were no deaths, treatment-related clinical signs or macroscopic changes.
- Marked reduction in bodyweight gain was noted in all treated groups.
- The number of corpora lutea, implantations and live embryos were significantly lower in treated females compared to controls.

Figure 35. Body weight



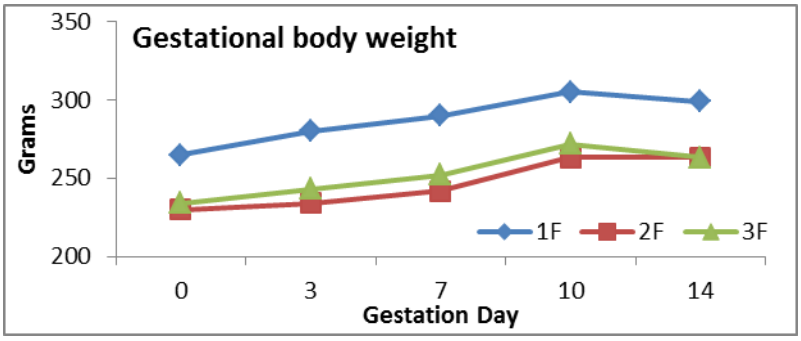


Table 142. Litter data (Applicant's table)

Group		Corpora lutea	Implantations	---------- Resorptions ---------- early	late	total	Live Embryos	Implantation % loss Pre-	Post-
Statistical Test:		Wi	Du	Av	Fe	Av	Sh	Kw	Kw
1	mean	15.6	15.2	1.4	0.0	1.4	14.1	4.7	8.6
	SD	2.1	1.6				1.4		
	n	15	15	12	12	12	12	15	12
2	mean	12.5**	11.5**	1.0	0.3	1.3	10.4**	7.5	11.3
	SD	1.9	1.7				3.3		
	n	15	15	12	12	12	12	15	12
3	mean	12.9**	13.2**	1.3	2.2**	3.5	9.9**	2.4	27.6
	SD	2.3	1.5				4.6		
	n	14	14	12	12	12	12	14	12

Group	:	1	2	3
Compound	:	Control	NNC 0113-0217	NNC 0113-0217
Dose (mg/kg/day)	:	0	0.2	0.4

Table 143. Plasma concentration (Applicant's table)

Sample ID	Treatment Description	Week	Nominal Time (h)	Batch ID	Dilution Factor	Concentration (nmol/L)	LLOQ
046	0 mg/ml	5	10	1	1	< 4.86	4.86nmol/L
047	0 mg/ml	5	10	1	1	< 4.86	4.86nmol/L
048	0 mg/ml	5	10	1	1	< 4.86	4.86nmol/L
049	0 mg/ml	5	10	1	1	< 4.86	4.86nmol/L
050	0.4 mg/ml	5	10	1	1	517	4.86nmol/L
051	0.4 mg/ml	5	10	1	1	524	4.86nmol/L
052	0.4 mg/ml	5	10	1	1	441	4.86nmol/L
053	0.4 mg/ml	5	10	1	1	496	4.86nmol/L
054	0.8 mg/ml	5	10	1	1	832	4.86nmol/L
055	0.8 mg/ml	5	10	1	1	844	4.86nmol/L
056	0.8 mg/ml	5	10	1	1	181	4.86nmol/L
057	0.8 mg/ml	5	10	2	2	953	4.86nmol/L

Embryo assessment

- Reductions in yolk sac, embryonic growth and development were seen in both cultured embryos and in vivo embryos on GD 11, 13 and 15.

- There were no embryonic deaths during culture (subgroup A). Increase in embryonic deaths was apparent on GD 13 at the high-dose and on GD 15 at both doses.

- Low incidences of morphological abnormalities were observed in cultured embryos (allantois defect, enlarged heart, and pericardial sac defect) on GD 9.5.

- On GD 11, pericardial sac defect and flexion to left side were noted.

- Heart abnormalities, irregular brain formation, deformed otic, optic, and olfactory systems, small head, absent or small forelimbs/hind limbs were observed on GD 13 and 15.

Table 144. Growth and development of cultured embryos, subgroup A (Applicant's table)

Group			1	2	3
		Statistical test			
Yolk sac diameter	Mean	Wi	4.46	3.54**	3.48**
(mm)	SD		0.30	0.25	0.26
	n		15	15	15
Crown-rump length	Mean	Wi	3.84	3.33**	3.34**
(mm)	SD		0.19	0.34	0.29
	n		15	15	15
Head length	Mean	Sh	1.91	1.65**	1.65**
(mm)	SD		0.08	0.21	0.21
	n		15	15	15
Somite number	Mean	Wi	25.9	24.6**	24.7**
	SD		1.0	1.2	1.2
	n		15	14	15
Morphological score	Mean	Sh	40.2	37.8**	38.3**
	SD		0.4	4.2	2.8
	n		15	15	15
No. of embryos evaluated			15	15	15
No. of embryos with observation			4	10	10
No. of embryos dead			0	0	0

Table 145. Summary of observations in cultured embryos, subgroup A (Applicant's table)

	Embryos			Litters		
Group	1	2	3	1	2	3
Total number	15	15	15	3	3	3
Number dead	0	0	0	0	0	0
Number excluded	0	0	0	0	0	0
Number evaluated	15	15	15	3	3	3
Number affected	4	10	10	2	3	3
	Percentage with observations:			Number with observations:		
Yolk sac vessel defect	0	6.7	6.7	0	1	1
Allantois defect	0	20.0	26.7	0	2	3
Flexion deficient	0	6.7	6.7	0	1	1
Heart enlarged	0	0	20.0	0	0	1
Heart enlarged in yolk sac	0	26.7	33.3	0	1	1
Pericardial sac defect	0	13.3	26.7	0	1	2
Pericardial sac, haemorrhage in	20.0	0	0	2	0	0
Caudal neural tube deformed	0	6.7	0	0	1	0
Craniofacial appearance abnormal	0	6.7	0	0	1	0
Head small	0	13.3	6.7	0	2	1
Otic vesicles deformed	0	6.7	6.7	0	1	1
Fore limb buds small	0	6.7	6.7	0	1	1
Somites irregular	6.7	0	6.7	1	0	1
Haemorrhages	6.7	0	0	1	0	0

Table 146. Growth and development at GD 11, subgroup B (Applicant's table)

Group		Statistical test	1	2	3
Yolk sac diameter (mm)	Mean	Sh	4.69	3.99**	4.13**
	SD		0.52	0.28	0.28
	n		41	38	39
Crown-rump length (mm)	Mean	Sh	3.72	3.42**	3.50**
	SD		0.70	0.34	0.21
	n		41	40	40
Head length (mm)	Mean	Sh	1.89	1.79**	1.84**
	SD		0.33	0.24	0.11
	n		41	40	40
Somite number	Mean	Wi	26.1	25.3	25.9
	SD		1.1	1.6	1.2
	n		36	40	40
Morphological score	Mean	Kw	40.0	39.7	40.0
	SD		0.9	2.8	0.8
	n		36	40	40
No. of embryos evaluated			46	40	42
No. of embryos with observation			28	4	6
No. of embryos dead			10	0	2

Table 147. Growth and development at GD 13, subgroup C (Applicant's table)

Group		Statistical test	1	2	3
Yolk sac diameter (mm)	Mean	Sh	9.96	7.70**	6.36**
	SD		1.28	0.57	0.88
	n		42	30	41
Crown-rump length (mm)	Mean	Sh	8.38	6.99**	5.52**
	SD		0.45	0.55	0.92
	n		42	30	41
Head length (mm)	Mean	Sh	4.87	4.10**	3.08**
	SD		0.25	0.35	0.67
	n		42	30	41
Somite number	Mean	Sh	47.8	46.9	39.1**
	SD		1.8	1.8	8.4
	n		42	30	41
Morphological score	Mean	Sh	70.2	69.6	61.0**
	SD		0.5	1.5	13.1
	n		42	30	41
No. of embryos evaluated			45	32	43
No. of embryos with observation			3	9	28
No. of embryos dead			3	2	14

Table 148. Growth and development at GD 15, subgroup D (Applicant's table)

Group		Statistical test	1	2	3
Yolk sac diameter (mm)	Mean	Wi	14.07	11.43**	9.59**
	SD		1.53	1.76	1.69
	n		41	24	22
Crown-rump length (mm)	Mean	Sh	13.30	8.81**	7.03**
	SD		0.49	3.31	2.38
	n		40	34	36
Head length (mm)	Mean	Sh	7.84	5.94**	4.60**
	SD		0.28	1.58	1.39
	n		40	28	30
Somite number	Mean	Sh	61.5	58.2	47.6**
	SD		3.0	6.6	7.8
	n		39	25	22
No. of embryos evaluated			42	34	36
No. of embryos with observation			2	33	36
No. of embryos dead			2	12	26

Table 149. Summary of observations on GD 11, subgroup B (Applicant's table)

	Embryos			Litters		
Group	1	2	3	1	2	3
Total number	50	41	42	3	3	3
Number dead	10	0	2	2	0	1
Number excluded[#]	4	1	0	2	0	1
Number evaluated	46	40	42	3	3	3
Number affected	28	4	6	3	2	2
	Percentage with observations:			Number with observations:		
Yolk sac vessel defect	0	2.5	0	0	1	0
Flexion deficient	6.5	2.5	0	3	1	0
Flexion to left side	0	0	2.4	0	0	1
Heart defect	0	2.5	0	0	1	0
Pericardial sac defect	2.2	0	4.8	1	0	1
Caudal neural tube deformed	0	7.5	0	0	2	0
Posterior neuropore open	2.2	0	0	1	0	0
Head small	0	5.0	0	0	1	0
Otic vesicles deformed	0	5.0	0	0	1	0
Optic vesicles deformed	4.3	2.5	2.4	1	1	1
Branchial bars small	0	2.5	0	0	1	0
Fore limb buds absent	2.2	0	0	1	0	0
Fore limb buds small	0	2.5	0	0	1	0
Subcutaneous blisters *	30.4	0	0	3	0	0
Early resorption	21.7	0	4.8	2	0	1

[#] Excluded from % observations, unable to evaluate due to technical damage

* Considered a procedural artefact due to chilling on cold plate

Table 150. Summary of observations on GD 13, subgroup C (Applicant's table)

	Embryos			Litters		
Group	1	2	3	1	2	3
Total number	45	32	43	3	3	3
Number dead	3	2	14	3	1	3
Number excluded	0	0	0	0	0	0
Number evaluated	45	32	43	3	3	3
Number affected	3	9	28	3	3	3
	Embryos			Litters		
Group	1	2	3	1	2	3
	Percentage with observations:			Number with observations:		
Yolk sac vessel defect	0	3.1	0	0	1	0
Yolk sac blood filled	0	0	2.3	0	0	1
Flexion deficient	0	0	7.0	0	0	1
Heart enlarged	0	0	2.3	0	0	1
Heart defect	0	3.1	7.0	0	1	3
Pericardial sac defect	0	12.5	2.3	0	3	1
Pericardial sac, haemorrhage in	0	6.3	2.3	0	1	1
Caudal neural tube deformed	0	0	4.7	0	0	1
Posterior neuropore deformed	0	0	2.3	0	0	1
Neural tube haemorrhagic	0	3.1	4.7	0	1	2
Brain irregular formation	0	0	7.0	0	0	2
Head small	0	0	14.0	0	0	1
Otic vesicles deformed	0	0	16.3	0	0	2
Optic vesicles deformed	0	3.1	20.9	0	1	2
Olfactory system absent	0	0	2.3	0	0	1
Olfactory system deformed	0	0	14.0	0	0	1
Branchial bars deformed	0	0	7.0	0	0	1
Maxillary process deformed	0	3.1	4.7	0	1	2
Mandibular processes deformed	0	0	2.3	0	0	1
Fore limbs absent	0	0	2.3	0	0	1
Fore limbs small	0	0	14.0	0	0	1
Fore limbs irregular	0	0	2.3	0	0	1
Hind limbs absent	0	0	2.3	0	0	1
Hind limbs small	0	3.1	11.6	0	1	2
Somites irregular	0	0	2.3	0	0	1
Haemorrhages	0	3.1	7.0	0	1	2
Early resorption	6.7	6.3	4.7	3	1	1
Late resorption	0	0	27.9	0	0	3

Table 151. Summary of observations on GD 15, subgroup D (Applicant's table)

	Embryos			Litters		
Group	1	2	3	1	2	3
Total number	42	34	36	3	3	3
Number dead	2	12	26	2	2	3
Number excluded	0	0	0	0	0	0
Number evaluated	42	34	36	3	3	3
Number affected	2	33	36	2	3	3
	Percentage with observations:			Number with observations:		
Yolk sac blood filled	0	2.9	5.6	0	1	2
Body wall/skin thin	0	64.7	58.3	0	3	3
Flexion dorsal kink	0	5.9	13.9	0	1	2
Craniofacial poor development	0	5.9	27.8	0	2	3
Craniofacial nares absent/unclear	0	5.9	5.6	0	2	1
Craniofacial vibrissae absent	0	0	5.6	0	0	1
Fore limb digits absent	0	5.9	25.0	0	2	3
Fore limb <5 digits	0	20.6	36.1	0	2	3
Hind limb bud	0	2.9	0	0	1	0
Hind limb digits absent	0	5.9	36.1	0	2	3
Hind limb <5 digits	0	38.2	30.6	0	3	3
Limbs digits unclear	0	2.9	0	0	1	0
Genitals absent	0	0	2.8	0	0	1
Genital defect	0	29.4	33.3	0	2	3
Tail kinked/short	0	8.8	25.0	0	2	3
Subcutaneous blisters	0	29.4	25.0	0	3	3
Haemorrhages	0	2.9	16.7	0	1	2
Early resorption	4.8	26.5	33.3	2	2	3
Late resorption	0	8.8	38.9	0	2	3

Study of effect on inverted yolk sac and embryonic development of the rat in vitro (Study # 210019)

Key findings

Reduction in yolk sac diameter and embryonic growth, and morphological abnormalities were observed.

Method

The effect of semaglutide on growth and development of yolk sacs and embryos was assessed in Day 9.5 embryos from rats. Semaglutide was added directly to the culture medium at concentrations of 0.85, 2.7, 8.5, 27, 85, 125, 575 and 1000 µM. Embryos were evaluated after approximately 48 hours in culture.

Due to a calculation error, the actual exposure levels were 1000-fold higher than intended.

Group	Nominal Dose (nM)	Molar concentration of dosing solution (µmol/mL)	Dose – volume (µL/mL)	Actual Dose (µM)
1	0	0	20	0
2	0.85	0.04035	21.07	0.85
3	2.7	0.4035	6.69	2.7
4	8.5	0.4035	21.07	8.5
5	27	4.035	6.69	27
6	85	4.035	21.07	85
7	125	4.035	30.98	125
8	575	40.35	14.25	575
9	1000	40.35	24.78	1000

Results

- There were no deaths during culture.
- Reductions in yolk sac diameter, crown rump length, and head length were apparent in all treated groups compared with control embryos, without clear dose relationship.
- Flexion deficient, heart enlarged in yolk sac, caudal neural tube open, hemorrhagic neural tube, irregular somites, small head, and deformed otic and optic vesicles were observed with clear dose relationship.

Table 152. Growth and development (Applicant's table)

Group		Statistical test	1	2	3	4	5	6	7	8	9
Yolk sac diameter (mm)	Mean	Wi	4.46	3.76**	3.70**	3.60**	3.62**	3.67**	3.65**	3.58**	3.33**
	SD		0.25	0.11	0.16	0.18	0.16	0.09	0.10	0.11	0.21
	n		10	10	10	10	10	10	10	10	10
Crown-rump length (mm)	Mean	Wi	3.63	3.49	3.53	3.44*	3.32*	3.45*	3.45*	3.21**	2.99**
	SD		0.20	0.18	0.16	0.21	0.18	0.15	0.11	0.19	0.32
	n		10	10	10	10	10	10	10	10	10
Head length (mm)	Mean	Wi	1.90	1.79*	1.82*	1.74**	1.71**	1.75**	1.76**	1.59**	1.47**
	SD		0.13	0.07	0.06	0.13	0.07	0.07	0.10	0.10	0.14
	n		10	10	10	10	10	10	10	10	10
Somite number	Mean		26.2	26.0	25.6	25.8	26.3	25.6	25.2	25.1	24.4
	SD		0.8	0.8	1.0	0.9	0.7	0.8	0.8	1.1	1.5
	n		10	10	10	10	10	10	10	10	10
Morphological score	Mean		39.9	38.8	39.9	39.5	40.0	39.9	39.5	37.7	36.9
	SD		0.5	2.9	0.4	1.3	0.4	0.5	0.3	3.7	3.5
	n		10	10	10	10	10	10	10	10	10
No. of embryos with observation			7	3	3	3	4	6	3	6	5
No. of embryos dead			0	0	0	0	0	0	0	0	0

Table 153. Summary of observations (Applicant's table)

Group	1	2	3	4	5	6	7	8	9
Number of embryos evaluated	10	10	10	10	10	10	10	10	10
Percentage of embryos with observations:	70	30	30	30	40	60	30	60	50
Dead	0	0	0	0	0	0	0	0	0
Allantois defect	0	10	0	0	0	10	0	0	0
Flexion deficient	10	10	0	20	0	10	0	30	30
Heart enlarged	0	0	0	0	0	0	10	0	0
Heart enlarged in yolk sac	0	0	20	0	10	0	20	10	10
Pericardial sac defect	40	0	0	10	30	20	10	0	10
Pericardial sac haemorrhage in	20	10	0	0	0	0	0	20	0
Caudal neural tube open	0	10	0	0	0	0	0	20	20
Neural tube haemorrhagic	0	0	0	0	10	20	0	10	0
Head small (and/or bent backwards)	0	10	0	0	0	0	0	10	30
Otic vesicles deformed	0	0	0	0	0	0	0	10	0
Optic vesicles deformed	0	10	0	0	0	0	0	20	20
Branchial bars deformed	0	0	10	0	0	0	0	0	0
Somites irregular	0	0	0	0	0	0	0	0	10
Subcutaneous blisters	0	0	0	0	0	10	0	0	10
Haemorrhages	30	0	0	0	0	0	0	0	0

Effects of semaglutide (NNC-0113-0217), unacetylated semaglutide (NNC-0113-0236), and native GLP-1 on rat embryo (Study # 211213, 211341, 211342)

Key findings

GLP-1R stimulation during GD9.5 to GD11.5 inhibited yolk sac growth.

Study design

Embryos from female SD rats were incubated at 37C for approximately 48h with GLP-1R antagonist exendin 9-39 followed by test substances (semaglutide, NNC 0113-0236-unacetylated semaglutide with shorter half-life, and native GLP-1). Further groups received the test substance alone or the GLP-1 receptor antagonist alone. After termination of culture, embryos were washed in saline, and the yolk sac diameter, crown-rump length and head length was measured using a micrometer disk in a focusing eyepiece. The number of somites was counted and each embryo was examined for the presence of a heartbeat and dysmorphogenesis, and the morphological score was assessed

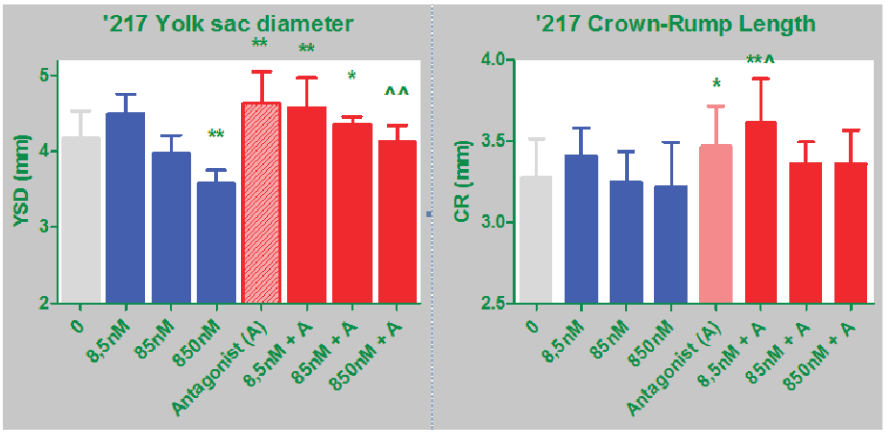
Species / Strain	Method of Administration	Duration of Dosing	Nominal Doses	Gender and No. per Group	Noteworthy Findings	Study ID
Whole Embryo Culture Studies						
Rat/Sprague Dawley NNC 0113-0217	*In vitro* (WEC)	48 h *in vitro*	0, 8.5, 85 and 850 nmol/L, and a 10-fold higher molar concentration of the GLP-1 receptor antagonist (exendin 9-39)	8 groups of 15 GD9.5 embryos	Reduced yolk sac diameter ≥85nmol/L. No apparent effect on embryonic growth. Additionally, evidence of heart enlargement and other malformations were observed at 850 nmol/L. Yolk sac effects were prevented by the prior addition of the GLP-1 receptor antagonist exendin 9-39. Examination by transmission electron microscopy showed a reduced number of pinocytotic vesicles in the yolk sac membrane.	211213
Rat/Sprague Dawley NNC0113-0236 *	*In vitro* (WEC)	48 h *in vitro*	50, 500 and 5000 nmol/L and a 10-fold higher molar concentration of the GLP-1 receptor antagonist.	8 groups of 15 GD9.5 embryos	NNC 0113-0236 caused reduced yolk sac diameter ≥50 nmol/L. An increased incidence of heart enlargement was apparent at 500 and 5000 nmol/L. These effects were prevented by prior treatment with the GLP-1R antagonist, exendin 9-39.	211341
Rat/Sprague Dawley Native GLP-1	*In vitro* (WEC)	48 h *in vitro*	10, 100 and 1000 nmol/L and a 10-fold higher molar concentration of the GLP-1R antagonist.	8 groups of 15 GD9.5 embryos	Reduced embryonic growth ≥100 nmol/L and above and on yolk sac diameter at 1000 nmol/L. These effects were prevented by prior treatment with the GLP-1R antagonist, exendin 9-39.	211342

Results

- All GLP-1 R agonists (semaglutide, un-acetylated semaglutide, and native GLP-1) decreased yolk sac diameter. This decrease was prevented in the presence of by GLP-1R antagonist exendin 9-39.

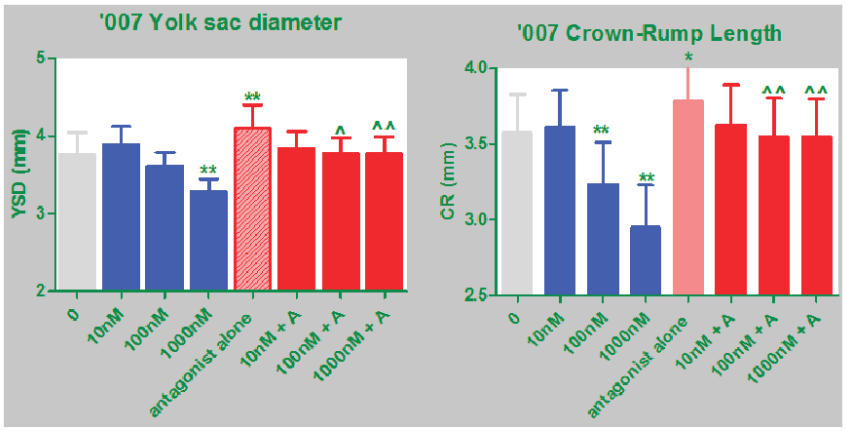
- Semaglutide and unacetylated semaglutide had no significant effect on crown-rump length at concentration up to 5000nM and 850nM, respectively. The lack of effect on crown-rump length is in contradiction to the findings in the pilot study (Study # 210019) and was considered due to semaglutide lower exposure concentration and an unusually low crown-rump length in the control group. Crown-rump length was decreased in the presence of native GLP-1 (at ≥100nM).

Figure 36. Yolk sac diameter and crown-rump in WEC study with semaglutide (Applicant's figure)



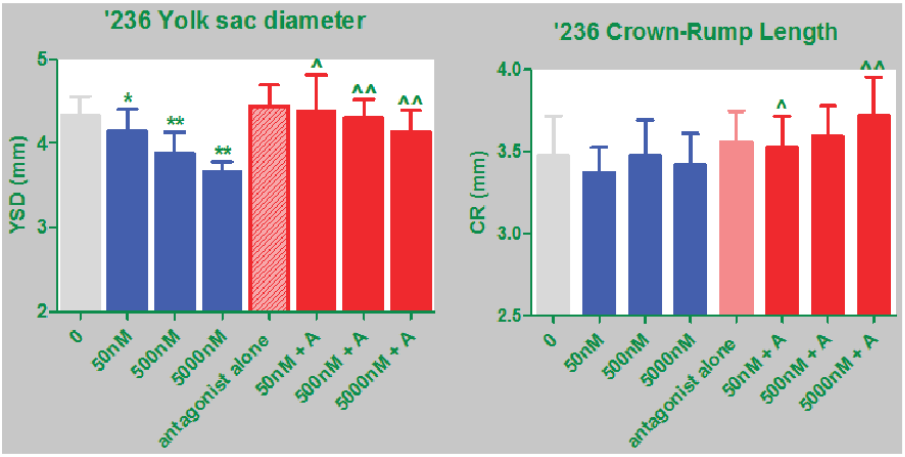
Yolk sac diameter (left panel) was significantly decreased at 850 nM semaglutide compared to control. Note that the concentration of semaglutide in this study was in nM contrary to the pilot study where the concentration s were uM. Exendin 9-39 alleviated the effect of semaglutide. Addition of exendin 9-39 alone resulted in an increased yolk sac diameter (left panel) and crown-rump length (right panel). '217 = semaglutide. A = Antagonist (exendin 9-39). Data presented as means ± SD. * p<0.05, ** p<0.01 vs control (0 nM); ^ p<0.05, ^^ p<0.01 (semaglutide exposed group vs. corresponding group with antagonist added).

Figure 37. Yolk sac diameter and crown-rump in WEC study with native GLP-1 (Applicant's figure)



Yolk sac diameter (left panel) was significantly decreased at 1000 nM native GLP-1 compared to control. The size of the embryo measured as crown-rump length (right panel) was reduced compared to control at concentrations of 100 nM and above. Exendin 9-39 alleviated the effect of native GLP-1. Addition of exendin 9-39 alone resulted in an increased yolk sac diameter (left panel) and crown-rump length (right panel). '007 = native GLP-1. A = Antagonist (exendin 9-39). Data presented as means ± SD. ** p<0.01 vs control (0 nM); ^ p<0.05, ^^ p<0.01 (GLP-1 exposed group vs. corresponding group with antagonist added). (Table 2.6.7.17.G/211342)

Figure 38. Yolk sac diameter and crown-rump in WEC study with NNC0113-0236 (Applicant's figure)



Yolk sac diameter (left panel) was significantly decreased at 50 nM NNC0113-0236 and above compared to control. Exendin 9-39 alleviated the effect of native GLP-1. '236 = NNC0113-0236. A = Antagonist (exendin 9-39). Data presented as means ± SD. * $p<0.05$, ** $p<0.01$ vs control (0 nM); ^ $p<0.05$, ^^ $p<0.01$ (GLP-1 exposed group vs. corresponding group with antagonist added). (Table 2.6.7.17.G/211341)

Investigative study for effects on embryo-fetal development in the CD rat by continuous intravenous infusion administration with a pre-pairing dosing period (Study # 211412)

The aim of this study was to investigate the link between the time when the embryo is dependent on yolk sac function for nutrition and the presence of fetal abnormalities in the rat.

Key findings

Skeletal malformations (short, bent, thickened long bones, bent scapula and bent clavicola) were observed when semaglutide was administered up to GD13 and GD17. These were no treatment related abnormalities where treatment was withdrawn on GD8.

Study design

Female rats (24/group) were treated with NNC 0113-0000-0236 (unacylated semaglutide peptide, with shorter half-life, to accurately target the specific fragmented periods of organogenesis of interest) by continuous intravenous infusion for ten days before mating up to GD 8 (Group 2), GD 13 (Group 3) and GD17 (Group 4). Control group received the vehicle for ten days before pairing and up to Day 17 after mating. All dams were killed on GD 20 and fetuses examined. Following a dose escalation to ensure tolerability, all treated groups received a continuous infusion dose of 2160 nmol/kg/day (90 nmol/kg/hr). The dose level was selected on basis of a DRF study to produce a clear effect on embryo-fetal growth and development.

Dose (nmol/kg/day)	Group 1	Group 2	Group 3	Group 4
Days 1-4 pre-pairing	0	288	288	288
Days 5-8 pre-pairing	0	1440	1440	1440
Day 9 pre-pairing to Day 8 after mating	-	2160	-	-
Day 9 pre-pairing to Day 13 after mating	-	-	2160	-
Day 9 pre-pairing to Day 17 after mating	0	-	-	2160

Results

Dams

- No treatment related mortality was observed.

- Piloerection, hunched posture and underactivity were observed amongst a large number of treated animals on Day 3 of dosing when the animals were receiving the initial dose of 288 nmol/kg/day. In most cases these signs were no longer evident on escalation of the dose on Day 5 of treatment to 1440 nmol/kg/day. Hunched posture or piloerection was first observed in a small number of females when the dose was escalated to 1440 nmol/kg/day (Days 6 – 8) or 2160 nmol/kg/day (Days 10 – 11) before pairing. There were no signs associated with treatment during the gestation phase.

- Marked reductions in bodyweight and food consumption was observed at 288 nmol/kg/day. On escalation of the dose there were no subsequent reductions in bodyweight or food consumption but values remained lower than that of the controls. Gravid uterine weight was lower at 2160nmol/kg/day compared to controls.

- Systemic exposure to NNC 0113-0236 in the dosed animals ranged between 40 to 48 nmol/l. The terminal half-life was in the range 1.9 to 4.5 hours.

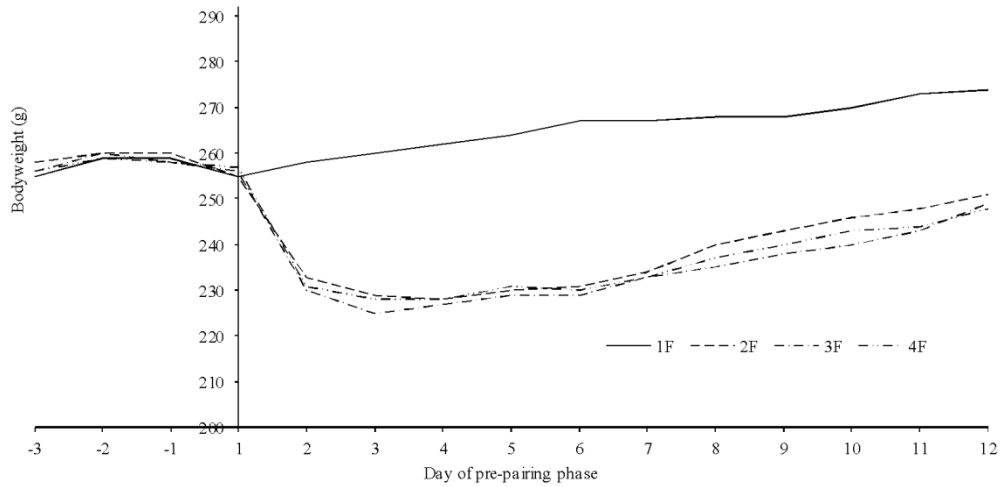
Table 154. Clinical signs (Applicant's table)

Clinical signs - group distribution of observations for females before pairing

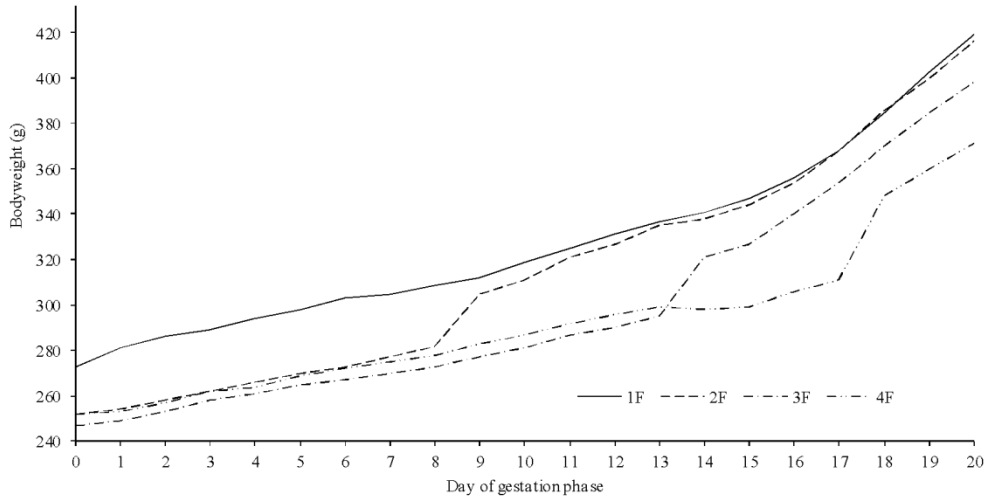
			Number of animals affected			
		Group/sex:	1F	2F	3F	4F
Category	Observation	Number in group:	24	24	24	24
Behaviour	Underactive		0	3	10	8
Coat	Piloerection		0	11	16	14
Posture	Hunched		0	4	9	8

Figure 39. Body weight (Applicant's figure)

Bodyweight - group mean values (g) for females before pairing



Bodyweight - group mean values (g) for females during gestation



Gravid uterine weight, adjusted bodyweight and bodyweight change - Day 20 of gestation

Group /Sex		Bodyweight on Day 0	Terminal Bodyweight on Day 20	Bodyweight Change Days 0-20	Gravid Uterine Weight	Adjusted Bodyweight Day 20	Adjusted Bodyweight Change Days 0-20
Statistical test:		Wi	Wi	Wi	Wi	Wi	Du
1F	Mean	273	419	146	85	334	61
	SD	20.2	36.8	24.0	21.4	29.7	12.4
	N	21	21	21	21	21	21
2F	Mean	252**	416	164	78	338	86**
	SD	23.1	31.8	21.9	16.2	25.4	14.3
	N	21	21	21	21	21	21
3F	Mean	247**	398	151	67**	331	83**
	SD	20.9	34.0	19.6	12.0	26.5	13.1
	N	21	21	21	21	21	21
4F	Mean	252**	370**	118**	47**	323	71*
	SD	16.0	35.2	23.1	12.9	26.4	14.5
	N	24	24	24	24	24	24

Table 155. NNC 0113-0236 plasma concentration (Applicant's table)

Group number	Target dose Day 9 pre-pairing until end of infusion	End of infusion	Steady state plasma concentration	Plasma concentration <LLOQ on GD 17
	Nominal dose level (nmol/kg/hr)	Day after mating	(nmol/l)	Hours after end of infusion
1	0	17	<LLOQ	<LLOQ
2	90	8	42.8	10
3	90	13	47.7	10
4	90	17	39.8	10

Litter data

- The numbers of corpora lutea and implantations were lower in all treated groups compared to controls. In Group 4 where treatment was withdrawn on Day 17 of gestation, there was a clear increase in late embryo-fetal deaths compared with controls and this resulted in a higher mean percentage post-implantation loss. There was no increase in post-implantation losses amongst the litters of dams where treatment was withdrawn on Day 8 or on Day 13 of gestation.

- Fetal and placental weights were similar in animals that had treatment withdrawn on Day 8 of gestation (Group 2) and controls. Fetal weight was decreased in animals that had treatment withdrawn on Day 13 (Group 3) and Day 17 (Group 4) of gestation, with the effect being most marked in Group 4. Mean placental weight was low in Group 4 compared with controls.

Table 156. Litter data (Applicant's table)

Litter data - group mean values for females on Day 20 of gestation

Group /Sex		Corpora Lutea	Implantations	Resorptions Early	Resorptions Late	Resorptions Total	Live Young Male	Live Young Female	Live Young Total	Sex ratio (%M)	Implantation Loss (%) Pre-	Implantation Loss (%) Post-
Statistical test:		Wi	Wi	Wc	Wc	Wc	Wi	Wi	Wi	Wa	Wa	Wa
1F	Mean	16.0	15.0	1.1	0.0	1.1	6.9	7.0	13.8	48.9	6.7	8.8
	SD	3.02	2.77				2.61	2.27	3.61			
	N	21	21	21	21	21	21	21	21	21	21	21
2F	Mean	14.3*	13.2*	1.0	0.0	1.0	6.0	6.3	12.3	48.6	8.1	7.7
	SD	2.17	2.68				2.26	2.05	2.72			
	N	21	21	21	21	21	21	21	21	21	21	21
3F	Mean	14.4*	13.3*	1.1	0.1	1.2	6.7	5.4*	12.1	55.0	7.8	9.0
	SD	1.89	1.93				2.39	2.16	1.95			
	N	21	21	21	21	21	21	21	21	21	21	21
4F	Mean	14.2*	13.2*	1.5	1.0**	2.5	5.7	5.0**	10.7**	53.8	6.8	19.8**
	SD	2.08	1.63				2.33	2.32	3.17			
	N	24	24	24	24	24	24	24	24	24	24	24

Placental, litter and fetal weights - group mean values (g) on Day 20 of gestation

Group /Sex		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
Statistical test:		lWi	Wi	Wi	Wi	Wi	Wi
1F	Mean	0.61	52.87	13.81	3.91	3.71	3.82
	SD	0.072	14.231	3.614	0.263	0.251	0.249
	N	21	21	21	21	21	21
2F	Mean	0.65	48.04	12.29	4.06	3.78	3.91
	SD	0.102	11.317	2.723	0.207	0.280	0.237
	N	21	21	21	21	21	21
3F	Mean	0.57	40.80**	12.10	3.47**	3.24**	3.36**
	SD	0.051	8.316	1.947	0.293	0.267	0.282
	N	21	21	21	21	21	21
4F	Mean	0.42**	25.65**	10.67**	2.48**	2.24**	2.38**
	SD	0.044	8.783	3.171	0.238	0.232	0.216
	N	24	24	24	24	23	24

Fetal malformation

- These were no treatment related abnormalities where treatment was withdrawn on Day 8 of gestation.

- Bent long bones were observed on GD 13 and 17, with time related incidence. Short/bent/thickened long bones, bent scapula and bent clavicola were noted on GD17. According to the applicant, the effects on GD 17 are likely caused by the continued inhibitory effect on the yolk sac function which persists even after the establishment of the CAP.

Table 157. Fetal malformations (Applicant's table)

Fetal examinations - major abnormalities - group incidences

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number Examined		290	258	254	256	21	21	21	24
Total Number Affected		2	0	14	70	2	0	9	20
Skeletal									
Limbs and Girdles	Short/bent/thickened long bones	0	0	0	42	0	0	0	15
	Bent scapula(e)	0	0	0	54	0	0	0	19
	Bent long bones	0	0	1	30	0	0	1	13
	Bent clavicle	0	0	0	2	0	0	0	2

Fetal examinations - minor skeletal abnormalities/variants - group incidences

		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number Examined		145	131	127	126	21	21	21	24
Vertebral element abnormality	sacral/caudal	0	0	1	0	0	0	1	0
	scoliosis minimal	0	0	1	0	0	0	1	0
Ribs	medially thickened/kinked	1	1	0	85	1	1	0	23
	partially fused	0	0	9	5	0	0	5	4
Costal cartilage	partially fused	0	0	8	4	0	0	3	3
Caudal elements	misaligned	0	0	18	25	0	0	12	15

Presence of functional GLP-1 receptors in yolk sac and embryo

Study of GLP-1 receptor localization in embryonic tissue of the rat (Study # 212301)

Design

The expression of GLP-1R was evaluated in rat embryonic tissue and yolk sac. GLP-1R protein expression was investigated by in situ ligand binding (ISLB) with ^{125}I-GLP-1 and ^{125}I-Exendin9-39. GLP-1R mRNA expression was investigated by in situ hybridization (ISH). The tissue used for ISLB was obtained from time mated, untreated rats. Embryos were dissected with (GD 6.5, 9.5, 11.5, and 13.5) and without (GD11.5, 13.5, and 16.5) membranes and cryo-fixed in OCT in 2-methylbutane on dry ice. In situ hybridization was performed with riboprobes transcribed from linearized rat GLP-1R cDNA plasmid (rGLP-1R-4, base pair 952-1285 corresponding to the rat GLP-1R cDNA). Antisense and sense (negative control) probes were labelled with [^{35}S]- Uridine 5`-(a-thio) triphosphate, (NEN, 1250 Ci/mmol) using T7 and T3 RNA polymerase. Sections adjacent to ISLB sections were used for ISH

Results

- In the yolk sac, GLP-1 receptor was identified from GD 9.5, with increasing levels of expression seen at GD 11.5 and GD 13.5.
- In embryonic tissue, GLP-1R expression was seen at GD 13.5 and GD 16.5.

Table 158. GLP-1R expression in embryonic tissue and yolk sac (Study # 212301, Applicant's table)

Age (GD)	Number of embryos used for analysis*	Blastocyst			Embryonic tissue			Yolk sac		
		ISLB (GLP-1)	ISLB (Exendin 9-39)	ISH	ISLB (GLP-1)	ISLB (Exendin 9-39)	ISH	ISLB (GLP-1)	ISLB (Exendin 9-39)	ISH
6.5	6	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
9.5	5 (5)	N/A	N/A	N/A	N/A	N/A	N/A	-	+	-
11.5	4 (2)	N/A	N/A	N/A	-	(-)	-	+	+	+
13.5	3 (1)	N/A	N/A	N/A	+	+	+	+	+	+
16.5	1	N/A	N/A	N/A	+	+	+	N/A	N/A	N/A

"+" indicates positive signal, "-" indicates no signal, "(-)" indicates weak, potential signal.
N/A: Not applicable.
*: number refers to samples containing embryonic tissue.
Number in ( ) refers to samples dissected with intact yolk sac.

Exploratory study for effects on embryo-fetal development in rats (Study # 212080)

Design

Female rats were dosed with semaglutide once daily by subcutaneous injection at doses of 0, 0.1, and 0.25 mg/kg/day. Treatment was initiated at least 9 days prior to mating, and continued throughout pregnancy and until the day before termination on GD 12 and GD 14. For GD 12 females, the pregnancy status and number of implantation sites were recorded, and yolk sacs and embryos were collected. From GD 14 females, the intact uterus was dissected out for further investigation of the yolk sac in Ussing chambers and for whole embryo imaging.

Group	Dose Day 1-4 (mg/kg/day)	Dose Day 5-7 (mg/kg/day)	Dose from Day 8 through mating and until Day of termination (mg/kg/day)	Animal Nos (GD 12)	Animal Nos (GD 14)
				Female	Female
1	0 (vehicle)	0 (vehicle)	0 (vehicle)	1-5, 29-40	21-28
2	0.03	0.10	0.10	6-10, 41, 44	42-43, 45-47
3	0.03	0.10	0.25	11-20, 53, 56-58	48-52, 54-55

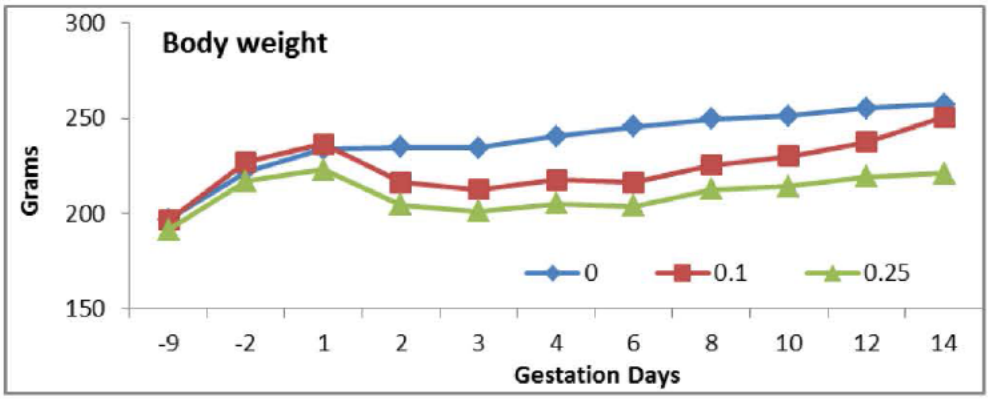
Results

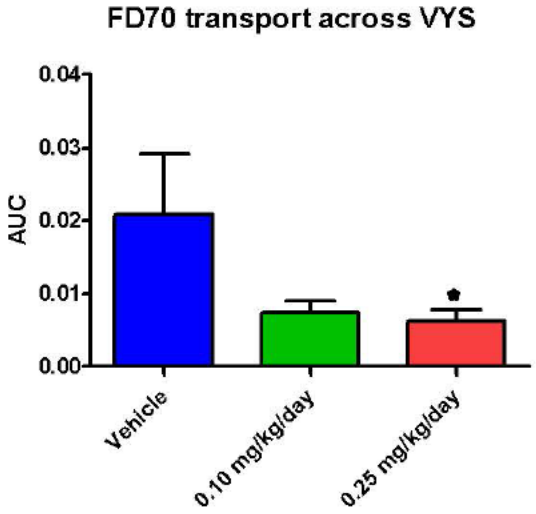
- Body weight loss (10%) was observed at the beginning of dosing. Despite a slight body weight gain from Day 4 of dosing, the body weights continued to be significantly lower as compared to the control group. On GD 12, a statistically significantly lower litter size was recorded for the HD group.

- GLP-1 receptor is expressed in rat yolk sac and embryos at GD12. The expression levels are approximately 4000 times lower in the embryos compared to the yolk sacs. Pre-treatment with semaglutide did not significantly change GLP-1 receptor expression in the yolk sacs. Both native GLP-1 and semaglutide stimulated cAMP release from the rat yolk sacs dose-dependently at GD12 (data not submitted).

- The investigations in the Ussing chamber showed that in vivo exposure of semaglutide to pregnant rats result in a reduction in pinocytotic transport of FD70 across the visceral yolk sac of the rat embryo.

- By single plane illumination microscopy (SPIM), and 3D digital reconstruction, embryos from pregnant females treated with 0.25 mg/kg/day were found to be significantly smaller than the vehicle treated embryos and various degrees of skeletal abnormalities could be observed in the developing fore limbs on semaglutide treated embryos. There was a clear correlation between the size of the semaglutide treated embryos and the degree of limb abnormalities, i.e. the smaller embryos had more severe malformations.





FD70 transport across visceral yolk sacs from Semaglutide treated rats. Bars represent the accumulated transport in percentage of the apical start volume of FITC dextran 70kD across the visceral yolk sac.

Table 159. Crown/rump (C/R) length (Applicant's table)

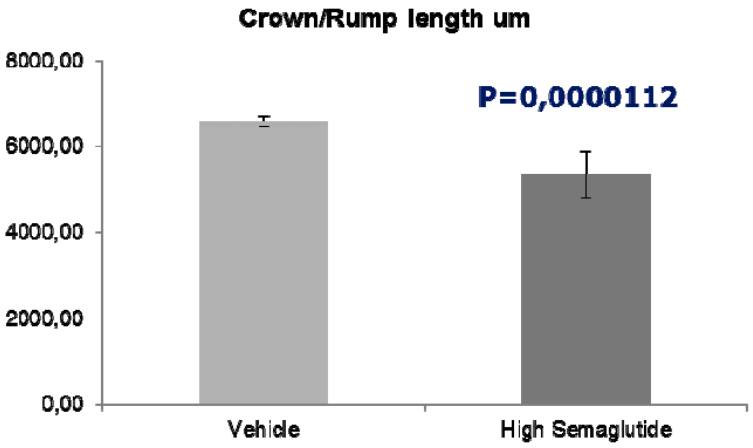
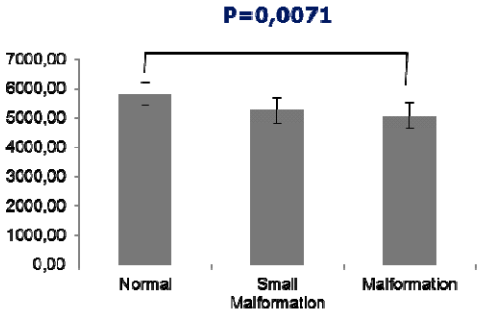


Figure 40. Limb defects and embryo size correlation (Applicant's table)

Dose	Size	LF	RF
Sema High	6249	Normal	Normal
Sema High	NA	Normal	Normal
Sema High	5971	Normal	damaged
Sema High	5437	Normal	Normal
Sema High	5532	Normal	Normal
Sema High	5442	Normal	Mild
Sema High	5222	Normal	Mild
Sema High	4586	Normal	Mild
Sema High	NA	Mild	Mild
Sema High	4562	Severe	damaged
Sema High	5405	Normal	Severe
Sema High	4297	Severe	Severe
Sema High	5077	Normal	Severe
Sema High	5103	Severe	Normal



GLP-1 receptor expression in cynomolgus monkey yolk sac tissue (Study # JHES150306)

Key findings

No GLP-1R expression could be detected in yolk sacs from cynomolgus monkeys using immunohistochemistry, in situ hybridisation or RT-PCR.

Design

Yolk sacs were isolated from four cynomolgus monkeys during early organogenesis (GD 34-42). Of each yolk sac, one half was fixed for immunohistochemistry and in situ hybridisation, while the other half was processed for RT-PCR. All three methods were subsequently employed to test for the presence of GLP-1R expression in the yolk sac

tissue. Maternal pancreas and kidney were used as positive control for GLP-1R detection.

Results

- No GLP-1R protein or mRNA was detected in the four yolk sacs examined.
- Using in situ hybridisation, no GLP-1R mRNA was detected in 3 yolk sacs, while tissue from one yolk sac had a weak signal in the endodermal layer of the membrane.
- Using RT-PCR, no expression of GLP-1R was detected.
- GLP-1R expression was detected in the maternal pancreas and kidney using each of the three modalities.

Distribution of semaglutide to the inverted yolk sac placenta

[^{3}H]-Oct-NNC 0113-0217: The study of distribution of radioactivity in the rat embryo following subcutaneous administration to the dam, using microautoradiography techniques (Study # 210018).

Key findings

Semaglutide distributed to the yolk sac membranes. Embryonic tissue exposure was low.

Design

Qualitative micro-autoradiography was performed on pregnant rats to determine the tissue distribution of semaglutide on GD9, GD10, GD11 and GD12 in the inverted visceral yolk sac and embryonic tissues following a single dose of radioactively labelled semaglutide (^{3}H-Octsemaglutide) to the pregnant dam. The dams had been dosed with "cold" semaglutide from 2 week before mating through the day of receiving the radioactive dose. Tissue sections were evaluated 24 hours after the radioactive dose was administered.

Dose group	Dosing	Sampling time	Number of animals
1	Day 8 of gestation	24 hours after dosing ~ day 9 of gestation	4*
2	Day 9 of gestation	24 hours after dosing ~ day 10 of gestation	4*
3	Day 10 of gestation	24 hours after dosing ~ day 11 of gestation	4*
4	Day 11 of gestation	24 hours after dosing ~ day 12 of gestation	4*

* - Although 4 animals were allocated to each dose group, only 3 per group were required for the scientific objectives of the study to be met. The additional animals were held in reserve in case any animals were found not to be pregnant at necropsy.

Results

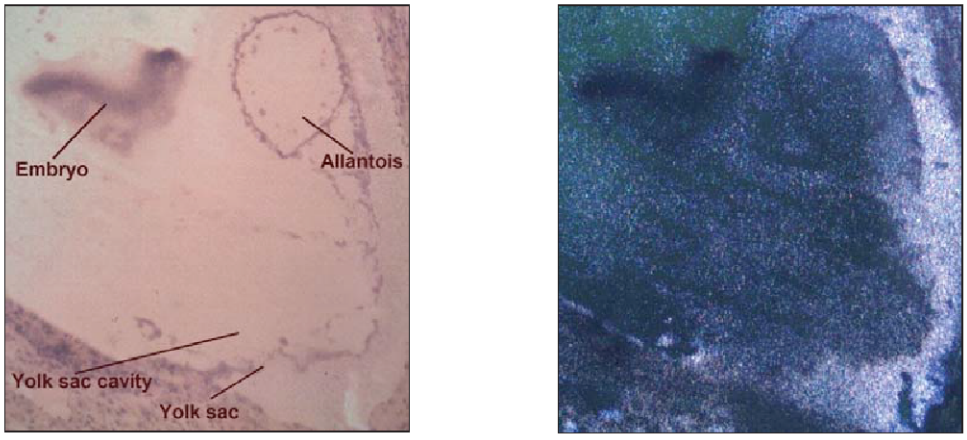
- At the earlier sampling times (days 9 and 10 of gestation) high levels of radioactivity were noted in maternal endometrium but not the uterine crypts. On GD 11 and 12 levels of radioactivity in the endometrium had fallen to moderate but high levels were recorded in the uterine crypts together with the presence of red blood cells. This observation reflects a normal developmental stage occurring in rat pregnancy whereby uterine blood sinuses rupture on approximately day 10.5 of gestation and release maternal blood into the uterine lumen via the crypts. Levels of radioactivity in the uterine glands remained low in all samples.

- In the decidual tissue, at all sampling times, there appeared to be a common gradient of radioactivity from high or moderate levels in the outer decidual to low levels in the inner regions close to the trophoblast.

- Levels of radioactivity in the yolk cavity, between the trophoblast and the visceral yolk sac, were elevated compared with the surrounding tissues. On GD 9 the level was considered to be moderate, but from GD 10, when the embryo was fully enclosed by the visceral yolk sac, high levels were recorded. This finding is suggestive of the visceral yolk sac acting as some form of barrier.

- In general, radioactivity in embryonic tissues was low. However, GD 11 and 12 higher levels of radioactivity were seen in the fluid within the brain ventricles but not within the neural tissue itself.

Figure 41. Micro-autoradiography image of a GD10 rat embryo and yolk sac membrane (Applicant's figure)



^{3}H-Oct-semaglutide is seen as light areas in the right picture. Magnification: x10 Objective (Study 210018)

Table 160. Subjective analysis of the levels of radioactivity in uterine and embryonic tissues of pregnant rats (Applicant's table)

Tissue	Gestation days 9 and-10	Gestation days 11 and 12
Uterine endometrium	++++	+++
Uterine glands	++	++
Uterine crypts	+ to ++	++++
Outer decidua	+++ to ++++	++++
Inner decidua	++	++
Trophoblast	++	++ to +++
Yolk sac	++	++
Chorio-allantoic placenta	Not present /formed	++
Embryo	++	++
Embryo (brain ventricle)	Not present/formed	+++
Amnion	++	++
Yolk cavity	+++ to ++++	++++
Yolk sac cavity	+++	+++ to ++++
Amniotic cavity	+++	+++

NS = tissue Not Sectioned
\+ = background levels of radioactivity
++ = low levels of radioactivity
+++ = moderate levels of radioactivity
++++ = high levels of radioactivity

Functional effects of semaglutide on inverted yolk sac pinocytosis

Glucagon was reported to cause embryotoxicity via an inhibition of pinocytosis in the yolk sac, and it was hypothesized that yolk sac pinocytosis was the cellular process affected following activation of the GLP-1R (Brown J et al. 1977). Therefore, the effect of semaglutide on yolk sac pinocytosis was evaluated.

Ex vivo trans-epithelial transport across the visceral yolk sac membrane (Study # 212241)

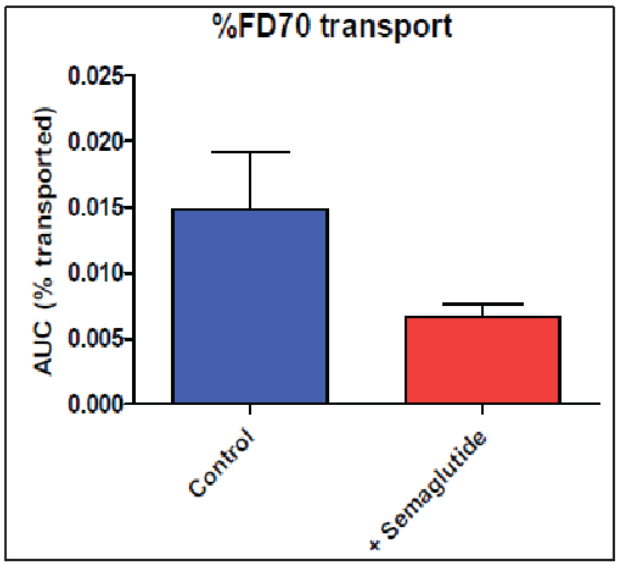
Design

The effect of semaglutide on trans-epithelial transport across the visceral yolk sac membrane was investigated by an Ussing chamber system. Untreated, pregnant SD rats were sacrificed on gestation day 13. The uterus was removed and moved to ice cold Krebbs-Ringar buffer, followed by immediate dissection of the visceral yolk sacs. The isolated yolk sacs were mounted onto the CHM8 chambers (World Presicion Instruments) and semaglutide was added to the apical side of the yolk sacs to a final concentration of 850 nM. Following 30 minutes equilibration, FITC-dextran 70kD (the non-receptor mediated trans-cellular transport of the macropinocytotic marker) or FITC-Dextran 4kD was added to the apical side of all the yolk sacs. 4 kD dextran has been suggested to be transported across the rabbit conjunctiva paracellularly (Horibe et al. 1997) and has been used as a paracellular marker in intestinal in vitro models (Elamin et al., 2012). The 4 kD dextran can thus provide information on membrane integrity. The larger 70 kD dextran is a classic macropinocytotic tracer (Falcone et al., 2006; Mercer and Helenius, 2009), which has successfully been used in a variety of tissues. Samples were collected basolaterally every ten minutes for the duration of the 60 minutes experiment. Apical samples were collected at 0 and 60 minutes.

Results

Semaglutide tended to reduce the non-receptor mediated trans-cellular transport of the macropinocytotic marker FITC-dextran 70kD. In contrast, the transport of the paracellular marker FITC-dextran 4kD remained unaffected by the semaglutide treatment. These results suggest that the effect of semaglutide on the visceral yolk sac is isolated to transcellular activities, rather than causing a general disturbance of the epithelial sheet.

Figure 42. FD70 transport across visceral yolk sacs from GD13 embryos



Ex vivo trans-epithelial transport across the visceral yolk sac membrane following long term exposure to semaglutide (Study # 212080)

Design

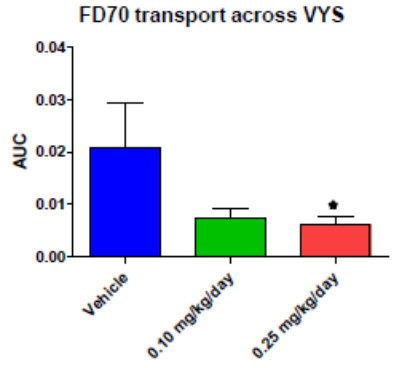
Rats were treated with semaglutide 0.10 and 0.25 mg/kg/day starting before mating and continuously throughout the pregnancy, until sacrifice on GD 14. The uterus was removed and visceral yolk sacs were dissected and mounted onto the CHM8 chambers. Following 30 minutes equilibration, 2.5 mg/ml FITC-dextran 70kD was added to the apical side of the yolk sacs. Samples were collected basolaterally every ten minutes for the duration of the 60 minutes experiment. Apical samples were collected at 0 and 60 minutes.

	Semaglutide (mg/kg/day)		
Group	Day 1-4	Day 5-7	Day 8-termination
Vehicle	0	0	0
Low dose	0.03	0.1	0.1
High dose	0.03	0.1	0.25

Results

Semaglutide reduced trans-cellular transport of FITC dextran across the visceral yolk sac and the number of pinocytotic vesicles and late endosomal compartments in the yolk sac membrane. The late endosome compartments are a result of prior pinocytosis, but while pinocytotic vesicles and early endosomes have a life time of less than a minute, late endosomes persist for longer. Therefore, an effect on the number of late endosomes in treated cells is indicative of a preceding effect on the pinocytotic activity in these cells.

Figure 43. FD70 transport across visceral yolk sacs (Applicant's figure)



11 Integrated Summary and Safety Evaluation

Semaglutide has been engineered to have a low clearance and thereby a long elimination half-life of approximately one week in humans, which makes the compound suitable for once-weekly administration.
The principal mechanism of protraction is albumin binding facilitated by a large fatty acid derived chemical moiety attached to the lysine in position 26. The specific modifications in the molecule are:

1. a modification in position 8 (alanine to 2-aminoisobutyric acid) of the peptide backbone in order to further increase stability against DPP-4, and a change in position 34 from a lysine to an arginine in order to only have one lysine in the sequence;
2. a large hydrophilic linker between the lysine in position 26 and the gamma glutamate whereto the fatty acid is attached
3. a C18 fatty diacid with a terminal acidic group. The latter two contribute to increased albumin binding which results in decreased renal clearance. In addition to slowed degradation in plasma and decreased renal clearance, delayed absorption from subcutis also contributes to a prolonged half-life of approximately 1 week making semaglutide suitable for once-weekly s.c. administration.

Safety Pharmacology

A standard battery of safety pharmacology studies was conducted to assess the effect of semaglutide on central nervous system, cardiovascular and respiratory system, and renal function.
In the Irwin's test in rats, abnormal gait (walking on toes), decreased touch response, passivity, increased urination, dirty muzzle, lethargy and piloerection were observed at the clinical exposure within the first 8h hours after dose. Similar CNS-related clinical signs were also noted in single-dose and 2-week toxicity studies in mice and rats. When doses were increased by titration for 3 weeks prior to study start, no adverse CNS signs were observed, indicating that tolerance to these effects can occur over time. Hunched posture and piloerection were observed in the chronic studies, but were generally well tolerated.

Semaglutide was shown to have a diuretic effect in rats at the clinical exposure, which consisted of increases in urinary excretion of sodium, potassium, chloride, and protein as well as increased urine volume and pH. These effects were most noteworthy during the first 8 hours after dosing. A similar effect was also noted in the chronic rat and monkey studies, as diuresis is a known pharmacological effect of GLP-1R agonists in the rat (Moreno C 2002; Larsen PJ 2001).

Semaglutide did not inhibit hERG tail current and had no effect on cardiac action potential parameters in cardiac Purkinje fibers isolated from New Zealand white rabbits at concentration up to ≥242X the clinical dose. There were no treatment related ECG changes in telemetered conscious monkeys following an acute dose up 14X MRHD. In the 52-week monkey study, a chronic left bundle-branch-block occurred in one high-dose female at an exposure 27-fold above the exposure at the maximum recommended human dose, based on AUC. Cardiac bundle-branch-blocks are occasional findings in monkeys and in humans and are, in most cases, a consequence of other underlying cardiac diseases (Bristow JD 1965; Francia P 2007). However, a relation to treatment could not be excluded.

There were no adverse effects on respiratory function.

General toxicity

The toxicity profile of semaglutide has been evaluated in mice, rats, and cynomolgus monkeys for up to 3-, 6- and 12-month duration, respectively. In all species, dose levels were limited by the pharmacological effects on food intake and body weight. When high dose levels were administered acutely, CNS-related effects such as decreased locomotor activity, abnormal gait (walking on tip toes), decreased touch response, passivity, lethargy, and/or piloerection were observed in rats and mice. However, when dose levels were escalated slowly over a few weeks, CNS-related signs and effects on body weight and food consumption were diminished, indicating that tolerance occurs after repeated dosing. A dose-escalation approach was utilized in all of the pivotal toxicology studies to minimize the initial treatment-related effects on body weight.

In a 3-month study in the mouse, body weight loss during the first week of dosing and decrease in body weight gain thereafter were observed at all doses. The thyroid was the main target of toxicity: mild focal C-cell hyperplasia, C-cell nests, and dilated ultimobranchial ducts were noted starting at the lowest dose tested (17X MRHD). Calcitonin levels were increased in all treated groups compared to control animals, without dose-relationship. At higher doses, liver necrosis and centrilobular hypertrophy were noted, mostly in the males (175X MRHD).

In a 6-month study in the rat, dose-related decrease in body weight and food consumption were observed at the clinical exposure. Minimal to moderate Brunner's gland hypertrophy was noted in nearly all treated rats. This finding was reversible and was considered non-adverse, given the absence of associated inflammatory or

degenerative changes. A dose-related increase in the incidence of fluid distension and luminal dilatation in the uterus was observed and was considered likely due to the stage of the sexual cycle. No treatment-related adverse microscopic lesions were observed in the pancreas or thyroid at any doses (up to 27X MRHD).

In the 12-month study in monkeys, there were no definitive signs of toxicity other than the expected effects on body weight and food consumption. ECG abnormalities (a bigeminal rhythm with two episodes of sinus tachycardia in Week 13 and a continuous left bundle branch block-like recording that persisted from Week 26 to Week 52) and slight multifocal myocardial vacuolation and degeneration, with karyomegaly, in the left ventricle were observed in one high-dose female and male, respectively (27X MRHD). A relationship to treatment could not be excluded. Increased mean absolute and relative thyroid weights in treated females and increased mean weights for prostate and testes were observed, without correlative microscopic findings.

Genotoxicity

A standard battery of in vitro and in vivo genetic toxicology studies indicated that semaglutide is devoid of mutagenic or clastogenic activity.

Carcinogenicity

The administration of semaglutide once daily by subcutaneous injection to CD-1 mice and SD rats for two years resulted in an increased incidence of thyroid C-cell adenoma and combined C-cell adenoma and carcinomas in all treated groups. Thyroid neoplasms occurred at the clinical exposure in rats, and at slightly higher than the clinical exposure in mice (2X and 5X in female and males, respectively). The incidence of C-cell carcinomas was statistically significant increased in male rats at ≥0.01 mg/kg/day (0.4X the clinical exposure). A numerical increase in C-cell carcinoma was noted in mice (n=2, 2, 2 in LD, MD and HD male mice; n=1, 2, 2 in LD, MD and HD female mice). Proliferative C-cell changes in rodents are a known class effect of long-acting GLP-1R agonists and have been reported in rodent carcinogenicity studies with liraglutide, exenatide, lixisenatide, and dulaglutide. Madsen et al. have demonstrated that C-cell hyperplasia and calcitonin release associated with liraglutide are GLP-1-receptor dependent (GLP-1R-KO mice do not secrete calcitonin and do not develop C-cell hyperplasia in response to GLP-1R agonists) and are not associated with the activation of the rearranged-during-transfection (RET) proto-oncogene, which is often seen in human medullary thyroid cancer. Evaluation of cell signaling pathways downstream from RET activation indicated liraglutide did not activate MEK1/2, but activated the mammalian target of rapamycin (mTOR) pathway which in turn results in downstream phosphorylation of ribosome S6 (50). In short term mouse and rat studies with semaglutide, plasma calcitonin levels were increased after a single dose in male and female mice, and after 6 week of dosing in male rats, but not female rats. There was no increase in plasma calcitonin and no C-cell hyperplastic or neoplastic changes in monkeys treated with semaglutide or other GLP-1R agonists. Based on the mechanistic data available for semaglutide and other GLP-1R agonists, the absence of GLP-1Rs on normal monkey or human thyroid C-cells (Pike C 2014; Waser B 2015), and the absence of changes in calcitonin levels or proliferative lesions in chronic monkey

studies, the applicant believes that the human relevance of rodent C-cell tumors is low. However, it is currently unclear whether a lack of calcitonin secretion in non-human primates and humans is a valid indicator that a mitogenic signal is not being initiated in these non-rodent species. Therefore, the human relevance of C-cell tumors is unknown.

DART

Developmental toxicity studies were performed in rats, rabbits, and monkeys. In combined fertility and embryonic development studies in rats, no effects were observed on male fertility. In females, an increase in estrus cycle length was observed at all doses, together with a small reduction in numbers of corpora lutea. Both findings occurred at the clinical exposure, but were likely an adaptive response secondary to the pharmacological effect of semaglutide on food consumption and body weight. Embryotoxicity (decrease in live litter size, and litter and fetal weights), skeletal (cranial bones, caudal vertebra, ribs, short tibia) and visceral malformations (retroesophageal aortic arch, double aortic arch, membranous ventricular septal defect) were observed at the clinical exposure. Malrotated, absent, shortened, curved, kinked, misaligned, partially fused, and/or displaced bones (scapula, long bones, ribs, digits, vertebrae and cranial bone(s) were observed at higher doses (3X MRHD). Although these findings were considered in part related to pharmacologically-mediated decrease in body weight gain, additional causative agents couldn't be excluded.

In pregnant rabbits administered semaglutide once daily by subcutaneous injection at doses of 0.001, 0.0025, and 0.0075 mg/kg/day (0.03, 0.3, 2X MRHD, respectively) from GD 6 through GD 19, maternal body weight loss during GD6-GD14 and decrease in body weight gain thereafter was noted at the clinical exposure. There was not a definitive treatment-related effect on the incidence of major abnormalities. There were marginally higher than expected incidences of fetuses/litters with minor skeletal abnormalities/variants starting at the mid-dose (additional sternebral centers, bridge of ossification/partially fused/fused sternebra, unossified/incompletely ossified metacarpals/phalanges) and visceral abnormalities/variants at the high-dose (dilated renal pelvis, additional liver lobe, and forepaw flexure). The dose-dependent increase in skeletal variations observed in rabbits is likely related to the marked maternal weight loss; however, a direct drug-related effect on fetal development as a contributing factor cannot be ruled out.

In pregnant Cynomolgus monkeys administered semaglutide at 0.015, 0.075, and 0.15 mg/kg (1, 5, and 15X MRHD) every third day by subcutaneous injection from GD16 to GD50, body weight loss was observed in all treatment groups throughout dosing. There was no apparent treatment related effect on fetal mortality, placental weights, fetal weights, or fetal development. Few sporadic abnormalities (blood accumulation under the skull causing misshapen right brain hemisphere in one high dose fetus; shift in alignment of the vertebrae, ribs, and first sternebra at the cervico-thoracic border in one mid-dose and high dose fetus) were observed at $\geq$5X clinical exposure. Because these findings exceeded the concurrent control values and the historical control range, a relationship to treatment could not be excluded. The NOAEL was established at 0.015 mg/kg (1X MRHD).

In the peri- and post-natal development study, pregnant Cynomolgus monkeys received semaglutide at 0.015, 0.075, and 0.15 mg/kg (~1, 3, and 7X MRHD) every third day by subcutaneous injection from GD16 to GD140. Body weight loss in all treatment groups throughout GD 50, and decrease in body weight gain thereafter resulted in increased incidence of early pregnancy loss at ≥0.075 mg/kg (3X MRHD). Infant weight was lower at birth in the high-dose group, but was similar to controls by postnatal day 91. There was no apparent treatment related effect on fetal or infant development.

In order to elucidate the mode of action behind the observed embryotoxicity in rats, a series of mechanistic studies was performed. The majority of fetal abnormalities observed in rats involved anatomical structures (tail, digits and major blood vessels) for which development is generally initiated between gestational day 10 and 13, which coincides with the period when the rat embryo is highly dependent on nutrition supplied via the inverted yolk sac (DeSesso JM 2012). Therefore, the mechanistic studies were designed to investigate the presence of functional GLP1-R in the yolk sac, the distribution of semaglutide in the yolk sac, and the timing and functional effects of semaglutide on the yolk sac. These studies demonstrated that GLP-1 receptors are expressed in the rat yolk sac tissue between GD9-13, and activation of these receptors by semaglutide resulted in the reduction of yolk sac diameter, number of pinocytotic vesicles, and rate of pinocytotic transport (nutrients transport) in vitro. These effects were prevented by addition of a specific GLP-1R antagonist. Continuous intravenous infusion of unacetylated (short-acting) semaglutide in rats from before mating up to GD 8, GD13, and GD17 induced skeletal malformations similar to those observed in the embryofetal toxicity studies in rats (short, bent, thickened long bones, bent scapula and bent clavicola) starting at ≥GD 13 when the rat embryo is dependent on the inverted yolk sac placenta for nutrition. Furthermore, there was a clear correlation between the size of the semaglutide treated embryos and the degree of limb abnormalities, i.e. the smaller embryos had more severe malformations.

In contrast to the rat, the yolk sac in primates does not invert to function as a placenta, and does not expressed GLP-1 receptors. Based on anatomical and functional differences between the rat and primate yolk sac, and similarity between the monkey and human structure, the applicant concluded that the rat embryotoxicity is unlikely to be of relevance in primates, but involvement of additional mechanisms leading to embryotoxicity in rats cannot be completely excluded.
The Division agrees the mechanistic data suggest a role for an effect on the visceral yolk sac; however, the data do not eliminate the possible involvement of other mechanisms and do not necessarily imply species (rat) specificity or a lack of human relevance, based on: 1) the increase in fetal deaths in the monkey peri- and post-natal developmental toxicity study; 2) ultrastructural similarities between human and primate yolk sacs and the rodent visceral yolk sac; 2) the important role of the human yolk sac in the histotrophic nutrition of early pregnancy; 3) the fact that yolk sac pathology has been associated with developmental toxicity and spontaneous abortion in humans; 4) the prototypical yolk sac poison, trypan blue, is reportedly teratogenic in multiple species, including dogs, pigs, and monkeys.

Semaglutide was absorbed and secreted in the milk after a single subcutaneous administration in rats at day 10 post-partum. Semaglutide levels in milk were 3 to 12 fold lower than in maternal plasma.

Local tolerance

In a single-dose study in pigs and rabbits, no treatment related effects at the injection sites were observed following subcutaneous, intramuscular, intravenous, and intra-arterial injections. Minimal to slight cellular infiltrate with or without edema in the pig, and minimal or slight inflammatory cell reaction, perivascular or vascular necrosis, intima proliferation and hemorrhage in the rabbits were observed in all study groups, including controls.

12 Appendix/Attachments

EXPERT STATEMENT

MINUTES FROM EXPERT PANEL MEETING IN

SAN FRANCISCO, CALIFORNIA (USA)

08 MARCH 2012

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/s/

FEDERICA BASSO
08/02/2017

RONALD L WANGE
08/02/2017
I concur with Dr. Basso's recommendation for approval.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 209637 **Applicant: Novo Nordisk** **Stamp Date: 12-5-2016**

Drug Name: Semaglutide Injection **NDA/BLA Type: 505(b)1**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		Four new impurities that arisied from a new manufacturing process were qualified in a 13-week toxicity study in the rat and in two in vitro genetic toxicology studies
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

FEDERICA BASSO
01/17/2017

RONALD L WANGE
01/17/2017