CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203313Orig1s000
203314Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 203314
Submission date: 9/29/2011

Drug: insulin degludec
Sponsor: Novo Nordisk
Indication: improvement of glycemic control in patients with diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Comments:
The pharm/tox reviewer and supervisor both found the nonclinical information adequate to support approval of insulin degludec for the intended indication.

Insulin degludec is a long acting basal insulin analog that is produced using recombinant DNA technology and chemical modification with a C-16 fatty acid (hexadecanedioic acid).

The pharmacology and toxicology of insulin degludec appears to be primarily related to the insulin activity.

Conclusions:
I agree that the information is adequate from a pharm/tox perspective to support approval of this NDA. No additional nonclinical studies are recommended at this time.

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/s/

PAUL C BROWN
02/07/2013

Tertiary Pharmacology/Toxicology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 203313
Submission date: 9/29/2011

Drug: insulin degludec/insulin aspart
Sponsor: Novo Nordisk
Indication: improvement of glycemic control in patients with diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Comments:
The pharm/tox reviewer and supervisor both found the nonclinical information adequate to support approval of insulin degludec/insulin aspart for the intended indication.

This product is a co-formulation of the long acting basal insulin (Insulin degludec) and the fast-acting prandial insulin (insulin aspart). Insulin degludec is reviewed under NDA 203314. Insulin aspart is an active component of marketed products.

The pharmacology and toxicology of insulin degludec/insulin aspart appears to be primarily related to the insulin activity.

Conclusions:
I agree that the information is adequate from a pharm/tox perspective to support approval of this NDA. No additional nonclinical studies are recommended at this time.

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/s/

PAUL C BROWN
02/07/2013

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 203313
Supporting document/s:	0000
Applicant's letter date:	Sept 29th 2011
CDER stamp date:	Sept 29th 2011
Product:	Insulin Degludec/Insulin Aspart (Ryzodeg™)
Indication:	Diabetes
Applicant:	Novo Nordisk Inc
Review Division:	DMEP
Reviewer:	Miyun Tsai-Turton, PhD, MS
Supervisor/Team Leader:	Karen Davis Bruno, PhD
Division Director:	Mary Parks, MD
Project Manager:	Rachel Hartford

Disclaimer

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1 Executive Summary

1.1 Introduction

Insulin degludec/insulin aspart (IDegAsp) is a co-formulation of the long acting basal insulin (IDeg) and the fast-acting prandial insulin (IAsp). IDeg is reviewed under NDA 203314. IAsp was an active component of the marketed products such as NovoRapid/NovoLog (NDA 20986) and NovoMix/NovoLog Mix. The safety and efficacy profile of IAsp has been well established,

IDegAsp is intended for once-daily or twice daily sc administration (injection to the abdominal wall, the upper arm, or the thigh). It will be administered with the main meals. IDegAsp will be formulated in one strength of 100 U/ml in a clear and colorless solution containing the drug substance 70% IDeg (420 nmol/ml) and 30% IAsp (180 nmol/ml). It is intended to be marketed either in a [redacted] (b) (4) pre-filled disposable PDS290 pen-injector.

The nonclinical development of IDegAsp focused on safety and efficacy evaluation of IDeg for which a complete nonclinical development program was conducted (under NDA 203314). Based on ICH M3 guideline on combination medical products, the efficacy of IDegAsp had been assessed in primary PD studies as well as tolerance and toxicity studies (repeat-dose and Seg II) following sc administration.

1.2 Brief Discussion of Nonclinical Findings

The non-clinical development of IDegAsp has focused on safety and efficacy evaluation of IDeg where a complete non-clinical development program was conducted (submitted under NDA 203314). Few additional studies were conducted to further evaluate the co-formulation of IDeg and IAsp.

- PD/PK profile

IDeg had a prolonged PK profile due to a slow and continuous delivery of IDeg from a sc injection site into the systemic circulation. By adjusting the Zn concentration in the co-formulation of IDeg and IAsp, the long PK profile of IDeg was not affected by the rapid absorption of IAsp.

- Tox profile

The single and repeated dose toxicity of insulin degludec were characterized in rats and dogs. The repeated dose toxicity of IDegAsp was further characterized in rats. In the 13 week toxicity study in rats (Study No 208337), SIAC 30 or IDegAsp (up to 75 nmol/kg/day insulin 454 + 32 nmol/kg/day insulin/kg/day) was tolerable. Reduced plasma glucose was the pharmacological action of insulin 454 and insulin aspart, resulted in few secondary findings (i.e. increased food intake, body weight gain, and adaptive change in water balance, effects upon fatty acid and protein metabolism).

These findings were partially or completely recoverable. There were 3 hypoglycemic-related deaths (1 HD, 1 NPH, and 1 MD). Antibody formation was seen with insulin 454 and insulin aspart but there was no apparent effect on the plasma concentration and glucose lowering effect of SIAC30. The TK profile for both insulins is generally gender- and dose-independent, with low accumulation over the 13 weeks of once daily SIAC 30 administration. These findings were consistent with those given NPH insulin. Based on these study findings, the NOAEL was determined at 36 nmol/kg/day SIAC 30 (as 25 nmol insulin 454 + 11 nmol insulin aspart). In addition, approx 1/3 rats developed antibodies towards IDeg or IAsp. However, these antibodies formed did not influence the study outcome of exposure to insulin and there was no inhibition of the glucose lowering effect.

The reproductive and development toxicity of insulin degludec were characterized in rats and rabbits. The embryo-fetal development (Seg II) toxicity of IDegAsp was further characterized in rats. In the Seg II study in rats (Study No 208334), treatment with IDegAsp did not affect embryo-fetal survival or development. Decreased maternal food consumption and body weight, periparturient maternal hypoglycaemia-related mortality, lowered live birth index and viability index, lower offspring body weight, and viability, skeletal changes in the offspring and delayed balano preputial separation were all considered secondary changes to the expected pharmacological effect on lowering the maternal blood glucose levels. These findings were also observed with IDeg and NPH insulin.

The impurities have been qualified in the nonclinical studies for IDeg and IDegAsp. To further qualify drug product related impurities present at end of shelf-life and in-use period, a 4 week study in rats comparing the toxicity profile of aged and non-aged IDegAsp was conducted. Only minor differences between aged and non-aged IDegAsp were observed (i.e. body weight, hematology, clinical chemistry and organ weights) and these were considered incidental.

The local toxicity of IDeg and IDegAsp were both characterized in pig/minipigs and rabbits. The local tissue reaction was mild and comparable to that of vehicle or NPH insulin

Based on all the nonclinical studies conducted for IDeg and IDegAsp, IDegAsp did not cause any unexpected adverse findings in animals. Therefore, the sc administration of IDegAsp should not pose any human safety concern other than hypoglycaemia-related adverse effects.

1.3 Recommendations

1.3.1 Approvability

Pharm/tox recommends approval.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Proposed by the applicant

(b) (4)

[redacted]

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(b) (4)

Suggested by the DMEP

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate well-controlled clinical studies of the use of insulin degludec/aspart in pregnant women. Patients should be advised to discuss with their health care provider if they intend to or if they become pregnant. Because animal reproduction studies are not always predictive of human response, insulin degludec/aspart should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is essential for patients with diabetes or a history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in these patients.

An open-label, randomized study compared the safety and efficacy of NovoLog (insulin aspart, the rapid-acting component of , (b) (4) versus human insulin in the treatment of pregnant women with Type 1 diabetes (322 exposed pregnancies (insulin aspart: 157, human insulin: 165). Two-thirds of the enrolled patients were already pregnant when they entered the study. Since only one-third of the patients enrolled before conception, the study was not large enough to evaluate the risk of congenital malformations. Mean HbA_{1c} of ~ 6% was observed in both groups during pregnancy, and there was no significant difference in the incidence of maternal hypoglycemia.

Subcutaneous reproduction and teratology studies have been performed with, insulin degludec/aspart, and human insulin (NPH) as a comparator in rats. In these studies, insulin degludec/aspart was given to rats during organogenesis. The effect of , insulin degludec/aspart was consistent with those observed with human insulin as both caused visceral/skeletal abnormalities in rats at dose of 30 U/kg/day (approximately 8 times the human subcutaneous dose of 1.08 U/kg/day based on U/body surface area).

Subcutaneous reproduction and teratology studies have been performed with insulin degludec (basal component of insulin degludec/aspart), and human insulin (NPH) as a comparator in rats and rabbits. In these studies, insulin was given to female rats before mating throughout pregnancy until weaning, and to rabbits during organogenesis. The effect of insulin degludec was consistent with those observed with human insulin as both caused pre- and post-implantation loses and visceral/skeletal abnormalities in rats at an insulin degludec dose of 21 U/kg/day (approximately 5 times the human subcutaneous dose of 0.75 U/kg/day, (b) (4)) and in rabbits at a dose of 3 U/kg/day (approximately 10 times the human subcutaneous dose of 0.75 U/kg/day (b) (4)). The effects are probably secondary to maternal hypoglycemia.

Subcutaneous reproduction and teratology studies have been performed with insulin aspart, the rapid-acting component of (b) (4) and regular human insulin in rats and rabbits. In these studies, insulin aspart was given to female rats before mating, during mating, and throughout pregnancy, and to rabbits during organogenesis. The effects of insulin aspart did not differ from those observed with subcutaneous regular human insulin. Insulin aspart, like human insulin, caused pre- and post-implantation losses and visceral/skeletal abnormalities in rats at a dose of 200 U/kg/day (approximately 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area) and in rabbits at a dose of 10 U/kg/day (approximately three times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area). The effects are probably secondary to maternal hypoglycemia at high doses. No significant effects were observed in rats at a dose of 50 U/kg/day and in rabbits at a dose of 3 U/kg/day. These doses are approximately 8 times the human subcutaneous dose of 1.0 U/kg/day for rats and equal to the human subcutaneous dose of 1.0 U/kg/day for rabbits, based on U/body surface area.

8.3 Nursing mothers

It is unknown whether insulin degludec/aspart is excreted in (b) (4) in human milk. Because many drugs, including human insulin, are excreted in human milk, caution should be exercised when Trade Name is administered to a nursing mother. (b) (4) with diabetes who are lactating may require adjustments in insulin dose, meal plan, or both.

In rats, the basal component of Trade Name, insulin degludec, was secreted in milk and the concentration in milk was (b) (4) plasma.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of insulin degludec/aspart.

In a 52-week study including human insulin (NPH insulin) as comparator, Sprague-Dawley rats were dosed subcutaneously with insulin degludec, the basal component of , insulin degludec/aspart at 3.3, 6.7, and 10 U/kg/day, resulting in 5 times the human exposure (AUC) when compared to a human subcutaneous dose of 1.08 U/kg/day Trade Name. Human insulin was dosed at 6.7 U/kg/day. No treatment-related increases in incidences of hyperplasia, benign or malignant tumors were recorded in female mammary glands from rats dosed with insulin degludec and no treatment related changes in the female mammary gland cell proliferation were found using BrdU incorporation. Further no treatment related changes in the occurrence of hyperplastic or neoplastic lesions were seen in any animals dosed with insulin degludec when compared to vehicle or human insulin.

In 52-week studies, Sprague-Dawley rats were dosed subcutaneously with insulin aspart, the rapid-acting component of insulin degludec/aspart , at 10, 50, and 200 U/kg/day (approximately 2, 8, and 32 times the human subcutaneous dose of 1.0 U/kg/day, based on U/body surface area, respectively). At a dose of 200 U/kg/day, insulin aspart increased the incidence of mammary gland tumors in females when compared to untreated controls. The incidence of mammary tumors found with insulin aspart was not significantly different from that found with regular human insulin. The relevance of these findings to humans is not known.

Genotoxicity testing of insulin degludec was not performed. Insulin aspart was not genotoxic in the following tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, *in vivo* micronucleus test in mice, and *ex vivo* UDS test in rat liver hepatocytes.

In a combined fertility and embryo-fetal study in male and female rats, treatment with insulin degludec up to 21 U/kg/day (approximately 5 times the human subcutaneous dose of 0.75 U/kg/day, based on U/body surface area) prior to mating and in female rats during gestation had no effect on mating performance.

In fertility studies with insulin aspart (Novolog) in male and female rats, at subcutaneous doses up to 200 U/kg/day (approximately 32 times the human subcutaneous dose, based on U/body surface area), no direct adverse effects on male and female fertility, or general reproductive performance of animals was observed.

2 Drug Information

2.1 Drug

CAS Registry Number 844439-96-9 (IDeg) and 116094-23-6 (IAsp)

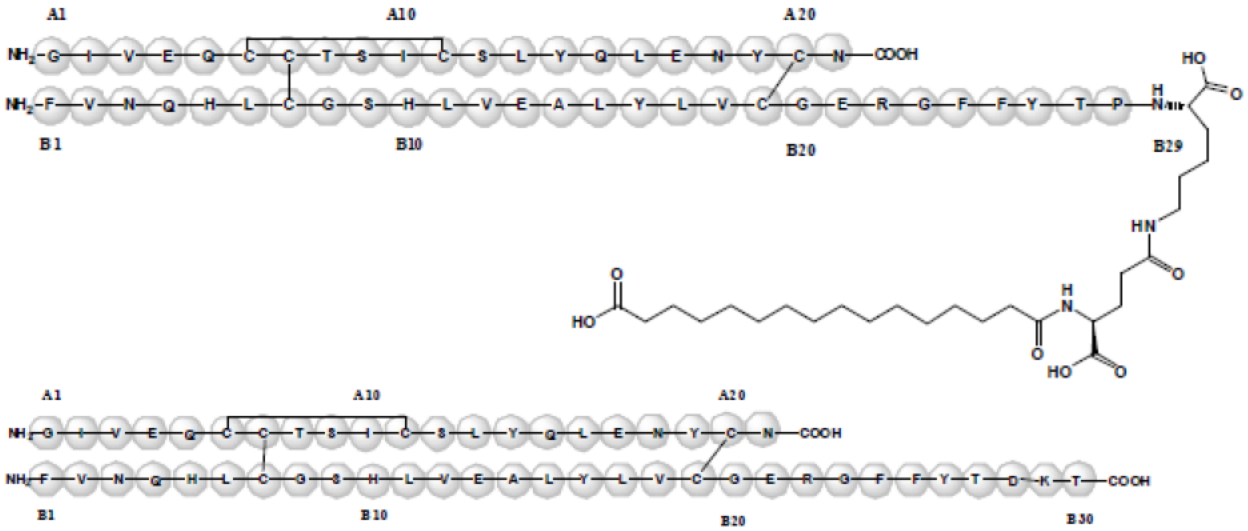
Generic Name insulin degludec and insulin aspart

Code Name Insulin 454/insulin aspart, SIAC and NN5401

Chemical Name LysB29(Nε-hexadecandioyl-γ-Glu) des(B30) human insulin
Asp^{B28} insulin human

Molecular Formula/Molecular Weight $C_{274}H_{411}N_{65}O_{81}S_6$ (610[(b) (4)]) and $C_{256}H_{381}N_{65}O_{79}S_6$ (5825.8)

Structure or Biochemical Description



Pharmacologic Class Long (for IDeg) and fast (for IAsp) acting insulin analogs

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 223314 (IDeg), IND 76496 (IDeg), NDA 20986 (Novolog), IND 73198 (IDeg/Asp)

2.3 Drug Formulation

The composition of IDegAsp 100 U/ml is listed below.

Composition of insulin degludec/insulin aspart 100 U/ml

Name of components	Quantity per ml	Function	Reference to standards
Active substance			
Insulin degludec	420 nmol	Drug substance	Novo Nordisk
Insulin aspart	180 nmol	Drug substance	Novo Nordisk
Excipients			
Phenol[1]	1.50 mg	(b) (4)	Ph Eur, USP, JP
Metacresol[1]	1.72 mg		Ph Eur, USP
Glycerol	19.0 mg		Ph Eur, USP, JP
Sodium chloride	0.58 mg		Ph Eur, USP, JP
Zinc	27.4 µg		Ph Eur, USP, JPE[2]
Hydrochloric acid[3]	q.s.	pH adjustment	Ph Eur, USP, JP
Sodium hydroxide[3]	q.s.	pH adjustment	Ph Eur, USP, JP
Water for injections	(b) (4)	(b) (4)	Ph Eur, USP, JP

(b) (4)

[3] To reach pH 7.4

2.4 Comments on Novel Excipients

None of the excipients were classified as novel excipients.

2.5 Comments on Impurities/Degradants of Concern

Three groups of IDeg and three groups of IAsp impurities and related substances were identified. In addition, HMWP were also identified. These impurities were qualified in the nonclinical studies during development of IDeg and IDegAsp.

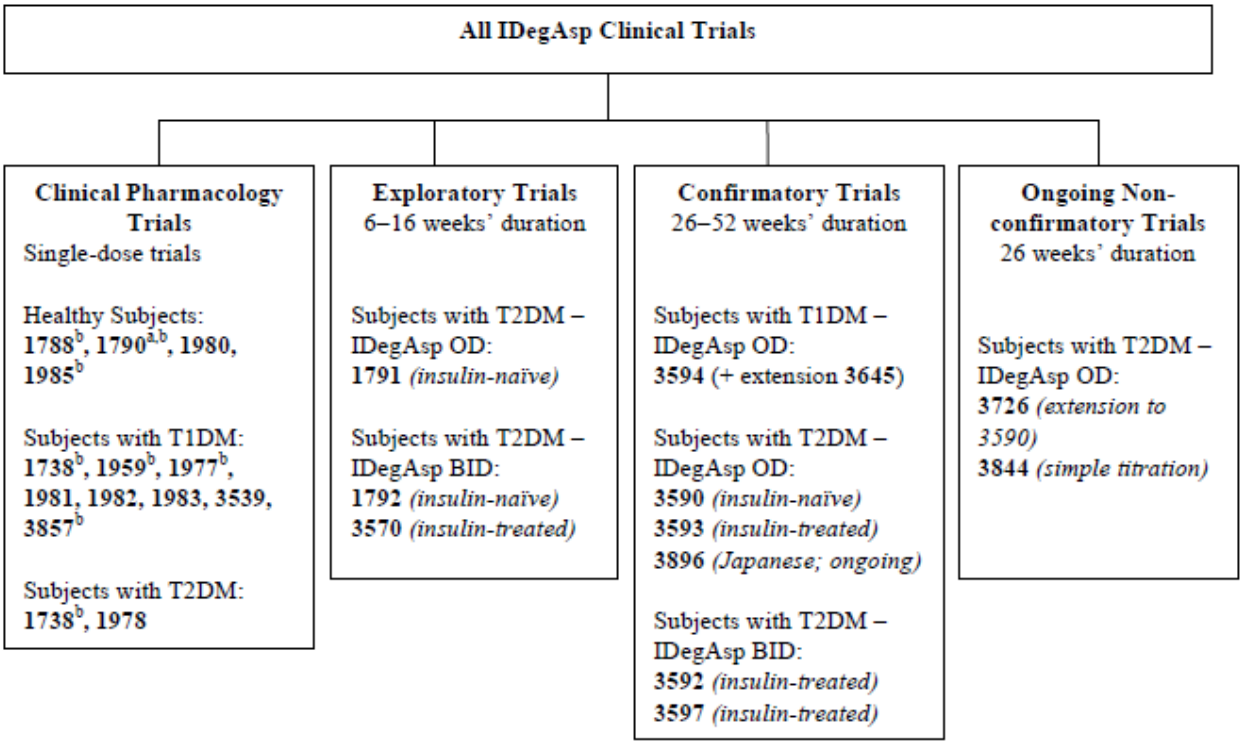
Based on 4-week rat study to qualify drug product related impurities (at the end of shelf life and in use period), no differences were seen between aged and non-aged IDeg.

Impurities in IDegAsp drug product

Drug substance	Impurities
Insulin degludec	(b) (4)
Insulin aspart	

2.6 Proposed Clinical Population and Dosing Regimen

This is intended for once-daily or twice-daily sc administration during main meal time in diabetic patients.



OD = once daily; BID = twice daily
[a] Clinical Pharmacology Trial 1790 was multiple-dose
[b] Clinical Pharmacology Trials also including IDeg

2.7 Regulatory Background

Timeline:

- March 2008 – IND submission
- Feb 2009 – EOP2 meeting
- June 2011 – pre-NDA meeting
- Sept 2011 – NDA submission

3 Studies Submitted

3.1 Studies Reviewed

PHARMACOLOGY
Study 6ulr051108-100-mar-2006: clamp study in pigs
Study ars-23-aug-2005: additive effect of the combo
Study UIR060904-0100 feb 2007: clamp study in pigs

PK/TK
Study 204383: absorption I in pigs
Study 205053: absorption II in pigs
Study 205220: absorption III in pigs
Study 205419: absorption IV in pigs

TOXICOLOGY
Study 208289: 4 week toxicity study in rats
Study 208337: 13 week toxicity (pivotal) study in rats
Study 208333: Seg II DRF rat
Study 208334: Seg II (pivotal) rat
Study 210228: 4-wk rat study for impurities

3.2 Studies Not Reviewed

Studies also included in NDA 203314 (IDeg) were not reviewed again here.

3.3 Previous Reviews Referenced

Some PD/PK/Tox studies were reviewed under IND73198 (IDegAsp) and NDA 203314 (IDeg).

4 Pharmacology

4.1 Primary Pharmacology

See NDA 203314 for IDeg.

Note: 3 PD studies for IDegAsp were described here in details: in vitro and in vivo (clamp) studies.

Study No 6ulr051108-100-mar-2006: clamp pig

PK/PD profiles of insulin 454/insulin aspart combinations. euglycaemic clamp in pigs [by Novo Nordisk]

Study design: This non-GLP study was to demonstrate the combination of a once daily basal insulin analog (insulin 454) with insulin aspart, without interfering with the individual activity profiles. In this study, 8 LYD-pigs were given test articles on 3 separate days with an interval of 1 week, in random order 3 different treatments according to the dosing schedule (see tables below). The insulin injections were given subcutaneously as 1 depot for combinations or 2 depots when insulin 454 and aspart injections were separated. Glucose was infused with variable rate to maintain euglycemia. Plasma glucose was measured at regular intervals (approx. 60 min to 24 hr). Blood samples were drawn and analyzed for insulin 454 (specific ab), insulin aspart (specific ab) and c-peptide.

TEST SUBSTANCES, item(s)
1:8, 3.38 Zn Aspart/454 Mix batch no 15278-131-I 6I
1:8, 6 Zn Aspart/454 mix batch no 15278-131-II 6J
Insulin 454 6 Zn batch no 15278-131-III 6K
Insulin Aspart 3Zn batch no 15278-090-IV 4E

Preparation and dosing scheme

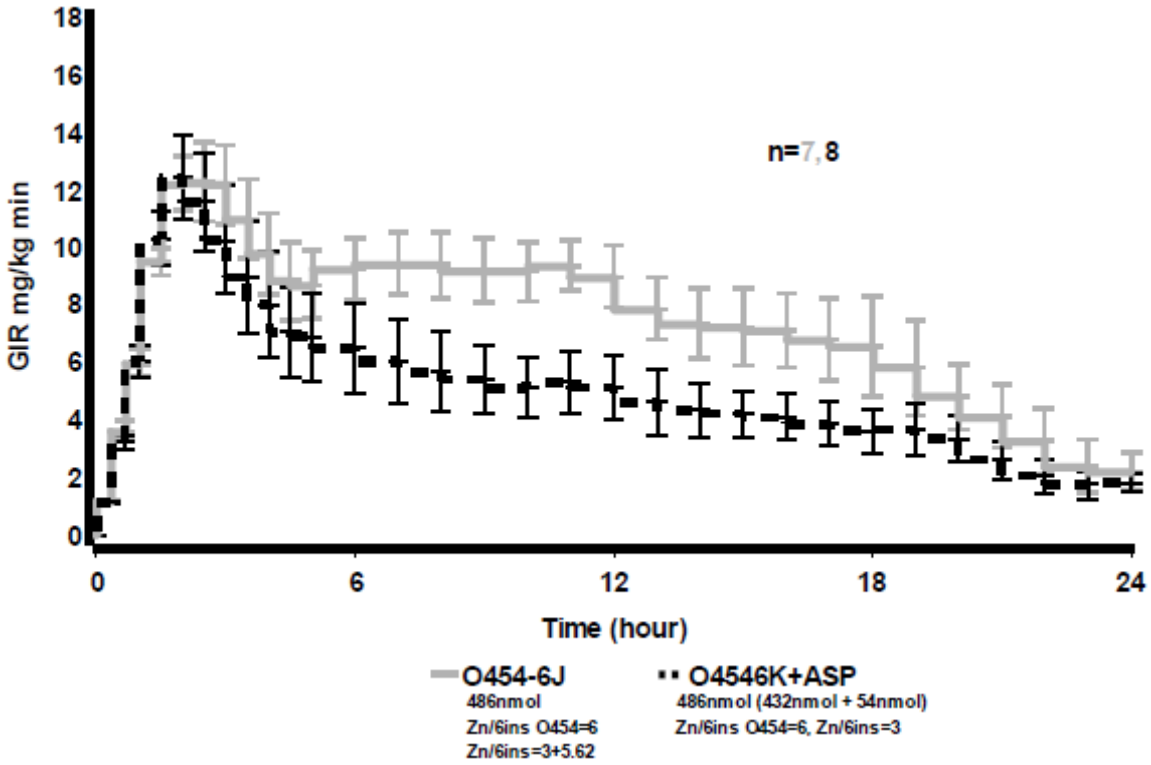
Code Analogues Batch no	Conc µM	Isotonic agent	Phe/Cre	Zn/6ins Zn/6ins454	[Zn]	Inj.vol µl	Dose nmol
15278-131-I aspart + 454 0100-0000-0454-6I	120 + 960	Glycerol 1.6% NaCl 10 mM	16 mM	3 + 3 3.38	540 µM	450	54 + 432
15278-131-II aspart + 454 0100-0000-0454-6J	120 + 960	Glycerol 1.6% NaCl 10 mM	16 mM	3 + 5.62 6	960 µM	450	54 + 432
15278-131-III 0454 0100-0000-0454-6K	1200	Glycerol 1.6% NaCl 10 mM	16 mM	6	1200 µM	360	432
In same pig 15278-090-IV aspart 0121-0000-0014-4E	600	Glycerol 1.6% NaCl 10 mM	16 mM	3	300 µM	90	54

Findings:

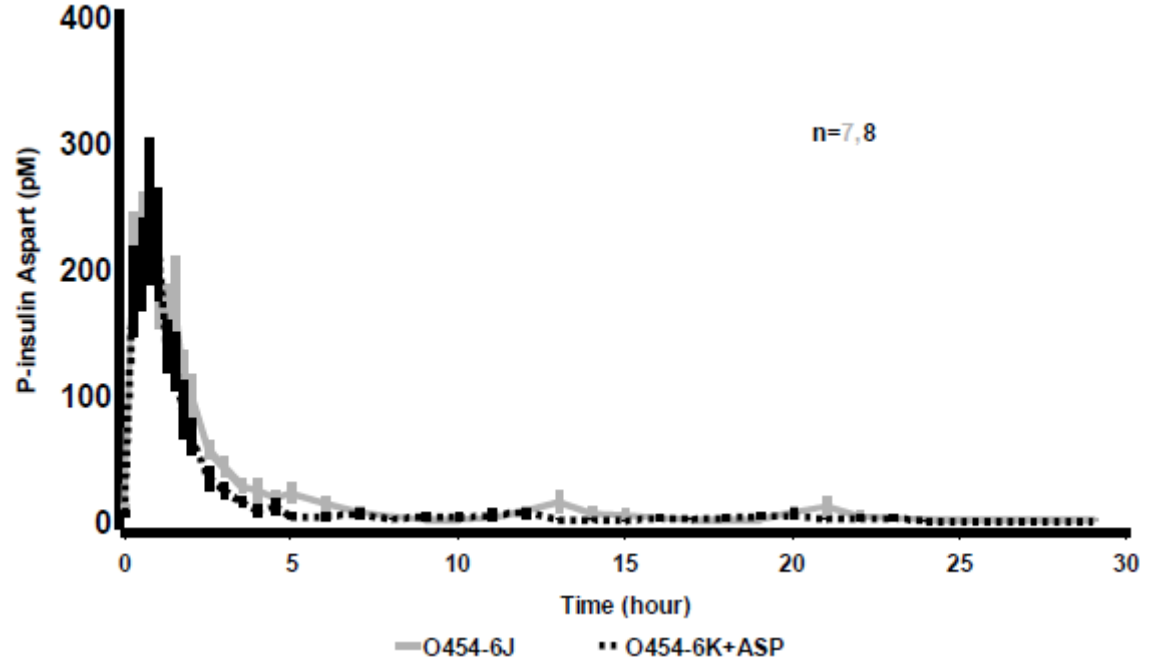
- At a 1:8 ratio of insulin aspart/insulin 454 in a formulation containing 6 zinc+6 insulin 454, no interference with the individual GIR or PK profiles was observed. However, at a 1:8 ratio of insulin aspart/insulin 454 in a formulation 3.38 zincs/6 insulin 454, significant interference with the individual action and PK profiles was observed.
- Protraction of insulin 454 becomes more pronounced with higher zinc and higher insulin 454 concentration.
- Insulin 454 can be formulated as a once-daily insulin.

Figure 1 Study No 6ulr051108-100-mar-2006 – Action/PK Profiles of Insulin 454 and/or Insulin Aspart

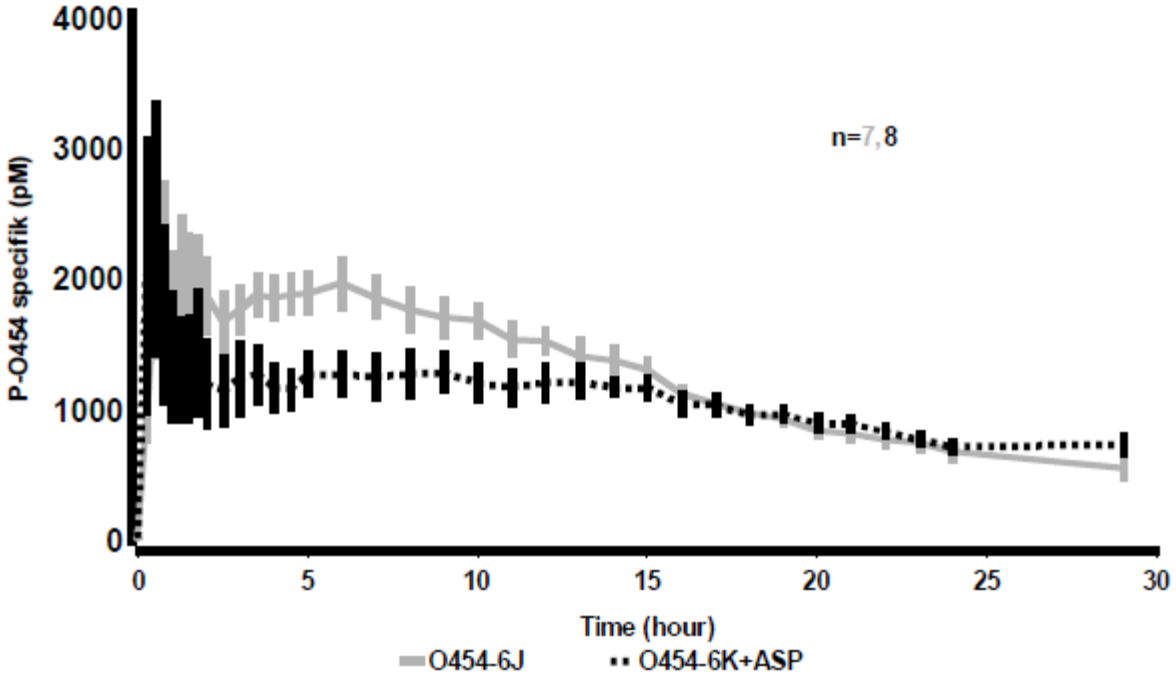
Action profile of insulin 454 and insulin Aspart administered separately or in a high zinc 1:8 combination



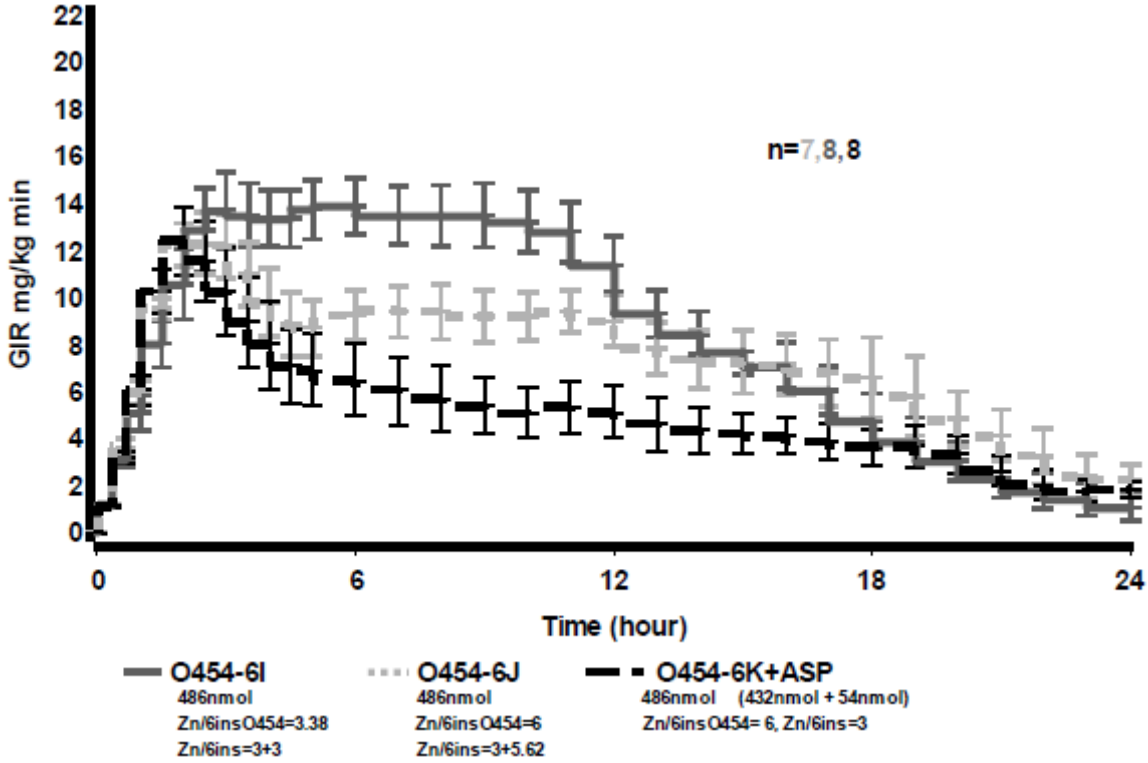
PK insulin Aspart profile when insulin Aspart is administered separately or in a high zinc 1:8 combination with insulin 454



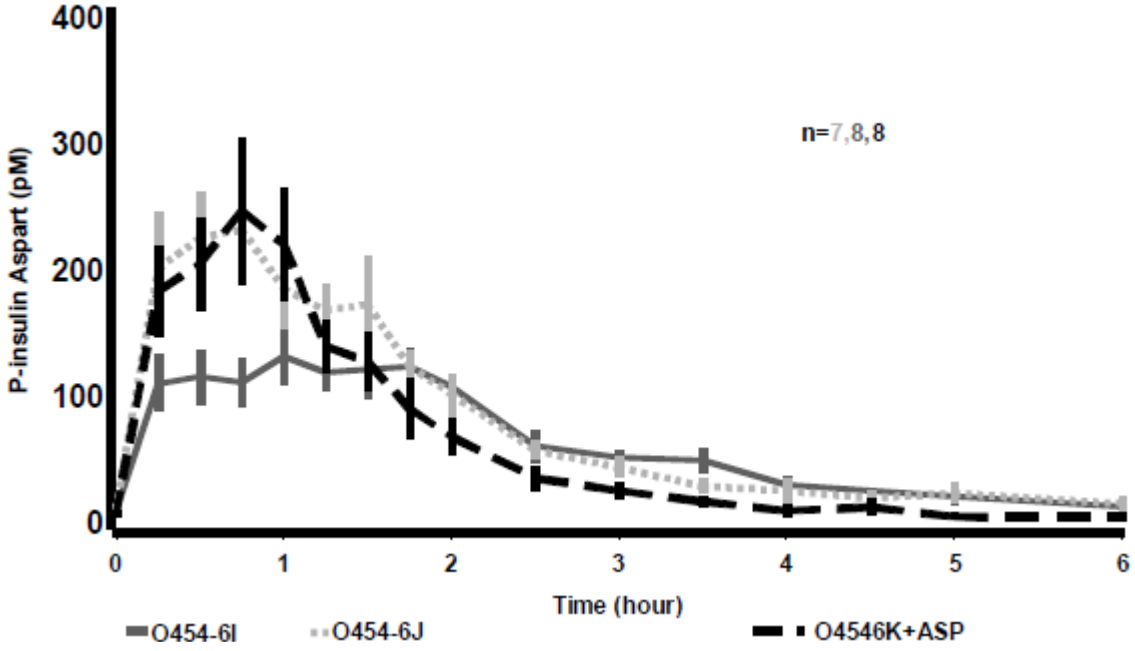
PK insulin 454 profile when insulin 454 is administered separately or in a high zinc 1:8 combination with insulin Aspart



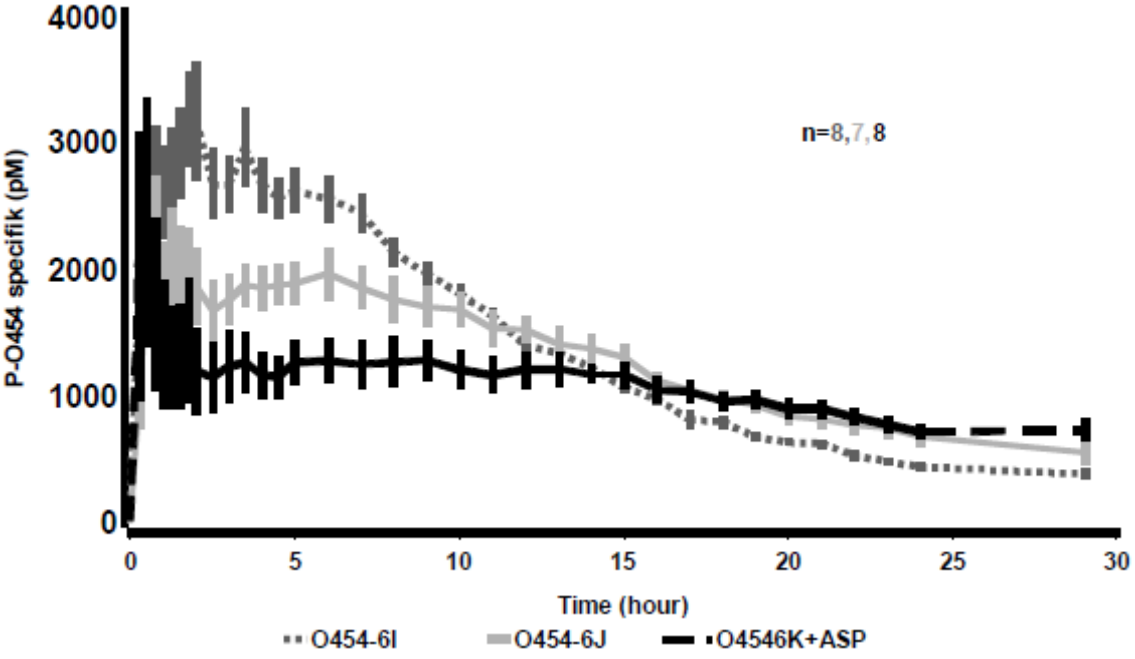
Action profile of Aspart-insulin 454 1:8 combinations with low or high zinc compared with high zinc insulin 454 and Aspart injected separately



PK insulin Aspart profile when insulin Aspart is administered separately or in a low or high zinc 1:8 combined with insulin 454



PK insulin 454 profile when insulin 454 is administered separately or in a low or high zinc 1:8 combined with insulin Aspart



Study No ars-23-aug-2005: additive effect of combo

Additive effect of combinations of insulin 454 and human insulin or insulin aspart [by Novo Nordisk]

Study design: This non-GLP study is to determine whether combinations of insulin 454 and human insulin or insulin aspart was additive in effect. Primary rat adipocytes were

incubated with combinations of insulin 454 and human insulin or insulin aspart. The ligopgenic effect was measured. This in vitro experiment had a compete factorial design of 8 dose levels of insulin 454 mixed with human insulin or insulin aspart.

TEST SUBSTANCES, item(s)
Human Insulin: Internal reference standard: Ins. 321.INS.99.2, Certified potency: 100.6 IU/ml
Insulin 454: Batch 2A and 4A
Insulin Aspart: Secondary reference material: 256.X14.01.2, Certified concentration: 609 µM
(All insulins were supplied by Novo Nordisk A/S)

Findings:

- The lipogenic response in primary rat adipocytes after 2 hrs stimulation with combinations of insulin 454 and human insulin or insulin aspart revealed no significant interaction between the ligands.
- The interaction parameter δ was in the combined result not different from 0, so neither synergism nor antagonism could be detected.
- The effect of having two different insulins would be additive. The apparent potency of insulin 454 depends on the concentration of albumin present during incubation. An increased amount of albumin would bind more insulin 454 and resulted in a lower amount of insulin 454 available (i.e. reduced potency of insulin 454) for insulin receptor binding.

Table 1 Study No ars-23-aug-2005 - The Effects of Having Insulin 454 and Insulin Aspart Co-formulated in the Presence of Albumin

Summary table of results

Assay no.	Test vs standard	HSA (%)	Potency (%)	95 % con lim		W	Synergy	95 % con lim	
1	Insulin Aspart vs HM Ins.321.INS.99.2	0.5	107.39	94.38	122.21	1254	0.0581	-0.0911	0.2074
2	Insulin Aspart vs HM Ins.321.INS.99.2	0.5	93.83	82.54	106.67	1273	0.1910	0.0389	0.3431
Combined result (average)		0.5	100.61	88.46	114.44		0.12	-0.03	0.28
3	Insulin 454-2A vs Insulin Aspart	0.5	1.20	1.01	1.43	716	0.1714	-0.0668	0.4097
5	Insulin 454-2A vs Insulin Aspart	0.5	1.32	1.20	1.45	2444	-0.0031	-0.1221	0.1158
7	Insulin 454-2A vs Insulin Aspart	0.5	1.60	1.44	1.79	1728	-0.0006	-0.1365	0.1352
Combined result (average)		0.5	1.38	1.22	1.56		0.06	-0.11	0.22
4	Insulin 454-2A vs Insulin Aspart	1	0.89	0.78	1.01	1173	0.0103	-0.1450	0.1656
6	Insulin 454-2A vs Insulin Aspart	1	0.65	0.60	0.71	2876	-0.0705	-0.1580	0.0170
8	Insulin 454-2A vs Insulin Aspart	1	0.82	0.74	0.91	1852	-0.0402	-0.1584	0.0781
Combined result (average)		1	0.79	0.70	0.88		-0.03	-0.15	0.09
9	Insulin 454-4A vs HM Ins.321.INS.99.2	0.5	1.76	1.59	1.95	1977	-0.0546	-0.1585	0.0492
11	Insulin 454-4A vs HM Ins.321.INS.99.2	0.5	1.81	1.63	2.01	1848	-0.0786	-0.1723	0.0151
Combined result (average)		0.5	1.78	1.61	1.98		-0.07	-0.17	0.03
10	Insulin 454-4A vs HM Ins.321.INS.99.2	1	1.06	0.96	1.18	2028	-0.0347	-0.1291	0.0598
12	Insulin 454-4A vs HM Ins.321.INS.99.2	1	1.06	0.96	1.17	2302	-0.1054	-0.2023	-0.0085
Combined result (average)		1	1.06	0.96	1.17		-0.07	-0.17	0.03

Combined result of combinations

Test vs standard	HSA (%)	Potency (%)	95 % con lim		Synergy (δ)	95 % con lim		n
Insulin Aspart vs HM Ins.321.INS.99.2	0.5	100.61	88.46	114.44	0.12	-0.03	0.28	2
Insulin 454-2A vs Insulin Aspart	0.5	1.38	1.22	1.56	0.06	-0.11	0.22	3
Insulin 454-2A vs Insulin Aspart	1	0.79	0.70	0.88	-0.03	-0.15	0.09	3
Insulin 454-4A vs HM Ins.321.INS.99.2	0.5	1.78	1.61	1.98	-0.07	-0.17	0.03	2
Insulin 454-4A vs HM Ins.321.INS.99.2	1	1.06	0.96	1.17	-0.07	-0.17	0.03	2

Study No UIR060904-0100 Feb-2007: clamp pig

Action profile of a SIAM 50/50 in a euglycaemic clamp in pigs [by Novo Nordisk]

Study design: This non-GLP study was to characterize an equipotent once daily basal insulin 454 and in addition to show if this insulin 454 could be used in neutral soluble combined formulation with fast acting insulin aspart without blunting the individual activity. Eight female LYD-pigs were given three treatments (SIAM 50/50, 300 nmol/ml insulin aspart + 300 nmol/ml insulin 454, 600 nmol/ml insulin aspart + 600 nmol/ml insulin 454) in random order. There were 5 insulin formulations used in this study. See table below. Plasma samples were drawn for glucose and insulin determination and a glucose solution was infused at variable rate (0-24h) to maintain fasting plasma glucose level.

TEST SUBSTANCES, item(s)

- Test item(s)
  - SIAM 50 (insulin aspart: insulin 454 1:1, both 300 nmol/ml) 600 nmol ins/ml, batch no 412 N06262
  - Insulin 454 6 Zn/6ins 600 nmol/ml, batch no 412 N06263
  - Insulin 454 6 Zn/6ins 300 nmol/ml, batch no 412 N06264
  - Insulin Aspart 3 Zn/6 ins 600 nmol/ml, batch no 412 N06265
  - Insulin Aspart 3 Zn/6 ins 300 nmol/ml, batch no 412 N0 6266

Details of Insulin formulations

	SIAM 50, 6 zn/6 ins. 454	Insulin 454 600 nmol/ml, 6Zn	Insulin 454 300 nmol/ml, 6Zn	Aspart 600 nmol, 3 Zn	Aspart 300 nmol/ml, 3 Zn
Insulin Aspart	300 nmol/ml	-	-	600 nmol	300 nmol
Insulin 454	300 nmol /ml	600 nmol	300 nmol	-	-
Phenol	1.50 mg/ml (16 mM)*	(b) (4)			
m-Cresol	1.72 mg/ml (16 mM)*				
Glycerol	(b) (4)				
Sodium Chloride	0.58 mg/ml (10 mM)				
Zinc	(b) (4)	(b) (4			
	(b) (4)				
Sodium hydroxide	q.s.**				
	(b) (4				
Water, WFI	(b) (4)				
pH	7.40				
Batch no.:	412_N06262	412_N06263	412_N06264	412_N06265	412_N06266
					(b) (4)

** For pH adjustment

(b) (4)

Findings:

- PK and PD profiles of insulin aspart and insulin 454 administered separately or pre-combined were studied in a euclycaemic clamp study in pigs. A total of insulin of 216 nmol/pig for either two insulins (given separately in two strengths or pre-combined) was injected subcutaneously.
- The glucose utilization and profile of the infusion rates to maintain euglycaemia were similar for the three treatments.
- Plasma insulin aspart profiles were superimposable.
- Plasma insulin 454 showed a trend towards a more rapid absorption and insulin 454 in the 300 nmol/ml formation compared to the 600 nmol/ml formation.
- The SIAM50/50 (pre-combination) gave raise to a plasma 454 insulin profile in between the profiles obtained with the 300 and 600 nmol/ml insulin 454 formulations.
- Pre-combination of insulin aspart and insulin 454 (SIAM50/50) in the formulation (containing 6 Zn/6 insulin 454) did result in a minor but not significant blunting of insulin aspart but did not influence the insulin 454 profile.
- Therefore, this study concluded that there was no blunting of either insulin aspart or insulin 454 when the two insulins were combined in a 50/50 ratio in a formulation containing 6 Zn/6 insulin 454.

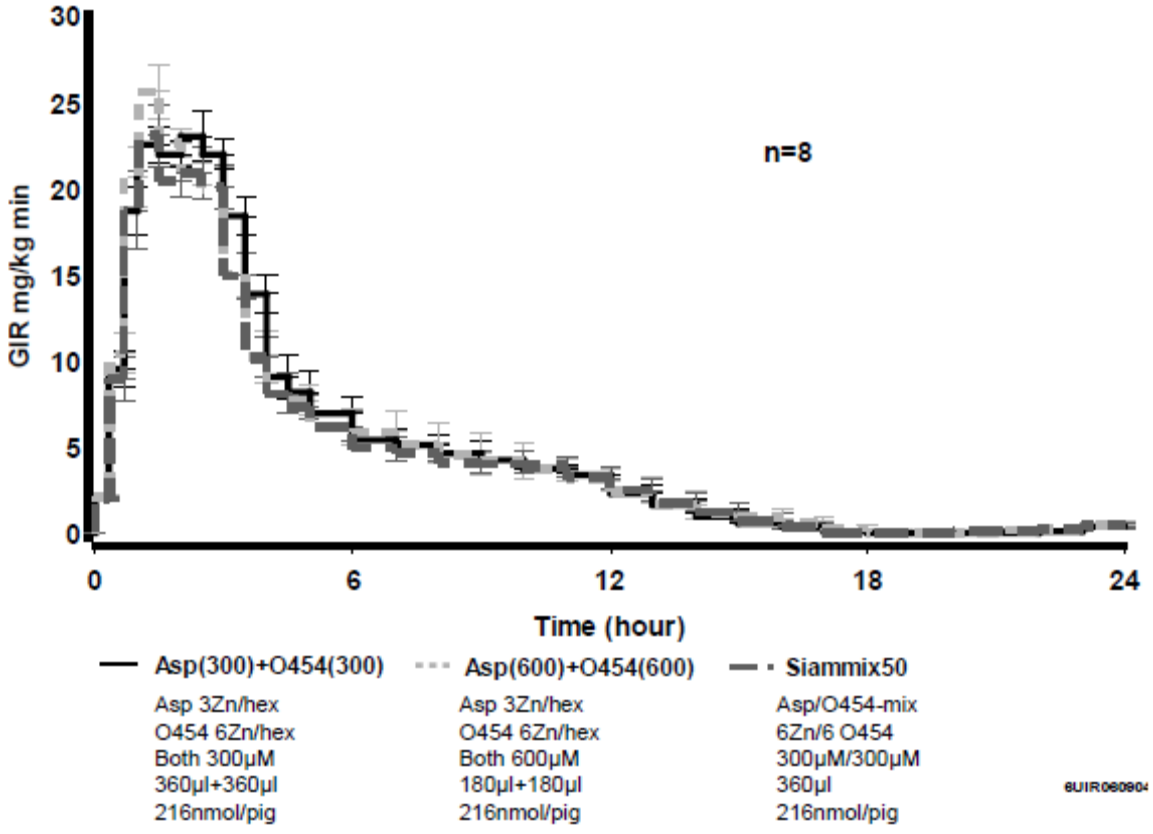
Table 2 Study No UIR060904-0100 Feb-2007 - The PD/PK Profiles of Insulin 454 and/or Insulin Aspart

Key pharmacokinetic and pharmacodynamic variables obtained from the three treatments

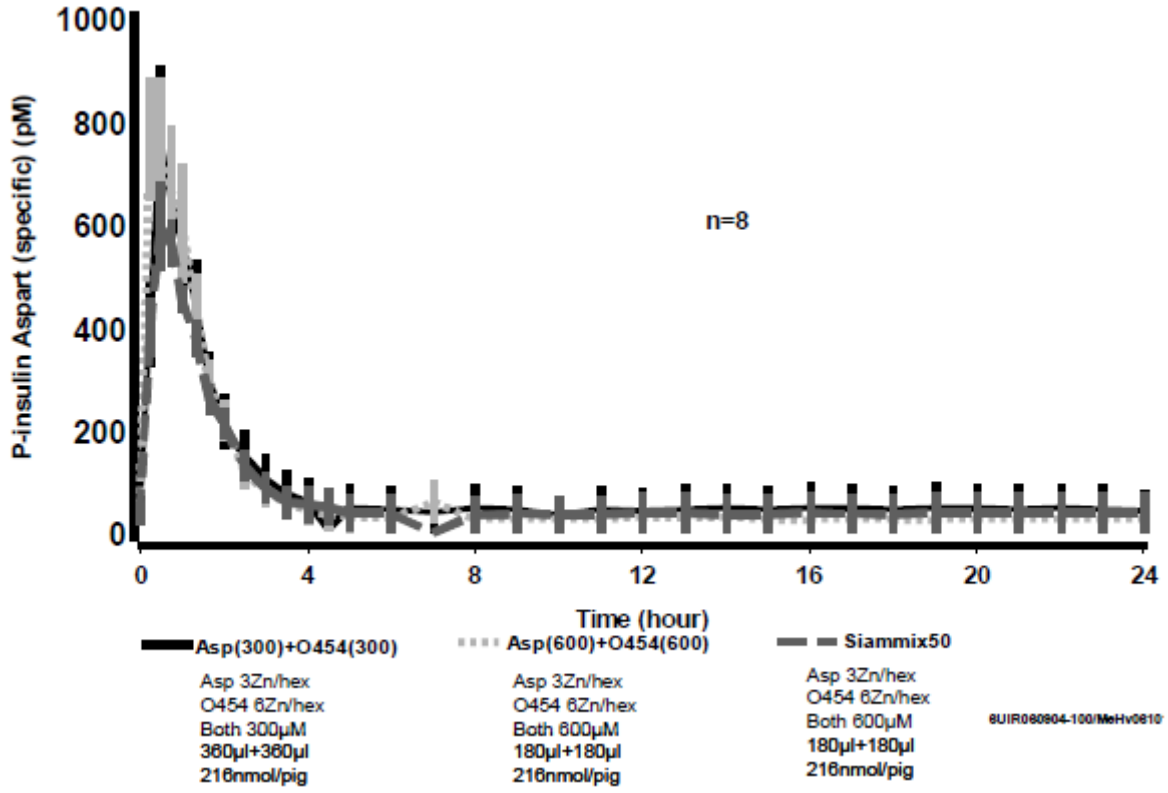
Treatment	Glucose utilization 0-24 h mg/kg	Glucose clamp level mM	Plasma insulin Aspart AUC 0-8 h pM h	Plasma insulin 454 AUC 0-8 h pM h	Plasma insulin 454 AUC 0-24 h pM h
Asp 300 454 300	7245 ± 1107	4.52 ± 0.12	1438 ± 1297	6696 ± 1160	10837 ± 719
Asp 600 454 600	7103 ± 2227	4.47 ± 0.08	1465 ± 775	6053 ± 1727	10347 ± 1139
SIAM combination 50:50	6740 ± 1520	4.51 ± 0.07	1233 ± 860	6132 ± 720	10328 ± 1249

Figure 2 Study No UIR060904-0100 Feb-2007 - Glucose Infusion Rats and Plasma Insulin Aspart or Insulin 454 Profiles after sc Administration of Aspart/Insulin 454 Co-formulation

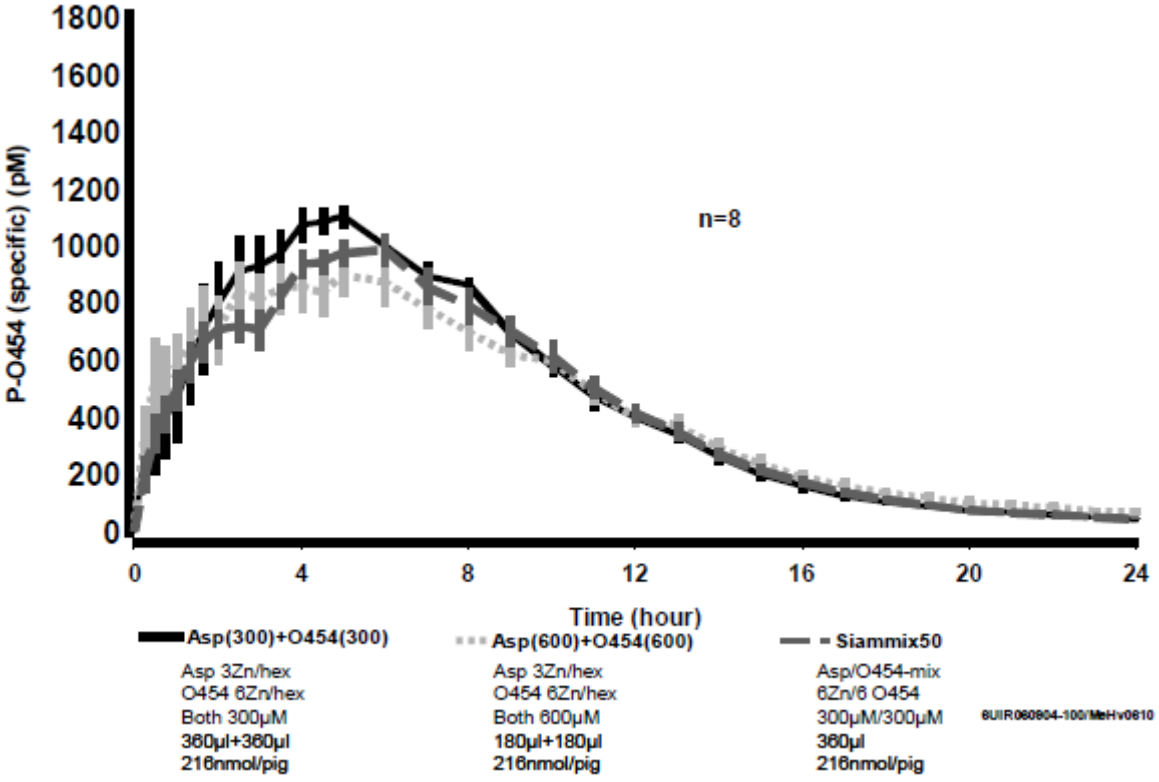
Glucose infusion rates 0-24 h after sc administration of Aspart/454 preparations

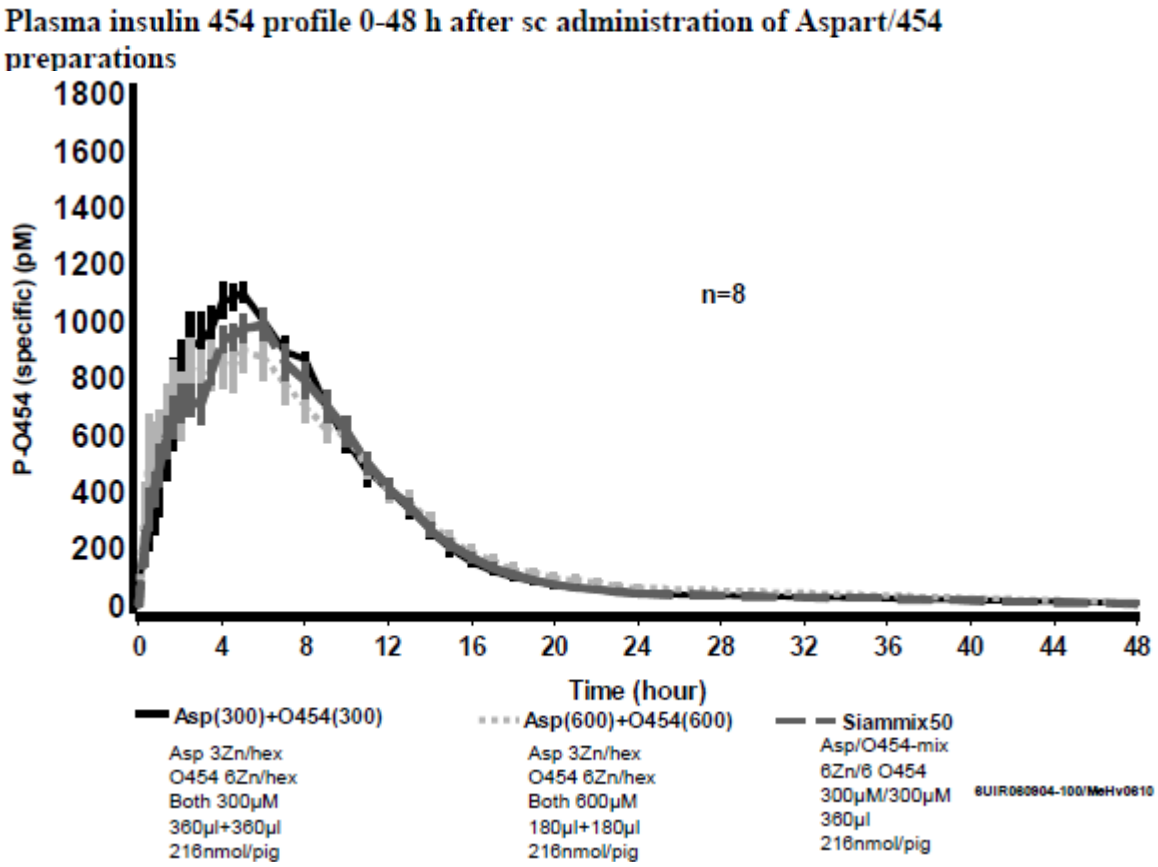


Plasma insulin aspart profiles after sc administration of Aspart/454 preparations



Plasma insulin 454 profile 0-24 h after sc administration of Aspart/454 preparations





4.2 Secondary Pharmacology

See NDA 203314 for IDeg.

4.3 Safety Pharmacology

See NDA 203314 for IDeg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

See NDA 203314 for IDeg.

Note: 4 PK studies for IDegAsp in pigs were described here in details.

Absorption

Study No 204383: PK in pigs (I)

Pharmacokinetics of insulin aspart and insulin 454 after sc administration in pigs of mixtures of the two insulin analogues (I) [by Novo Nordisk]

Study design: This non-GLP study was to evaluate the PK of insulin aspart (600 µM) with insulin 454 (857 µM), in proportions of 30% insulin aspart compared to insulin aspart alone. This study also evaluated the effect of increasing pH and determined the PK of insulin 454 in presence of insulin at increasing pH and alone at 3 and 4 Zn per 6 insulin 454. There were 5 preparations administered to 8 pigs in a cross over design. The insulin aspart was 0.9 nmol/kg. The administration was performed with a NovoPen® 3.0 with a 28G needle mounted with a stopper allowing the needle to be introduced 5 mm. Blood was sampled at 0, 20, 40, 60 min, and 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hrs following dosing.

The following batches of insulin aspart mixed with insulin 454 were produced at Preformulation and Delivery in Diabetes Protein Engineering.

One letter code	Code Analogues Batch no	Conc. of IAsp and I454 (µM)	Zn/6ins	Isotonic	Phe/Cre	Tris	Dose (nmol/kg)
A	**DQ** aspart + 454 14794/012DQ	180 +600	3	Glycerol 1.6% NaCl 10 mM	16 mM	7 mM pH 7.5	0.9 + 3.0 nmol
B	**DR** aspart + 454 1479/012DR	180 + 600	3	Glycerol 1.6 % NaCl 10 mM	16 mM	7 mM pH 7.9	0.9 + 3.0 nmol
C	**DS** 454 14794/012DS	600 O454	3	Glycerol 1.6 % NaCl 10 mM	16 mM	7 mM pH 7.5	0 + 3.0 nmol
D	**DT** 454 14794/012DT	600 O454	4	Glycerol 1.6% NaCl 10 mM	16 mM	7 mM pH 7.5	0 + 3.0 nmol
E	**DU** Aspart 14794/012DU	600 IAsp	3	Glycerol 1.6% NaCl 10 mM	16 mM	7 mM pH 7.5	0.9 + 0 nmol

Tris = (trishydroxymethylaminomethan)

Eight female LYD pigs were included in the study.

Pharmacokinetic study No.	Group No 1	Group No 2	Group No 3	Group No 4	Group No 5
	Animals 1 + 6	Animals 2 + 7	Animals 3 + 8	Animal 4	Animal 5
1	A	B	C	D	E
2	B	C	D	E	A
3	C	D	E	A	B
4	D	E	A	B	C
5	E	A	B	C	E

Findings:

- The PK parameters of insulin aspart showed no statistically significant difference as to t_{max}, C_{max}, plasma half-life, AUC, V/F, CL/F, or MRT when administered from formulation A (insulin aspart co-formulated with insulin 454 containing 3 Zn/6 insulin 454 at pH 7.5), from formulation B (same with formulation A except at pH 7.9), or formulation E (reference containing 3 Zn/insulin aspart at pH 7.5).
- The PK parameters of insulin 454 following sc administration to pigs of 3.0 nmol/kg insulin 454 showed 1) sc absorption and elimination from plasma, and 2) no significant difference as to t_{max}, C_{max}, plasma half-life, AUC, V/F, CL/F, or MRT when administered from formulation A (insulin aspart co-formulated with insulin 454 containing 3 Zn/6 insulin at pH 7.5) when compared to formulation C (insulin 454 alone containing 3 Zn/6 insulin at pH 7.5).
- The data showed that absorption and elimination from plasma of insulin aspart seemed unaffected by presence of insulin 454, of the Zn level being 3 or 4 Zn/6 insulin 454, or the pH being 7.5 or 7.9. Similarly, absorption and elimination from plasma of insulin 454 seemed unaffected by presence of insulin aspart, of the Zn level being 3 or 4 Zn/6 insulin 454, or the pH being 7.5 or 7.9

Table 3 Study No 204383 - PK Profiles of Insulin Aspart and Insulin 454 after SC Administration

Summary of pharmacokinetics of insulin aspart in pigs after SC dose of 0.9 nmol/kg of preparations A, B and E

Formulation	Pig_No.	t_{max} (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUCINF (hr*pmol/L)	AUCExtrap (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
A	Mean		677	1.2409		850	5	1.0148	1.1948	1.2
	SD		206	0.3334		352	2	0.3804	0.4000	0.2
	Min	0.3	384	0.8906		501	1	0.4029	0.6056	1.0
	Median	0.3	688	1.1234		722	5	1.1801	1.2460	1.2
	Max	0.7	961	1.8808		1486	8	1.3991	1.7966	1.5
	Harmonic Mean				0.56					
B	Mean		880	1.1612		900	8	1.6013	1.2721	1.4
	SD		700	0.4508		534	11	1.9457	0.5692	0.6
	Min	0.3	184	0.3569		424	1	0.4218	0.4996	1.0
	Median	0.7	614	1.1237		619	5	0.9065	1.4550	1.2
	Max	1.0	2075	1.7218		1801	33	5.9498	2.1235	2.7
	Harmonic Mean				0.60					
E	Mean		698	1.6346		854	4	0.8740	1.2891	1.1
	SD		492	0.4436		541	3	0.4130	0.4712	0.2
	Min	0.3	317	1.1010		518	1	0.2029	0.4651	0.9
	Median	0.7	522	1.6524		672	3	0.9960	1.3640	1.2
	Max	1.5	1681	2.2924		1935	10	1.3754	1.7377	1.3
	Harmonic Mean				0.42					

Summary of pharmacokinetics of insulin 454 in pigs after SC dose of 3.0 nmol/kg of preparations A, B, C and D

Formulation	Pig_No.	t_{max} (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUCINF (hr*pmol/L)	AUCExtrap (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
A	Mean		3648	0.1398		34995	8	0.6572	0.0869	8.6
	SD		1120	0.0322		4489	7	0.1960	0.0107	2.1
	Min	1.0	2815	0.0894		29955	1	0.3800	0.0710	6.0
	Median	2.5	3192	0.1417		33228	5	0.6437	0.0903	8.5
	Max	6.0	5964	0.1867		42279	21	1.0291	0.1002	12.4
	Harmonic Mean				5.0					
B	Mean		3972	0.1403		33994	4	0.6380	0.0888	8.0
	SD		523	0.0146		3003	1	0.0798	0.0074	0.7
	Min	1.0	3251	0.1222		30433	3	0.5478	0.0754	6.9
	Median	2.0	4107	0.1378		33007	5	0.6279	0.0909	8.5
	Max	6.0	4499	0.1659		39765	6	0.7464	0.0986	8.6
	Harmonic Mean				4.9					
C	Mean		4380	0.1453		36280	5	0.6501	0.0863	8.2
	SD		1845	0.0368		8155	3	0.2927	0.0196	1.8
	Min	1.0	2557	0.1023		24441	1	0.3377	0.0591	5.5
	Median	2.0	4767	0.1454		35197	4	0.6254	0.0852	7.9
	Max	5.0	7554	0.2055		50750	10	1.1995	0.1227	10.8
	Harmonic Mean				4.8					
D	Mean		3674	0.1287		34977	6	0.6863	0.0874	8.7
	SD		701	0.0097		5345	1	0.1365	0.0128	0.6
	Min	1.0	2610	0.1168		29455	4	0.5266	0.0706	7.8
	Median	2.5	3845	0.1284		31934	5	0.6937	0.0939	8.6
	Max	4.0	4476	0.1410		42518	8	0.8704	0.1018	9.5
	Harmonic Mean				5.4					

- When compared the PK profiles for two insulin analogues, insulin aspart and insulin 454, the absorption and elimination of insulin aspart took place within the 1st 4 hrs following sc administration, while insulin 454 was circulating still 24 hrs following administration.
- These two insulins distributed differently. Insulin aspart had a smaller fraction appearing in blood plasma (i.e. moderated Vd and high CL) and relatively low

plasma concentration (698 pM ± 492 pM). Insulin 454 was present in plasma (i.e. smaller Vd and low CL) and high plasma concentration values (3674 pM ± 701 pM), which complied with the relatively high albumin binding of insulin 454.

Study No 205053: PK in pigs (II)

Pharmacokinetics of insulin aspart and insulin 454 after sc administration in pigs of mixtures of the two insulin analogues (II) [by Novo Nordisk]

Study design: This GLP study was to evaluate the PK of insulin aspart and insulin 454 when co-administered as a mixture (0.9 nmol/kg insulin aspart and 3.0 nmol/kg insulin 454) at pH 7.5 and pH 7.9. This study also evaluated a mixture of 0.9 nmol/kg insulin aspart and 1.5 nmol/kg insulin 454 at 3 and 4 Zn per 6 insulin 454, compared to insulin aspart alone at 3 Zn per 6 insulin 454 after sc dosing in pigs. There were 5 preparations administered to 8 pigs in a cross over design. The insulin aspart was 0.9 nmol/kg. The administration was performed with a NovoPen® 3.0 with a 28G needle mounted with a stopper allowing the needle to be introduced 5 mm. Blood was sampled at 0, 20, 40, 60 min, and 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hrs following dosing.

The following batches of insulin aspart mixed with insulin 454 were produced at Preformulation & Delivery in Diabetes Protein Engineering.

One letter code	Code Analogues Batch no	Conc. of IAsp and 0454 (μM)	Zn / 6ins	Isotonic	Phe/Cre	Tris/pH	Dose (nmol/kg
I	14794-054-**I**, aspart + 0454 0100-0000-0454-**4F**	180+600	4	Glycerol 1.6% NaCl 10 mM	16 mM	7 mM pH7.5	0.9 + 3.0 nmol
II	14794-054-**II**, aspart + 0454 0100-0000-0454-**4G**	180+600	4	Glycerol 1.6 % NaCl 10 mM	16 mM	7 mM pH 7.9	0.9 + 3.0 nmol
III	14794-054-**III**, aspart + 0454 0100-0000-0454-**4H**	300+500	3	Glycerol 1.6 % NaCl 10 mM	16 mM	7 mM pH 7.5	0.9 + 1.5 nmol
IV	14794-054-**IV**, aspart + 0454 0100-0000-0454-**4I**	300+500	4	Glycerol 1.6% NaCl 10 mM	16 mM	7 mM pH 7.5	0.9 + 1.5 nmol
V	14794-054-V, aspart 0121-0000-0014-**3B**	600	3	Glycerol 1.6% NaCl 10 mM	16 mM	Phosphate 7 mM pH 7.5	0.9 + 0 nmol

Tris = (trishydroxymethylaminomethan)

Eight female LYD pigs were included in the study.

Pharmacokinetic study No.	Group No 1	Group No 2	Group No 3	Group No 4	Group No 5
	Animals 1 + 6	Animals 2 + 7	Animals 3 + 8	Animal 4	Animal 5
1	I	II	III	IV	V
2	II	III	IV	V	I
3	III	IV	V	I	II
4	IV	V	I	II	III
5	V	I	II	III	IV

Findings:

- The PK parameters of insulin aspart showed no statistically significant difference as to t_{max}, C_{max}, plasma half-life, AUC, V/F, CL/F, or MRT when administered from formulations I-IV.
- The absorption and elimination from plasma of insulin aspart seemed unaffected by presence of insulin 454 when co-formulated (180 µM + 600 µM) in TRIS buffer containing 4 Zn/6 insulin at pH 7.5 and pH 7.9 respectively, and co-formulated (300 µM + 500 µM) in TRIS buffer with 3 and 4 Zn/6 insulin respectively at pH 7.5, in comparison with insulin aspart from conventional 3 Zn, glycerol phosphate buffered formulation.
- The PK parameters of insulin 454 showed that formulations I and II had a non-significant change at 4 Zn per 6 insulins from pH 7.5 to 7.9 (more "flat" plasma concentration time profile, with lower C_{max} and slightly longer half life). The administration of insulin 454 (1/2 the amount compared to that from formulations I/II) in presence of increased amounts of insulin aspart at pH 7.5 from formulations III/IV, likely resulted in more flat and protracted plasma concentration time profiles. The AUC following administration of insulin 454 at 1.5 nmol/kg were proportionally in agreement with those being obtained following administration of 3.0 nmol/kg.

Table 4 Study No 205053 - PK Profiles of Insulin Aspart and Insulin 454 after SC Administration

Summary of pharmacokinetics of insulin aspart in pigs after SC dose of 0.9 nmol/kg of preparations I, II, III, IV and V

Formulation	Pig_No.	t_{max} (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUC (hr*pmol/L)	AUCExtr (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
I	Mean		590	1.2294		719	4	1.0828	1.2808	1.2
0.9	SD		125	0.2889		120	1	0.2666	0.2053	0.2
nmol/kg	Min	0.5	429	0.8143	0.4	553	2	0.7925	0.9505	0.9
	Median	0.5	591	1.2209	0.6	704	4	1.0099	1.2809	1.3
	Max	1.0	786	1.7468	0.9	947	7	1.5115	1.6281	1.5
	Harmonic Mean			1.1711	0.6					
II	Mean		561	1.1737		653	5	1.2515	1.4179	1.2
0.9	SD		130	0.2946		124	2	0.2586	0.2393	0.3
nmol/kg	Min	0.5	410	0.7411	0.5	540	3	0.8112	1.0096	1.0
	Median	0.5	560	1.0634	0.7	603	5	1.3023	1.4953	1.2
	Max	1.0	763	1.5263	0.9	891	8	1.5602	1.6655	1.9
	Harmonic Mean			1.1078	0.6					
III	Mean		547	1.3180		632	5	1.1873	1.5517	1.2
0.9	SD		149	0.3033		161	3	0.3248	0.5953	0.2
nmol/kg	Min	0.5	267	0.9620	0.4	305	2	0.7188	1.0847	1.0
	Median	0.5	587	1.2614	0.6	647	4	1.1837	1.3910	1.1
	Max	1.5	710	1.8718	0.7	830	11	1.6367	2.9552	1.4
	Harmonic Mean			1.2625	0.5					
IV	Mean		628	1.5271		699	6	0.9040	1.2974	1.2
0.9	SD		186	0.3476		64	5	0.2925	0.1222	0.2
nmol/kg	Min	0.5	393	1.0383	0.3	590	2	0.6045	1.1472	0.9
	Median	0.5	574	1.5485	0.4	692	4	0.8237	1.3009	1.1
	Max	1.0	911	1.9826	0.7	785	18	1.4691	1.5253	1.5
	Harmonic Mean	0.5	585	1.4523	0.5	694	4	0.8354	1.2877	1.1
V	Mean		777	1.2190		799	5	1.0569	1.1954	1.2
0.9	SD		501	0.3244		224	3	0.3899	0.2945	0.3
nmol/kg	Min	0.5	327	0.8448	0.4	544	2	0.3748	0.7138	0.8
	Median	0.5	620	1.1313	0.6	780	4	0.9846	1.1544	1.2
	Max	0.5	1944	1.9043	0.8	1261	10	1.6634	1.6531	1.6
	Harmonic Mean				0.6					

Summary of pharmacokinetics of insulin 454 in pigs after SC dose of 3.0 nmol/kg of preparations I and II and 1.5 nmol/kg from preparation III and IV

Formulation	Pig No.	t_{max} (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUCINF (hr*pmol/L)	AUCExtrap (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
I	Mean		3082	0.0954		36856	12	0.9159	0.0828	11.6
3.0	SD		414	0.0181		4862	5	0.3145	0.0119	1.8
nmol/kg	Min	0.5	2580	0.0657	6.0	28291	8	0.5927	0.0680	9.6
	Median	3.8	2940	0.1020	6.8	37633	10	0.7772	0.0797	11.2
	Max	6.0	3744	0.1147	10.5	44135	20	1.5282	0.1060	14.9
	Harmonic Mean				7.3					
II	Mean		2518	0.0793		32217	21	1.2494	0.0949	13.9
3.0	SD		796	0.0178		4643	11	0.3060	0.0144	4.7
nmol/kg	Min	0.5	1264	0.0422	7.4	25109	10	0.9058	0.0788	10.5
	Median	1.0	2680	0.0848	8.2	31959	15	1.2024	0.0939	12.1
	Max	8.0	3380	0.0942	16.4	38069	38	1.8824	0.1195	25.0
	Harmonic Mean				8.7					
III	Mean		2085	0.1211		19216	12	0.6776	0.0801	8.9
1.5	SD		589	0.0212		3231	11	0.1524	0.0138	2.0
nmol/kg	Min	0.5	1240	0.0810	4.8	14671	3	0.4454	0.0634	7.3
	Median	1.5	1954	0.1265	5.5	18987	5	0.6686	0.0791	8.0
	Max	4.0	3032	0.1445	8.6	23657	29	0.9380	0.1022	13.2
	Harmonic Mean				5.7					
IV	Mean		1523	0.1060		18478	13	0.8455	0.0870	10.6
1.5	SD		383	0.0181		5165	8	0.2744	0.0243	1.9
nmol/kg	Min	0.5	1060	0.0784	5.4	12104	5	0.5037	0.0586	8.0
	Median	1.0	1423	0.1090	6.4	17381	10	0.9053	0.0878	10.3
	Max	8.0	2057	0.1284	8.8	25598	31	1.2664	0.1239	13.8
	Harmonic Mean				6.5					

Study No 205220: PK in pig (III)

Pharmacokinetics of insulin aspart and insulin 454 after sc administration in pigs of mixtures of the two insulin analogues (III) [by Novo Nordisk]

Study design: This GLP study was to evaluate the PK of insulin aspart and insulin 454 after sc administration of a mixture of 0.9 nmol/kg and 3.0 nmol/kg respectively, at pH 7.5 and pH 7.9. This study also evaluated a mixture of 0.9 nmol/kg insulin aspart and 1.5 nmol/kg insulin 454 at 3 and 4 Zn per 6 insulin 454, compared to insulin aspart alone at 3 Zn per 6 insulin 454 after sc dosing in pigs. There were 6 preparations administered to 8 pigs in a cross over design. The insulin aspart was 0.9 nmol/kg where as the insulin 454 was 3.0 nmol/kg or 1.5 nmol/kg. The administration was performed with a NovoPen® 3.0 with a 28G needle mounted with a stopper allowing the needle to be introduced 5 mm. Blood was sampled at 0, 20, 40, 60 min, and 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 30 and 48 hrs following dosing.

The following batches of insulin aspart mixed with insulin 454 were produced at Preformulation and Delivery in Diabetes Protein Engineering.

Preparation	Code Analogues Batch no	Conc (IAsp + I 454)	Zn/6ins	Isotonic Agent	Phe/Cre	Phosphate	Inj.vol	Dose (nmol/kg)
I	14794-112-**I** aspart + 454-**4S**	180+600 µM	3+5.1	Glycerol 1.6% NaCl 10mM	16 mM	7 mM pH7.5	300 µl/60kg	0.9 + 3.0 nmol
II	14794-112-**II** aspart + 454-**4T**	180+600 µM	3+5.1	Glycerol 1.6% NaCl 10mM	16 mM	7 mM pH 7.9	300 µl/60kg	0.9 + 3.0 nmol
III	14794-112-**III** aspart + 454-**4U**	180+600 µM	3+5.1	Glycerol 1.6% NaCl 10mM	16 mM	TRIS 7 mM pH 7.9	300 µl/60kg	0.9 + 3.0 nmol
IV	14794-112-**IV** aspart + 454-**4V**	300+500 µM	3+4.2	Glycerol 1.6% NaCl 10mM	16 mM	7 mM pH 7.5	180 µl/60kg	0.9 + 1.5 nmol
V	14794-112-V Aspart	600 µM (IAsp)	3	Glycerol 1.6% NaCl 10mM	16 mM	7 mM pH 7.5	90 µl/60kg	0.9 + 0 nmol
VI	in same pig: 14794-112-**VI** 04544**X**	600 µM (I 454)	6				300 µl/60kg	0 + 3.0 nmol

Eight female LYD pigs were included in the study. At start of acclimatisation period the body weight of the pigs was approximately 55-66 kg.

Pharmacokinetic study No.	Group No 1	Group No 2	Group No 3	Group No 4	Group No 5
	Animals 1 + 6	Animals 2 + 7	Animals 3 + 8	Animal 4	Animal 5
1*	I	II	III	IV	V and VI
2	II	III	IV	V and VI	I
3	III	IV	V and VI	I	II
4	IV	V and VI	I	II	III
5	V and VI	I	II	III	IV
6	I	II	III	IV	V and VI

*The first study was repeated as no. 6, as the body weight of the pigs had not been correct for dosing

Findings:

- The PK of insulin aspart was marginally affected by the difference in pH in formulation I (pH 7.5) and formulation II (pH 7.9). The values of AUC and CL/f were marginally affected, with higher AUC or lower CL/f with increasing pH.
- In contrast, the PK of insulin 454 was not affected by the difference in pH in formulations I and II.
- The PK of insulin aspart and insulin 454 was not affected by difference in buffer, observed when compared formulation II (phosphate buffer) and formulation III (TRIS buffer)
- The administration of insulin 454 (formulation IV with half the dose administration from half the concentration compared to that of formulation I) did have an effect on the PK of insulin aspart, resulting in non-significant lower plasma levels (significant lower apparent Vd) for insulin aspart. However, plasma C_{max} and half life were not significantly affected.
- The PK of insulin apart was not affected by the presence of insulin 454, comparing formulations I and V).
- The PK of insulin 454 was not affected by the presence of insulin aspart, comparing formulation I and VI (except T_{max}).

Table 5 Study No 205220 - PK Profiles of Insulin Aspart and Insulin 454 after SC Administration

Summary of pharmacokinetics of insulin aspart in pigs after SC dose of 0.9 nmol/kg from formulations I, II, III, IV and V

Formulation		t_{max} (h)	C_{max} (pmol/l)	λ_{el} (h^{-1})	$t_{½}$ (h)	AUC (h·pmol/l)	AUCExtrap (%)	V_z/f (l/kg)	CL/f (l/h/kg)	MRT (h)
I	Mean	0.5	415	1.2947	0.6	498	7	1.513	1.878	1.1
0.9 nmol/kg	SD	0.2	101	0.4082	0.2	95.4	5	0.298	0.431	0.3
	Min	0.3	287	0.7629	0.3	324	2	1.019	1.449	0.9
	Median	0.3	393	1.2195	0.6	512	5	1.498	1.761	1.1
	Max	0.7	604	1.9911	0.9	621	19	1.908	2.781	1.7
	Harmonic Mean	0.4	396	1.1824	0.5	479	5	1.459	1.808	1.1
II	Mean	0.6	620	1.2431	0.6	623	5	1.300	1.475	1.1
0.9 nmol/kg	SD	0.4	249	0.4200	0.2	94.9	2	0.430	0.230	0.2
	Min	0.3	344	0.7118	0.4	479	2	0.829	1.161	0.7
	Median	0.5	523	1.2136	0.6	624	5	1.300	1.449	1.1
	Max	1.5	1040	1.8614	1.0	775	10	1.908	1.880	1.4
	Harmonic Mean	0.5	549	1.1190	0.6	610	4	1.175	1.444	1.1
III	Mean	0.5	534	1.0763	0.7	670	6	1.405	1.453	1.3
0.9 nmol/kg	SD	0.3	132	0.2834	0.3	208	2	0.379	0.416	0.4
	Min	0.3	373	0.4774	0.5	467	3	0.799	0.935	1.0
	Median	0.3	548	1.1696	0.6	640	5	1.389	1.406	1.2
	Max	1.0	773	1.3557	1.5	963	9	2.016	1.929	2.1
	Harmonic Mean	0.4	508	0.9675	0.6	619	5	1.307	1.344	1.2
IV	Mean	0.5	507	1.5896	0.4	502	5	1.176	1.854	1.0
0.9 nmol/kg	SD	0.3	117	0.2021	0.1	90.5	2	0.247	0.408	0.1
	Min	0.3	366	1.1463	0.4	326	2	0.877	1.462	0.8
	Median	0.3	488	1.6570	0.4	511	4	1.137	1.765	0.9
	Max	1.0	668	1.7471	0.6	616	9	1.604	2.759	1.2
	Harmonic Mean	0.4	484	1.5619	0.4	485	4	1.134	1.791	0.9
V	Mean	0.4	485	1.6544	0.4	491	6	1.247	1.919	1.0
0.9 nmol/kg	SD	0.2	176	0.4159	0.1	111	4	0.482	0.448	0.2
	Min	0.3	300	0.9752	0.3	334	3	0.672	1.330	0.8
	Median	0.3	495	1.6324	0.4	488	3	1.093	1.845	1.0
	Max	0.7	785	2.2534	0.7	677	15	1.946	2.693	1.4
	Harmonic Mean	0.4	431	1.5528	0.4	469	4	1.095	1.832	0.9

Summary of pharmacokinetics of insulin 454 in pigs after SC dose of 3.0 nmol/kg from formulations I, II, III, and VI and 1.5 nmol/kg of formulation IV

Formulation		t_{max} (h)	C_{max} (pmol/l)	λ_z (h^{-1})	$t_{½}$ (h)	AUC (h·pmol/l)	AUCExtrap (%)	V_z/f (l/kg)	CL/f (l/h/kg)	MRT (h)
I	Mean	3.2	1947	0.1204	6.0	21240	1	1.3089	0.1563	8.8
3.0 nmol/kg	SD	1.1	624.5	0.0298	1.3	5642.1	1	0.4174	0.0676	1.9
	Min	0.7	1078	0.0872	3.9	9453.9	0	0.7302	0.1123	6.0
	Median	3.5	1888	0.1124	6.2	22218	1	1.2399	0.1351	8.7
	Max	4.0	3094	0.1797	8.0	26721	2	2.1298	0.3173	12.4
	Harmonic Mean	2.3	1781	0.1150	5.8	19197	1	1.1999	0.1412	8.5
II	Mean	1.6	2462	0.1239	5.9	25471	1	1.0189	0.1209	9.4
3.0 nmol/kg	SD	1.5	432.7	0.0338	1.1	4596.7	0	0.2500	0.0201	1.7
	Min	0.3	1951	0.1032	3.4	20598	0	0.6284	0.0899	7.1
	Median	0.8	2422	0.1084	6.4	23777	1	1.0623	0.1262	9.0
	Max	4.0	3010	0.2013	6.7	33363	1	1.2878	0.1456	12.1
	Harmonic Mean	0.6	2396	0.1182	5.6	24806	1	0.9573	0.1178	9.1
III	Mean	2.7	1969	0.1041	6.8	24844	1	1.2047	0.1227	10.8
3.0 nmol/kg	SD	2.7	495.7	0.0138	0.9	3357.8	1	0.2715	0.0168	1.9
	Min	0.3	1462	0.0848	5.5	20230	0	0.8037	0.1021	7.5
	Median	2.0	1886	0.1060	6.5	24523	1	1.1848	0.1223	10.7
	Max	8.0	2813	0.1270	8.2	29393	2	1.6535	0.1483	13.7
	Harmonic Mean	1.0	1874	0.1025	6.7	24452	1	1.1511	0.1208	10.5
IV	Mean	2.1	1273	0.1349	5.6	10551	2	1.2212	0.1500	8.7
1.5 nmol/kg	SD	1.6	258.0	0.0364	1.9	2674.4	2	0.6445	0.0360	2.2
	Min	0.3	1002	0.0737	4.2	7326.3	1	0.7802	0.0988	6.9
	Median	2.0	1240	0.1570	4.4	9815.8	1	1.0742	0.1546	8.4
	Max	4.0	1688	0.1643	9.4	15182	5	2.7789	0.2047	13.5
	Harmonic Mean	0.9	1230	0.1242	5.1	10000	1	1.0645	0.1422	8.3
VI	Mean	1.9	2251	0.1178	6.2	25822	1	1.0726	0.1228	9.2
3.0 nmol/kg	SD	1.0	446.4	0.0327	1.2	6412.1	0	0.3073	0.0325	2.3
	Min	1.0	1425	0.0953	3.8	17075	0	0.7476	0.0887	6.4
	Median	1.8	2404	0.1079	6.4	25233	1	0.9523	0.1202	9.3
	Max	3.0	2637	0.1836	7.3	33821	1	1.6079	0.1757	13.1
	Harmonic Mean	1.5	2155	0.1125	5.9	24435	1	1.0103	0.1162	8.8

Study No 205419: PK in pig (IV)

Pharmacokinetics of insulin aspart and insulin 454 after sc administration in pigs of high and low mixtures (with 3 and 6 Zn per 6-insulins) of the two insulin analogues (III) [by Novo Nordisk]

Study design: This GLP study was to evaluate the PK of insulin aspart and insulin 454 after sc administration of a mixture of 0.9 nmol/kg and 7.3 nmol/kg, with 3.4 Zn and 6 Zn

per 6 insulin 454, respectively. This study also evaluated a mixture of 0.9 nmol/kg insulin aspart and 3.0 nmol/kg insulin 454 with 3.9 Zn and 6 Zn per 6 insulin 454, respectively. Two reference formulations were also given. There were 6 preparations administered to 8 pigs in a cross over design. The insulin aspart was 0.9 nmol/kg where as the insulin 454 was 7.3 nmol/kg or 3.0 nmol/kg. The administration was performed with a NovoPen® 3.0 with a 31G needle mounted with a stopper allowing the needle to be introduced 5 mm. Blood was sampled at 0, 15, 30, 45, 60 min, and 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 30 and 48 hrs following dosing.

The following batches of insulin aspart mixed with insulin 454 were produced at Preformulation and Delivery in Diabetes Protein Engineering.

Formulation	Conc µM	Isotonic	Phe/Cre	Zn/6ins 454	Inj.vol	Dose/kg
I Insulin aspart + Insulin 454	90+729	Glycerol 1.6% NaCl 10 mM	16 mM	3.4	600 µl/60kg	0.9 + 7.29 nmol
II Insulin aspart + Insulin 454	90+729	Glycerol 1.6 % NaCl 10 mM	16 mM	6	600 µl/60kg	0.9 + 7.29 nmol
III Insulin aspart + Insulin 454	180+600	Glycerol 1.6% NaCl 10 mM	16 mM	3.90	300 µl/60kg	0.9 + 3.0 nmol
IV Insulin aspart + Insulin 454	180+600	Glycerol 1.6 % NaCl 10 mM	16 mM	6	300 µl/60kg	0.9 + 3.0 nmol
V Insulin 454	857	Glycerol 1.6 % NaCl 10 mM	16 mM	6	510 µl/60kg	7.29 nmol
VI Insulin aspart	600	Glycerol 1.6% NaCl 10 mM	16 mM	3	90 µl/60kg	0.9 nmol

Eight female LYD pigs were included in the study. At start of acclimatisation period the body weight of the pigs was 55-66 kg.

Pharmacokinetic study No.	Group No 1	Group No 2	Group No 3	Group No 4	Group No 5
	Animals 1 + 6	Animals 2 + 7	Animals 3 + 8	Animal 4	Animal 5
1	I	II	III	IV	V and VI
2	II	III	IV	V and VI	I
3	III	IV	V and VI	I	II
4	IV	V and VI	I	II	III
5	V and VI	I	II	III	IV

Findings:

- The PK of insulin aspart (independent of Zn or high mix or low mix insulin aspart/insulin 454 ratio from insulin 454) was not affected following administration of mixtures of insulin aspart/insulin 454 to pigs.
- The PK of insulin 454 (independent of Zn or high mix or low mix insulin aspart/insulin 454 ratios from insulin aspart) was only marginally affected following administration of mixtures of insulin aspart and insulin 454 to pigs.
- Mean residence time from insulin 454 delivered from reference was marginally longer (11.5±1.7 vs. 10±1.2 hr in comparison with insulin 454) when delivered from the low mix formulation with 6 Zn per 6 insulin.

Table 6 Study No 205419 - PK Profiles of Insulin Aspart and Insulin 454 after SC Administration

Summary of pharmacokinetics of insulin aspart in pigs after SC dose of 0.9 nmol/kg of formulations I, II, III, IV and VI

Formulation		t_{max} (h)	C_{max} (pmol/l)	λ_z (h^{-1})	$t_{½}$ (h)	AUC (h·pmol/l)	AUCExtrap (%)	V_z/f (l/kg)	CL/f (l/h/kg)	MRT (h)
I	Mean	0.9	331	0.6746	1.5	576	11	3.327	1.913	2.6
0.9 nmol/kg	SD	0.3	324	0.4292	1.0	287	5	1.526	0.872	1.3
	Min	0.5	170	0.2185	0.6	322	6	2.278	0.896	1.3
	Median	0.9	211	0.5398	1.4	509	10	2.745	1.929	2.5
	Max	1.5	990	1.2238	3.2	1000	17	6.277	2.798	4.5
	Harmonic Mean	0.8	227	0.4607	1.0	470	9	2.940	1.562	2.1
II	Mean	0.7	274	0.7481	1.0	446	12	3.028	2.198	1.7
0.9 nmol/kg	SD	0.2	147	0.1322	0.2	132	3	1.105	0.741	0.3
	Min	0.5	162	0.5578	0.8	247	6	1.550	1.403	1.5
	Median	0.8	226	0.7683	0.9	400	14	2.819	2.248	1.7
	Max	1.0	596	0.9057	1.2	641	15	4.735	3.638	2.2
	Harmonic Mean	0.6	235	0.7260	0.9	409	11	2.676	2.019	1.7
III	Mean	0.6	424	0.9128	0.8	538	8	2.463	2.160	1.4
0.9 nmol/kg	SD	0.4	256	0.3166	0.3	225	4	1.537	1.529	0.4
	Min	0.3	131	0.6031	0.5	164	4	1.137	1.030	1.0
	Median	0.5	356	0.9029	0.8	569	8	1.753	1.582	1.5
	Max	1.0	869	1.5084	1.1	874	14	5.178	5.480	1.9
	Harmonic Mean	0.4	305	0.8325	0.8	417	7	1.904	1.673	1.4
IV	Mean	0.6	295	0.9912	0.8	435	8	2.405	2.403	1.4
0.9 nmol/kg	SD	0.2	90.8	0.2989	0.3	155	2	0.632	1.152	0.4
	Min	0.3	158	0.5071	0.5	192	6	1.588	1.418	1.0
	Median	0.8	323	0.9820	0.7	487	9	2.568	1.846	1.3
	Max	1.0	397	1.5247	1.4	635	11	3.091	4.697	2.3
	Harmonic Mean	0.5	267	0.9043	0.7	375	8	2.254	2.070	1.3
VI	Mean	0.7	287	0.9519	0.9	349	12	3.951	2.873	1.5
0.9 nmol/kg	SD	0.2	160	0.4159	0.4	131	10	2.955	1.004	0.6
	Min	0.3	127	0.5326	0.5	223	4	1.489	1.651	1.1
	Median	0.8	281	1.0474	0.7	335	6	2.146	2.686	1.2
	Max	0.8	540	1.5191	1.3	545	25	7.326	4.039	2.3
	Harmonic Mean	0.5	226	0.8057	0.7	313	7	2.523	2.580	1.4

Summary of pharmacokinetics of insulin 454 in pigs after SC dose of 7.3 nmol/kg of formulations I, II and V and 3.0 nmol/kg of formulations III and IV

Formulation		t_{max} (h)	C_{max} (pmol/l)	λ_z (h^{-1})	$t_{½}$ (h)	AUC (h·pmol/l)	AUCExtrap (%)	V_z/f (l/kg)	CL/f (l/h/kg)	MRT (h)
I	Mean	1.1	7800	0.0570	12.7	63500	3	2.829	0.147	10.7
7.3 nmol/kg	SD	0.6	2570	0.0120	2.7	24000	2	2.753	0.109	3.1
	Min	0.3	3140	0.0428	9.7	17800	1	1.570	0.072	7.6
	Median	1.3	8390	0.0578	12.0	63200	2	1.793	0.116	10.1
	Max	2.0	10800	0.0713	16.2	101000	7	9.601	0.411	16.6
	Harmonic Mean	0.7	6740	0.0547	12.2	49800	2	2.022	0.115	10.1
II	Mean	0.5	6760	0.0803	8.8	55200	1	1.791	0.141	10.0
7.3 nmol/kg	SD	0.5	1820	0.0109	1.2	14100	0	0.568	0.042	1.2
	Min	0.3	3640	0.0645	7.2	31600	1	1.030	0.094	8.9
	Median	0.3	7160	0.0795	8.7	54400	1	1.821	0.134	9.5
	Max	1.5	8830	0.0964	10.7	78000	2	2.887	0.231	11.9
	Harmonic Mean	0.3	6220	0.0790	8.6	51600	1	1.638	0.132	9.9
III	Mean	2.1	2330	0.0674	11.1	19900	2	2.702	0.167	9.5
3.0 nmol/kg	SD	1.8	1010	0.0242	2.8	5790	1	1.548	0.065	1.3
	Min	0.3	889	0.0476	5.6	9800	1	1.601	0.106	7.4
	Median	1.5	2240	0.0618	11.2	21100	2	2.158	0.142	9.7
	Max	6.0	4450	0.1232	14.6	28300	3	6.377	0.303	11.0
	Harmonic Mean	0.9	1950	0.0622	10.3	18000	2	2.288	0.151	9.4
IV	Mean	2.3	2870	0.0724	10.3	24100	1	2.009	0.132	9.4
3.0 nmol/kg	SD	2.2	975	0.0201	3.2	6030	1	0.866	0.034	1.3
	Min	0.3	1460	0.0435	6.8	15400	1	0.864	0.088	8.0
	Median	1.9	2780	0.0791	8.8	22900	1	2.113	0.132	8.9
	Max	6.0	4310	0.1017	15.9	34100	2	3.464	0.195	11.5
	Harmonic Mean	0.6	2570	0.0671	9.6	22700	1	1.665	0.125	9.2
V	Mean	0.5	5630	0.0848	8.4	58400	2	1.549	0.128	11.5
V	SD	0.5	1750	0.0150	1.7	9710	1	0.401	0.021	1.7
7.3 nmol/kg	Min	0.3	4000	0.0602	6.9	47600	0	1.212	0.102	9.1
	Median	0.3	5110	0.0823	8.4	59800	2	1.487	0.122	11.2
	Max	1.5	8860	0.1006	11.5	71800	4	2.405	0.153	14.7
	Harmonic Mean	0.3	5260	0.0823	8.2	57000	1	1.482	0.125	11.3

5.2 Toxicokinetics

See NDA 203314 for IDeg.

The TK data for IDegAsp is being discussed in repeat-dose toxicity studies (4 wk and 13 wk rat studies) and Seg II study (preliminary rat study).

6 General Toxicology

6.1 Single-Dose Toxicity

See NDA 203314 for IDeg.

6.2 Repeat-Dose Toxicity

See NDA 203314 for IDeg.

Note: 2 studies for IDegAsp were described here in details: 4 wk and 13 wk toxicity studies in rats.

Study No 208289: DRF rat

SIAC 30 (B) and SIAC 45 (B): Dose range finding study by subcutaneous administration to Han Wistar rats for 4 weeks [CRO: (b) (4)]

Study design: This GLP-study was to see if there would be any systemic toxic potential with 2 fixed combinations of insulin aspart and insulin 454 - SIAC 30 (B) (batch no. TLDP010, with 96.4% and 96.5% purity for insulin 454 and insulin aspart respectively) and SIAC 45 (B) (batch no. TLDP011, with 95.7% and 96.6% purity for

insulin 454 and insulin aspart respectively). SIAC 30(B) was provided in (b) (4) (vials) containing 420 nmol/ml insulin 454, whereas SIAC 45(B) was provided in the same vial type where the concentration was 330 nmol/ml insulin 454.

The Wistar rats were given test articles over a period of 5 weeks by sc administration. The control group received the vehicle at the same dose volume of 0.5 mg/kg body weight. For TK analysis, the blood samples were collected from TK animals. During the study, the clinical condition (twice daily), body weight (twice weekly), food consumption (weekly), hematology (end of the 4-week), blood chemistry (end of the 4-week), plasma glucose determination (Days 1 and 29), TK (Days 1 and 29), organ weight (end of the 4-week), macropathology, and limited histopathology were done.

Group	Treatment	Dose concentration (nmol/ml)		Volume dose (ml/kg)
		Insulin 454	Insulin aspart	
1	Control	0	0	0.5
2	SIAC 30 (B)	50	22	0.5
3	SIAC 30 (B)	150	64	0.5
4	SIAC 30 (B)	300	128	0.5
5	SIAC 45 (B)	50	40	0.5
6	SIAC 45 (B)	100	82	0.5
7	SIAC 45 (B)	200	164	0.5

Findings:

- **Mortality/clinical signs:**

For SIAC 30(B), MD and HD males were underactive approx. 1 hr after dosing on Day 2. In addition, there was 1 MD male and 6 HD males had hypoglycaemic shock (prostration, underactivity, and poor righting reflex) on Day 8 or 15, resulting in euthanasia of one of the HD males on Day 8.

For SIAC 45(B), all animals were underactive approx 1 hr after dosing on Day 2. One MD male had hypoglycemia shock on Days 8 and 9, resulted in a premature sacrifice on Day 9.

- **Body weight/food consumption:**

For SIAC 30(B), body weight gains were seen in all treated groups, particularly in males, in a dose-dependent manner. The effect on body weight occurred throughout the treatment period in males but was confined to the latter part of the treatment period in females. Food consumption was high throughout the treatment period for HD animals and in Weeks 1 and 3 for MD males.

For SIAC 45(B), body weight gains were seen in males at all doses, which occurred consistently throughout the treatment period at MD or HD and from Day 8 at LD. Females, however, were not affected. There was no effect upon food consumption.

- **Hematology:**

For SIAC 30(B), slightly lower lymphocyte counts were seen in HD animals and slightly lower neutrophil and eosinophil counts were observed in females in all dose groups, resulting in low total WBC counts in HD animals.

For SIAC 45(B), slightly low lymphocyte counts were seen in HD males and slightly low neutrophil and eosinophil counts were observed in females at all dose, resulting in slight low total WBC counts in HD males and HD females.

- **Clinical chemistry:**

For SIAC 30(B), low triglyceride concentrations were seen in HD males and in females at all doses, in a dose-dependent manner. High phosphorus concentrations were detected at all doses. Low sodium and chloride, as well as α-1 globulin levels were observed in MD and HD females.

For SIAC 45(B), low triglyceride levels were seen in MD or HD males and in females at all doses. High phosphorus levels were seen in all doses. Low α-1 globulin levels were seen in HD females. Reduced total protein concentrations were observed in HD animals, particularly in females.

- **Glucose analysis:**

For SIAC 30(B), reduced plasma glucose levels at all doses and at intervals after dosing on Days 1 and 29. This was dose-dependent in extent and duration of effect, attributed to the pharmacological action of the test article.

- **Organ weight:**

For SIAC 30(B), liver weights were slightly reduced at all doses. Axillary lymph node weights were marginally higher in MD and HD males.

For SIAC 45(B), liver weights were slightly reduced at all doses. Axillary lymph node weights were marginally high in MD or HD males. Spleen weights were low in HD males.

- **Histological findings:**

There were no treatment-related macroscopic or histopathological findings with either SIAC 30 or SIAC 45.

- **Dose formulation analysis:**

Samples were ranged between -12 and +5% of nominal concentrations.

- **TK analysis:**

All satellite animals dosed with SIAC 30 and SIAC 45 were systemically exposed to insulin 454 and insulin aspart during the study. The TK profiles of insulin 454 and insulin aspart appeared to be generally gender and dose independent over the studied dose-range, although there was a tendency of a larger increase in exposure compared to the corresponding increase in dose observed for insulin aspart between the mid-dose (Groups 3 and 6) and high dose (Groups 4 and 7). There was little accumulation to

either insulin 454 or insulin aspart (< 2 fold) after once daily dosing for 29 days. This was consistent with the apparent rapid systemic elimination of these insulins.

For insulin 454, Cmax were generally reached at 3 hrs after dose and were similar for SIAC 30 and SIAC 45 on both Days 1 and 29. The t ½ was generally 3 hrs after a single dose of either SIAC 30 or SIAC 45. After once daily sc dosing, a slightly longer half-life was observed on Day 29 (approx 4-5 hrs).

For insulin aspart, Cmax were observed at 1 hr for all rats on both Days 1 and 29. The vast majority of insulin aspart appeared to be cleared systemically by 3 hrs post dosing, despite a relatively long terminal phase seen for several profiles between 3-24 hrs post dose. There was no explanation for such long phase, although It did not seem to have an influence on the total exposure of insulin aspart.

Table 7 Study No 208289 - TK Profiles of Insulin Aspart and Insulin 454 on Days 1 and 29 after daily sc Administration of SIAC30 or SIAC 45 for 4 Weeks to Rats

Estimated toxicokinetic parameters for insulin 454 on Day 1 and Day 29 after once daily s.c. administration of SIAC 30 or SIAC 45 for 4 weeks to rats.

Day	Group	Dose insulin 454 (nmol/-kg/day)	Dose insulin aspart (nmol/-kg/day)	Sex	C_{max} (nM)	t_{max} (h)	$AUC_{(0-9h)}$ (h*nM)	$AUC_{(0-24h)}$ (h*nM)	AUC (h*nM)	AUC_{extra} (%)	t½ (h)	Rac_{obs}
1	2	25	11	F	59.0	1	253	NR	NC	NC	NC	NA
				M	56.8	1	243	274	276	0.69	3.5	NA
	3	75	32	F	133	3	680	766	770	0.53	3.0	NA
				M	136	3	785	956	961	0.53	3.0	NA
	4	150	64	F	349	3	1500	1780	1790	0.76	3.3	NA
				M	260	3	1490	1820	1840	1.2	3.5	NA
	5	25	20	F	50.1	3	197	NR	NC	NC	NC	NA
				M	46.8	3	201	237	242	1.9	4.5	NA
	6	50	41	F	85.0	3	386	446	449	0.79	3.4	NA
				M	121	3	504	568	570	0.47	3.0	NA
	7	100	82	F	197	3	951	1140	1150	0.46	3.0	NA
				M	159	3	1020	1270	1280	0.69	3.0	NA
29	2	25	11	F	52.0	3	245	297	306	2.9	5.1	1.0[a]
				M	52.1	3	254	320	331	3.3	5.0	1.2
	3	75	32	F	138	3	749	890	904	1.6	4.0	1.2
				M	107	3	686	960	984	2.4	4.1	1.0
	4	150	64	F	310	3	1570	2010	2050	1.7	3.9	1.1
				M	240	3	1670	2470	2520	2.2	4.0	1.4
	5	25	20	F	58.1	1	280	317	321	1.3	4.1	1.4[a]
				M	54.0	3	281	358	370	3.2	4.8	1.5
	6	50	41	F	86.3	3	459	587	596	1.5	3.8	1.3
				M	91.3	3	559	744	755	1.5	3.6	1.3
	7	100	82	F	254	3	1280	1560	1580	0.87	3.3	1.4
				M	188	6	1260	1780	1800	1.2	3.3	1.4

F – female, M – male, NC – not calculated, NA – not applicable, a – based on $AUC_{(0-9h)}$, NR – Not reported as data only available to 9 hours post dose

Estimated toxicokinetic parameters for insulin aspart on Day 1 and Day 29 after once daily s.c. administration of SIAC 30 or SIAC 45 for 4 weeks to rats.

Day	Group	Dose insulin 454 (nmol/kg/day)	Dose insulin aspart (nmol/kg/day)	Sex	C_{max} (nM)	t_{max} (h)	$AUC_{(0-3h)}$ (h*nM)	$AUC_{(0-6h)}$ (h*nM)	$AUC_{(0-24h)}$ (h*nM)	Rac_{obs}
1	2	25	11	F	0.342	1	0.488	0.807	1.99	NA
				M	0.979	1	1.09	1.32	2.33	NA
	3	75	32	F	1.68	1	1.78	1.95	2.38	NA
				M	6.25	1	5.94	6.15	6.68	NA
	4	150	64	F	23.4	1	20.5	20.7	21.6	NA
				M	16.1	1	15.0	15.3	15.9	NA
	5	25	20	F	1.89	1	2.00	2.37	3.36	NA
				M	1.53	1	1.56	1.83	2.42	NA
	6	50	41	F	10.1	1	8.72	8.94	9.62	NA
				M	5.91	1	5.35	5.62	6.13	NA
	7	100	82	F	25.4	1	22.5	22.8	23.3	NA
				M	30.6	1	26.5	26.9	27.3	NA
29	2	25	11	F	0.370	1	0.404	0.478	0.828	0.4
				M	0.944	1	1.04	1.12	1.55	0.7
	3	75	32	F	10.8	1	9.62	9.72	10.2	4.3
				M	11.6	1	10.8	10.9	11.5	1.7
	4	150	64	F	25.5	1	21.5	21.6	22.1	1.0
				M	27.9	1	25.2	25.5	26.4	1.7
	5	25	20	F	3.48	1	3.53	3.66	4.15	1.2
				M	4.91	1	4.47	4.62	5.23	2.2
	6	50	41	F	13.1	1	10.9	11.0	11.7	1.2
				M	17.6	1	14.9	15.0	15.7	2.6
	7	100	82	F	32.8	1	28.1	28.3	28.7	1.2
				M	35.1	1	32.3	32.8	33.5	1.2

NA – Not applicable

Study No 208337 (PIVOTAL): 13 wk rat (4 wk recovery)

Study title: SIAC 30 - Toxicity study by subcutaneous administration to Han Wistar rats for 13 weeks followed by a 4 week recovery period

Study no.:	208337
Study report location:	Novo Nordisk, Denmark
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Jan 9 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	412_N08702 (purity for insulin 454: 99.0% and purify for insulin aspart: 98.6%)

Key study findings

- Daily sc administration of SIAC30 was tolerated at doses up to 75 nmol/kg/day insulin 454 + 32 nmol/kg/day insulin aspart. The dose selection was based on 4 week DFR study in rats.
- Three animals had hypoglycaemic shock: 1 HD insulin 454 and 1 insulin NPH, and 1 MD insulin 454 (eventually died). These were all attributed to exaggerated pharmacological effect of insulin.
- Treatment-related effect on reduced plasma glucose was associated with the pharmacological effect of insulin 454 and insulin aspart, leading to secondary findings, including increased food intake, body weight gain, adaptive change in water balance, and effects on fatty acid and protein metabolism. All these findings were partially recoverable.
- There were antibody formation towards insulin 454 and insulin aspart, there were no apparent effect on the plasma concentration vs. time profiles or glucose lowering effect of SIAC30. The PK profile of SIAC30 was generally sex and dose independent for both insulin 454 and insulin aspart (with daily sc administration for 13 weeks).
- Based on 1 death (satellite female) at MD group and hypoglycaemia-related clinical signs at MD/HD groups, the NOAEL was established at 36 nmol/kg/day IDegAsp.
  Note: the applicant established the NOAEL as 107 nmol/kg/day IDegAsp (75 nmol/kg/day insulin 454 + 32 nmol/kg/day insulin aspart).

Methods

Doses:	0, 50, 100, and 150 nmol/ml
Frequency of dosing:	Once daily
Route of administration:	Sc injection
Dose volume:	0.5 ml/kg/day
Formulation/Vehicle:	Phenol 1.50 mg/ml, *m*-cresol 1.72 mg/ml, glycerol 19.0 mg/ml, sodium chloride 0.58 mg/ml and water for injection (b) (4), pH 7.40
Species/Strain:	Han Wistar rats
Number/Sex/Group:	10/sex/group
Age:	42 days of age
Weight:	150-175 for males; 131-155 for females
Satellite groups:	Recovery group
Unique study design:	n/a
Deviation from study protocol:	Minor deviations

Study design

Group	Treatment	Dose# (nmol/kg/day)	Main Study					
			No. of animals		Animal numbers		Cage numbers	
			Male	Female	Male	Female	Male	Female
1	Control	0	10	10	1-10	129-138	1-2	28-29
2	Low-dose SIAC 30	25;11	10	10	27-36	155-164	7-8	34-35
3	Mid-dose SIAC 30	50;21	10	10	51-60	179-188	12-13	39-40
4	High-dose SIAC 30	75;32	10	10	75-84	203-212	17-18	44-45
5	NPH insulin	75	10	10	109-118	237-246	24-25	51-52

for Groups 2, 3 and 4 the dose refers to the insulin 454;insulin aspart content (SIAC 30). SIAC 30 as supplied contained 420 nmol insulin 454/ml and 180 nmol insulin aspart/ml.

Group	Treatment	Dose# (nmol/kg/day)	Recovery phase (13 weeks + 4 weeks recovery)					
			No. of animals		Animal numbers		Cage numbers	
			Male	Female	Male	Female	Male	Female
1	Control	0	10	10	11-20	139-148	3-4	30-31
2	Low-dose SIAC 30	25;11	-	-	-	-	-	-
3	Mid-dose SIAC 30	50;21	-	-	-	-	-	-
4	High-dose SIAC 30	75;32	10	10	85-94	213-222	19-20	46-47
5	NPH insulin	75	10	10	119-128	247-256	26-27	53-54

for Groups 2, 3 and 4 the dose refers to the insulin 454;insulin aspart content (SIAC 30). SIAC 30 as supplied contained 420 nmol insulin 454/ml and 180 nmol insulin aspart/ml.

Observations and Results

- **Mortality/Clinical Signs:** twice daily

All HD males and several MD males were transiently underactive approx. 3 hrs after dosing on one occasion in Weeks 4 and 5, respectively. In those HD animals, this was associated with partially closed eyelids.

Three animals had an exaggerated response (i.e. 1 HD female had hypoglycemic shock in Week 13, 1 satellite MD female had died 3 hrs post dose in Week 7, 1 NPH male had hypoglycemia shock on Day 5).

- **Body Weights/food consumption:** weekly

There was no adverse effect of treatment upon body weight or food intake. In MD or HD males, there was a small increase of weight gain up to Week 7 and a trend towards high food intake during the treatment period.

- **Ophthalmoscopy:** prior to treatment and Wk 13

No adverse finding was found.

- **ECG:** n/a

- **Hematology:** Weeks 6 and 13 (main) and Week 4 (recovery)

using a Bayer Advia 120 haematology analyser

Haematocrit (Hct)
Haemoglobin concentration (Hb)
Erythrocyte count (RBC)
Reticulocyte counts (Retic)
Mean cell haemoglobin (MCH)
Mean cell haemoglobin concentration (MCHC)
Mean cell volume (MCV)
Total white cell count (WBC)
Differential WBC count
- Neutrophils (N)
- Lymphocytes (L)
- Eosinophils (E)
- Basophils (B)
- Monocytes (M)
- Large unstained cells (LUC)

Platelet count (Plt)

In Week 6, there were slightly lower neutrophil and lymphocytes counts in all treated females and slightly lower basophil and monocytes counts in MD/HD females. These changes lead to a slightly lower total WBC counts in SIAC30 treated females. In Week 13, there were no effects observed in females or males. During 4 week recovery, lymphocyte, basophil, and total WBC counts for HD females were similar to those of the control.

Summary of haematological changes- group mean values (control) and percent of control (Groups 2-5):

Group/sex	1F	2F	3F	4F	5F
Dose (nmol/kg/day)	0	25~	50~	75~	75
Week 6					
WBC ($x10^9$/L)	5.61	-9	-19	-19	-14
Neutrophils ($x10^9$/L)	0.844	-15	-25	-35	-28
Lymphocytes ($x10^9$/L)	4.59	-8	-19	-16	-11
Basophils ($x10^9$/L)	0.012	-17	-33	-33	-8
Monocytes ($x10^9$/L)	0.085	-8	-19	-21	-18
Week R4					
WBC ($x10^9$/L)	4.26	N/A	N/A	-2	+3
Neutrophils ($x10^9$/L)	0.768	N/A	N/A	-23	-16
Lymphocytes ($x10^9$/L)	3.33	N/A	N/A	+4	+7
Basophils ($x10^9$/L)	0.008	N/A	N/A	+25	0
Monocytes ($x10^9$/L)	0.081	N/A	N/A	-23	-7

~ SIAC 30 dose in terms of nmol insulin 454/kg/day
N/A Not applicable

- **Clinical Chemistry**

Using a Roche P Modular Analyser:

Alkaline phosphatase (ALP)
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Total bilirubin (Bili)
Urea
Creatinine (Creat)
Glucose (Gluc)
Total cholesterol (Chol)
Triglycerides (Trig)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Calcium (Ca)
Inorganic phosphorus (Phos)
Total protein (Total Prot)

Several changes were seen with clinical chemistry parameters: 1) a dose-related increase in glucose concentration in females at all doses and in MD or HD males, 2) a dose-related decrease in urea level in MD or HD males in Week 6 and in all males in Week 13, 3) low triglyceride levels in both sexes at all doses in Week 6 and in MD or HD females in Week 13, 4) marginally low calcium levels in females at all doses in Week 6 and in MD or HD males, 5) high phosphorus levels in both HD males and females in Weeks 6 and 13, 6) low protein levels (mainly reduced albumin and α-1 globulin) in females at all doses in Weeks 6 and 13. These changes were also seen in animals given NPH insulin, suggesting that they were attributable to insulin administration. All these changes were recoverable.

Summary of plasma biochemical changes - group mean values (controls) and percent of controls (Groups 2-5):

Group/sex	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (nmol/kg/day)	0	25~	50~	75~	75	0	25~	50~	75~	75
Week 6										
Glucose (mmol/l)	10.4	-	+13	+20**	+14	8.4	+21***	+30***	+32***	+27
Urea (mmol/l)	5.79	-	-11	-17**	-10	-	-	-	-	-
Triglycerides (mmol/l)	2.17	-5	-18	-17	-7	2.19	-37**	-56***	-43***	-45
Calcium (mmol/l)	2.80	-	-4	-4	-5	2.80	-3*	-5***	-4**	-4
Phosphorus (mmol/l)	1.71	-	-	+13	+14	1.54	-	-	+8	+17
Total protein (g/l)	-	-	-	-	-	71.6	-5*	-7**	-7**	-6
Albumin (g/l)	-	-	-	-	-	38.8	-6*	-6*	-7*	-6

~ SIAC 30 dose in terms of nmol insulin 454/kg/day
- No noteworthy findings

Summary of plasma biochemical changes (cont) - group mean values (control) and percent of control (Groups 2-5):

Group/sex	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (nmol/kg/day)	0	25~	50~	75~	75	0	25~	50~	75~	75
Week 13										
Glucose (mmol/l)	8.97	-	+14*	+18**	+13	7.64	+14	+31***	+33***	+40
Urea (mmol/l)	5.83	-8	-10	-14	-15	-	-	-	-	-
Triglycerides (mmol/l)	-	-	-	-	-	1.47	-	-12	-18	-26
Calcium (mmol/l)	-	-	-	-	-	2.76	-	-	-3	-1
Phosphorus (mmol/l)	1.46	-	-	+12	+21	1.37	-	-	+11	+14
Total protein (g/l)	-	-	-	-	-	77.3	-6*	-7**	-6**	-6
Albumin (g/l)	-	-	-	-	-	43.9	-10**	-7*	-5	-5
Week R4										
Glucose (mmol/l)	10.20	N/A	N/A	0	+8	9.20	N/A	N/A	+6	0
Urea (mmol/l)	5.34	N/A	N/A	+8	+6	-	N/A	N/A	-	-
Triglycerides (mmol/l)	1.84	N/A	N/A	-8	-29	1.50	N/A	N/A	-12	-5
Calcium (mmol/l)	2.64	N/A	N/A	0	-2*	2.68	N/A	N/A	0	0
Phosphorus (mmol/l)	1.42	N/A	N/A	+6	-3	0.97	N/A	N/A	+14	+20
Total protein (g/l)	-	N/A	N/A	-	-	73.1	N/A	N/A	-3	-1
Albumin (g/l)	-	N/A	N/A	-	-	41.1	N/A	N/A	-3	-2

~ SIAC 30 dose in terms of nmol insulin 454/kg/day
- No noteworthy findings
N/A Not applicable

- **Glucose analysis:**

The plasma glucose levels at intervals after dosing on Day 1 and in Weeks 7 and 13 suggested that reduced levels at all doses in a dose-dependent manner. The greatest effect was generally seen at 1 hr post-dose, where group mean plasma glucose levels ranged between approx. 1 and 3 mmol/l. These changes were due to the pharmacological action of test article.

- **Urinalysis:** Week 12 (main) and Week 4 (recovery)

Appearance (App) - by visual assessment using the following abbreviations:

PY Pale yellow CPY Cloudy pale yellow
MY Medium yellow

Volume (Vol)
pH - using a Radiometer PHM 92 pH meter
Specific gravity (SG) - using Atago UR-1 digital refractometer
Protein (Prot), sodium (U-Na), potassium (U-K) and chloride (U-Cl) - using Roche P Modular Clinical Chemistry Analyser
Glucose (Gluc), ketones (Keto), bile pigments (Bili), blood pigments (UBld)

Low urinary volume and high specific gravity were seen in LD males and in MD or HD males/females in Week 12. Urinary pH was slightly low in MD females and HD males/females. These changes were also seen in NPH treated animals, suggesting were attributable to insulin administration. Such changes were partially or completely recoverable.

Summary of urinary changes- group mean values (control) and percent of control (Groups 2-5):

Group/sex	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (nmol/kg/day)	0	25~	50~	75~	75	0	25~	50~	75~	75
Week 12										
Volume (ml)	3.84	-30	-36*	-48**	-60	2.51	-	-39	-53*	-59
pH	8.18	-	-	-13**	-11	8.38	-6	-12	-20**	-18
Week R4										
Volume (ml)	2.86	N/A	N/A	-35	-17	2.88	N/A	N/A	-16	-26
pH	8.41	N/A	N/A	-1	-2	7.54	N/A	N/A	+6	+3

~ SIAC 30 dose in terms of nmol insulin 454/kg/day
- No noteworthy findings N/A Not applicable

Summary of urinary appearance:

Group/sex	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dose (nmol/kg/day)	0	25~	50~	75~	75	0	25~	50~	75~	75
Week 12										
Pale yellow	9	8	5	7	3	10	10	9	6	8
Medium yellow	1	2	5	3	7	0	0	1	4	2
No. examined	10	10	10	10	10	10	10	10	10	10
Week R4										
Pale yellow	10	N/A	N/A	10	10	10	N/A	N/A	10	10
No. examined	10			10	10	10			10	10

~ SIAC 30 dose in terms of nmol insulin 454/kg/day
N/A Not applicable

- **Gross Pathology:**

No test article-related lesions were observed.

- **Organ Weights:** Week 13

Adrenals	Prostate
Brain	Salivary glands - submandibular#
Epididymides	Salivary glands - sublingual#
Heart	Seminal vesicles
Kidneys	Spleen
Liver	Testes
Lungs with mainstem bronchi	Thymus
Lymph node - accessory axillary	Thyroid with parathyroids*
Ovaries and oviducts	Uterus with cervix
Pituitary	

* Weighed after partial fixation
\# Submandibular and sublingual glands weighed together; reported as salivary glands

There were low absolute as well as body weight and brain weight liver weights in HD females, to a lesser extent, in MD females (not sign.). This was recoverable after 4 week recovery period. No such effect was seen in males. In addition, the body weight relative liver weights for NHP females were reduced (similar magnitude to those of MD/HD females). There were also small increases in body weight relative epididymides and testes weights with NPH treated males.

Summary of organ weight changes- group mean values (controls) and percent of controls (Groups 2-5):

Group/sex		1F	2F	3F	4F	5F
Dose (nmol/kg/day)		0	25~	50~	75~	75
Week 13						
Liver	Absolute (g)	8.74	-	-7	-10	-3
	Bwt-rel (%)	3.51	-	-7	-9**	-7
	Brain rel (%)	470	-	-8	-12*	-7
Week R4						
Liver	Absolute (g)	8.91	N/A	N/A	-4	+3
	Bwt-rel (%)	3.42	N/A	N/A	+1	+3
	Brain rel (%)	453	N/A	N/A	-2	+5

~ SIAC 30 dose in terms of nmol insulin 454/kg/day
- No noteworthy findings
N/A Not applicable

- **Antibody Formation:**

After 13 weeks treatment with SIAC 30, 46/143 (32%) were positive for antibodies towards insulin 454 and 44/143 (31%) were positive for antibodies towards insulin aspart. After 4 week recovery period, 5/20 (25%) animals were positive for insulin 454 antibodies and 4/20 (20%) for insulin aspart antibodies. As for NPH insulin, 12/20 (60%) were positive for antibodies after 13 weeks treatment and 11/20 (55%) were positive for antibodies after 4 weeks recovery period.

Summary of insulin 454 antibodies

Group	Treatment	Dose insulin 454 (nmol/kg/day)	Main Study Positive/Total number	Satellite Study Positive/Total number	Recovery Phase (13 weeks+ 4 weeks) Positive/Total number
1	Vehicle	0	0/20	0/12	0/20
2	SIAC 30	25	6/20	10/28	N/A
3	SIAC 30	50	10/20	6/27	N/A
4	SIAC 30	75	6/20	8/28	5/20

N/A Not applicable

Summary of insulin aspart antibodies

Group	Treatment	Dose insulin 454 (nmol/kg/day)	Main Study Positive/Total number	Satellite Study Positive/Total number	Recovery Phase (13 weeks+ 4 weeks) Positive/Total number
1	Vehicle	0	0/20	0/12	0/20
2	SIAC 30	25	6/20	9/28	N/A
3	SIAC 30	50	10/20	6/27	N/A
4	SIAC 30	75	6/20	7/28	4/20

N/A Not applicable

Summary of human insulin antibodies

Group	Treatment	Dose NPH insulin (nmol/kg/day)	Main Study Positive/Total number	Satellite Study Positive/Total number	Recovery Phase (13 weeks+ 4 weeks) Positive/Total number
1	Vehicle	0	0/15	N/A	0/20
5	NPH insulin	75	12/20	N/A	11/20

N/A Not applicable

- **TK analysis:**

The TK profile after once daily sc administration of SIAC 30 to rats for 13 weeks was generally sex- and dose-independent, with low accumulation in systemic exposure seen. Such low accumulation was attributed to rapid elimination. Max plasma levels of insulin aspart were reached after 0.5 hrs while peak serum levels insulin 454 were seen 3 hrs post-dosing. The plasma t ½ for insulin aspart was 0.2-0.6 hr while the serium t ½ for insulin 454 was 3-5 hrs.

Table 8 Study No 208337 - TK Profiles of Insulin Aspart and Insulin 454 on Day 1 and Week 13 after Repeated Once Daily sc Administration of SIAC 30 for 13 Weeks to Rats

Estimated toxicokinetic parameters for insulin 454 on Day 1 and in Week 13 after repeated once daily s.c. administration of SIAC 30 for 13 weeks to rats.

Period	Group	Dose insulin 454 (nmol/kg/day)	Sex	C_{max} (nM)	t_{max} (h)	$AUC_{(0\text{-}9h)}$ (h*nM)	$AUC_{(0\text{-}24h)}$ (h*nM)	AUC (h*nM)	AUC_{extra} (%)	t½ (h)	Rac_{obs}
Day 1	2	25	F	64.3	1	256	NA	NR	NR	NR	NA
			M	53.9	3	263	317	319	0.76	3.5	NA
	3	50	F	107	3	486	545	548	0.44	3.0	NA
			M	137	3	518	581	584	0.52	3.3	NA
	4	75	F	190	3	853	964	967	0.34	2.9	NA
			M	192	3	973	1140	1150	0.67	3.2	NA
Week 13	2	25	F	68.3	3	356	551	NR	NR	NR	1.4[a]
			M	72.9	3	346	436	NR	NR	NR	1.4
	3	50	F	131	3	607	694	NR	NR	NR	1.3
			M	132	3	700	914	925	1.2	3.5	1.6
	4	75	F	354	3	1200	1420	1440	1.4	4.0	1.5
			M	150	3	701	999	1050	4.4	5.1	0.87

F – female, M – male, NA – not applicable, NR – Not reported as as only 2 points available for linear regression, a – based on $AUC_{(0\text{-}9h)}$

Estimated toxicokinetic parameters for insulin aspart on Day 1 and in Week 13 after repeated once daily s.c. administration of SIAC 30 or for 13 weeks to rats.

Period	Group	Dose insulin aspart (nmol/kg/day)	Sex	C_{max} (nM)	t_{max} (h)	$AUC_{(0-1h)}$ (h*nM)	$AUC_{(0-3h)}$ (h*nM)	$AUC_{(0-24h)}$ (h*nM)	AUC (h*nM)	AUC_{extra} (%)	$t_{½}$ (h)	Rac_{obs} [a]
Day 1	2	11	F	3.65	0.5	1.74	NA	NA	NR	NR	NR	NA
			M	3.29	0.5	1.64	2.07	NA	2.09	0.98	0.40	NA
	3	21	F	15.6	0.5	8.75	NA	NA	NR	NR	NR	NA
			M	13.6	0.5	8.32	10.5	10.6	10.6	0.089	0.24	NA
	4	32	F	21.1	0.5	14.0	19.1	NA	19.1	0.11	0.28	NA
			M	18.8	0.5	13.2	18.2	NA	18.2	0.063	0.26	NA
Week 13	2	11	F	3.99	0.5	2.47	3.16	3.19	3.19	0.13	0.25	1.4
			M	3.39	0.5	2.02	3.08	3.31	3.35	1.3	0.49	1.2
	3	21	F	16.5	0.5	10.3	13.4	13.6	13.6	0.082	0.26	1.2
			M	11.9	0.5	8.80	13.9	14.1	14.1	0.10	0.55	1.1
	4	32	F	30.4	0.5	19.2	30.8	33.4	33.5	0.11	0.51	1.4
			M	21.9	0.5	14.2	21.0	22.6	22.6	0.12	0.39	1.1

F – female, M – male, NA – Not applicable; NR – Not reported as as only 2 points available for linear regression; a – based on $AUC_{(0-1h)}$

- **Histopathology:**

Adequate Battery: Yes

Peer Review: Yes

Adrenals
Aorta – thoracic
Brain
Caecum
Colon
Duodenum
Epididymides
Eyes with lens
Femurs+
Harderian glands
Head#
Heart
Ileum
Injection sites
Jejunum
Kidneys
Lachrymal glands
Larynx
Liver
Lungs including bronchi
Lymph nodes - mandibular
- mesenteric
- inguinal
- axillary
Oesophagus
Optic nerves
Ovaries and oviducts
Pancreas
Peyers' patches
Pituitary
Prostate
Rectum
Salivary glands - submandibular+
- parotid+
- sublingual+
Sciatic nerves+
Seminal vesicles
Skeletal muscle +
Skin with mammary gland (inguinal area)
Spinal cord
Spleen
Sternum
Stomach
Testes
Thymus
Thyroid with parathyroids
Tongue
Trachea
Ureters
Urinary bladder
Uterus and cervix
Vagina

\+ Only one processed for examination
\# Not processed for examination

Some hemorrhage and inflammatory changes (minimal to marked severity) in the skin and subcutis were seen at the parenteral sites in all groups, including controls, after 13 weeks. This was considered to be associated with the sc route of administration. No other treatment related findings were identified after 13 weeks.

Table 9 Study No 208337 - Summary Tables of Histopathology Findings

Histopathology - group distribution of findings

Group	:	1	2	3	4	5
Compound	:	Control	SIAC 30	SIAC 30	SIAC 30	NPH insulin
Dose (nmol/kg/day)	:	0	25	50	75	75

Request ID: 172836/172837

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
Trachea	Number Examined:	10	0	0	10	10	10	0	0	10	10
Inflammatory Cells, Mucosa/Submucosa		0	0	0	0	0	1	0	0	0	0
Lungs + Bronchi	Number Examined:	10	3	4	10	10	10	8	6	10	10
Aggregations of Alveolar Macrophages		1	3	4	2	0	4	5	5	3	5
Aggregations of Alveolar Macrophages Associated with Hyperplasia		0	0	0	1	0	0	0	0	0	0
Arterial Mural Mineralisation		0	0	0	0	0	0	1	0	0	0
Haemorrhage, Alveolar		1	0	0	0	0	0	0	0	0	0
Haemorrhage, Interstitial		0	0	2	0	0	0	0	0	0	0
Liver	Number Examined:	10	0	0	10	10	10	0	0	10	10
Hepatocyte Vacuolation		0	0	0	0	2	0	0	0	0	0
Inflammation, Focal		0	0	0	0	1	2	0	0	0	4
Pericholangial Inflammation		0	0	0	1	0	0	0	0	0	0
Pigment in Hepatocytes		0	0	0	0	0	1	0	0	0	0
Pancreas	Number Examined:	10	0	0	10	10	10	0	0	10	10
Inflammation		0	0	0	1	0	0	0	0	0	1
Periductal Inflammation / Fibrosis		0	0	0	0	0	1	0	0	0	0
Phocal Periductal Atrophy		0	0	0	1	0	0	0	0	0	0

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
LN Mesenteric	Number Examined:	10	0	0	10	10	10	0	0	10	10
Lymphatic Ectasia		0	0	0	0	0	1	0	0	0	0
Lymphoid Hyperplasia		0	0	0	0	1	0	0	0	0	0
Macrophage Aggregates		0	0	0	0	1	0	0	0	0	0
Mastocytosis		1	0	0	3	4	4	0	0	2	4
Plasmacytosis		0	0	0	0	0	1	0	0	1	0
Sinus Erythrocytosis/Erythrophagocytosis		0	0	0	0	1	1	0	0	0	0
Kidneys	Number Examined:	10	0	1	10	10	10	0	1	10	10
Chronic Progressive Nephropathy		0	0	0	0	0	0	0	0	1	0
Cortical Tubular Basophilia		1	0	0	1	0	0	0	0	0	0
Interstitial Inflammation		0	0	0	1	1	0	0	0	0	0
Mineralisation, Corticomedullary		0	0	0	0	0	0	0	0	2	1
Mineralisation, Papilla		0	0	0	0	0	0	0	0	0	1
Pelvic Dilatation		1	0	0	0	0	0	0	0	0	0
Tubular Casts		0	0	0	1	0	0	0	0	0	0
Heart	Number Examined:	10	0	0	10	10	10	0	0	10	10
Focal Myocardial Inflammation		0	0	0	0	2	0	0	0	0	1

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
Spleen	Number Examined:	10	0	0	10	10	10	0	0	10	10
Extramedullary Haemopoiesis		0	0	0	0	2	5	0	0	0	1
Haemosiderosis		0	0	0	1	2	4	0	0	0	5
Thymus	Number Examined:	10	3	3	10	10	10	0	1	10	10
Epithelial Hyperplasia		0	0	0	0	0	3	0	0	0	3
Haemorrhage		1	3	3	5	7	1	0	1	0	2
Thyroids	Number Examined:	10	0	1	10	10	10	0	0	10	9
Prominent Ultimobranchial Cyst(s)		0	0	0	0	0	1	0	0	1	0
Adrenals	Number Examined:	10	0	0	10	10	10	1	0	10	10
Cortical Vacuolation		0	0	0	1	1	0	0	0	0	0
Ectopic Bone in the Cortex		0	0	0	0	1	0	0	0	0	0
Prom. Zona Reticularis		0	0	0	0	0	0	1	0	0	0
Pituitary	Number Examined:	10	0	0	9	10	10	0	0	10	10
Developmental Cyst(s)		0	0	0	1	0	0	0	0	0	0

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
LN Mandibular	Number Examined:	9	0	1	10	10	10	0	0	10	10
Lymphoid Hyperplasia		1	0	0	1	4	0	0	0	1	0
Mastocytosis		0	0	0	1	2	1	0	0	0	0
Plasmacytosis		6	0	0	8	9	8	0	0	1	6
Sinus Erythrocytosis/Erythrophagocytosis		2	0	1	2	5	0	0	0	2	1
Salivary Glands	Number Examined:	10	0	0	10	10	10	0	0	10	10
Basophilic Hypertrophy, Focal		0	0	0	0	0	0	0	0	1	0
Harderisation		1	0	0	0	0	0	0	0	0	0
Parotid S.G.	Number Examined:	10	0	0	10	10	10	0	0	10	10
Acinal Cell Vacuolisation		1	0	0	0	0	0	0	0	0	0
Inflammation		0	0	0	0	0	0	0	0	1	0
Tongue	Number Examined:	10	0	0	10	10	10	0	0	10	10
Haemorrhage		2	0	0	2	5	4	0	0	4	4
Inflammation		0	0	0	0	1	3	0	0	1	1
Ileum	Number Examined:	10	0	0	10	10	10	0	0	10	10
Granuloma, Foreign Type		0	0	0	1	0	0	0	0	0	0

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
Skin	Number Examined:	10	0	0	10	10	10	0	0	10	10
B-Basal Cell Tumour		0	0	0	0	0	1	0	0	0	0
Epidermal Hyperplasia		0	0	0	0	1	0	0	0	0	0
Skeletal Muscle	Number Examined:	10	0	0	10	10	10	0	0	10	10
Myofibre Degeration/Necrosis		0	0	0	0	1	0	0	0	0	0
Lachrymal Glands	Number Examined:	10	0	0	10	10	10	0	0	10	10
Glandular Atrophy		0	0	0	1	0	0	0	0	0	0
Inflammation		0	0	0	2	0	0	0	0	0	0
Eyes	Number Examined:	10	0	0	10	10	10	0	0	10	10
Retinal Rosettes/Folds		0	0	0	0	1	0	0	0	0	0
Harderian Glands	Number Examined:	10	0	0	10	10	10	0	0	10	10
Acinar Atrophy		0	0	0	0	0	0	0	0	0	1
Lymphoid Aggregates		1	0	0	2	1	1	0	0	3	2
Porphyrin Concretions		0	0	0	0	1	0	0	0	0	0
Regeneration of Secretory Epithelium		0	0	0	0	1	0	0	0	0	0

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
Femur inc. Joint	Number Examined:	10	0	0	10	10	10	0	0	10	10
Chondroid Degeneration		0	0	0	1	1	0	0	0	0	0
Synovitis		0	0	0	0	0	0	0	0	0	1
Prostate	Number Examined:	10	0	0	10	10	-	-	-	-	-
Inflammation		1	0	0	0	1	-	-	-	-	-
Lymphoid Aggregates		1	0	0	1	2	-	-	-	-	-
Epididymides	Number Examined:	10	0	1	10	10	-	-	-	-	-
Spermatozoa Absent		0	0	1	1	0	-	-	-	-	-
Testes	Number Examined:	10	0	1	10	10	-	-	-	-	-
Atrophy		0	0	0	1	0	-	-	-	-	-
Degeneration of Seminiferous Tubular Epithelium		0	0	1	1	0	-	-	-	-	-
Vagina	Number Examined:	-	-	-	-	-	10	0	0	10	10
Dioestrus		-	-	-	-	-	5	0	0	2	3
Metoestrus		-	-	-	-	-	0	0	0	0	1
Oestrus		-	-	-	-	-	2	0	0	3	3
Proestrus		-	-	-	-	-	2	0	0	4	3

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
Uterus	Number Examined:	-	-	-	-	-	10	2	0	10	10
Luminal Dilatation		-	-	-	-	-	3	1	0	3	3
Larynx	Number Examined:	10	0	0	10	10	10	0	0	10	10
Inflammatory Cells, Mucosa/Submucosa		0	0	0	0	2	0	0	0	1	0
Lt Upper Flank	Number Examined:	10	0	0	10	10	10	0	0	10	10
Haemorrhage, Subcutis	Slight:	0	0	0	1	0	0	0	0	0	0
	Total:	0	0	0	1	0	0	0	0	0	0
Inflammation	Minimal:	0	0	0	1	0	0	0	0	0	0
	Slight:	1	0	0	0	0	0	0	0	0	0
	Total:	1	0	0	1	0	0	0	0	0	0
Myofibre Atrophy	Slight:	1	0	0	0	0	0	0	0	0	0
	Total:	1	0	0	0	0	0	0	0	0	0
Rt Upper Flank	Number Examined:	10	0	0	10	10	10	0	1	10	10
Haemorrhage, Subcutis	Moderate:	0	0	0	0	0	0	0	0	0	1
	Total:	0	0	0	0	0	0	0	0	0	1
Inflammation, Cutis	Minimal:	0	0	0	0	1	0	0	0	0	0
	Total:	0	0	0	0	1	0	0	0	0	0
Scab	Marked:	0	0	0	0	0	0	0	1	0	0
	Total:	0	0	0	0	0	0	0	1	0	0

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
Lt Lower Flank	Number Examined:	10	3	1	10	10	10	2	1	10	10
Haemorrhage, Subcutis	Minimal:	0	0	1	0	0	0	0	0	0	0
	Slight:	2	1	0	1	1	0	1	0	0	1
	Moderate:	0	1	0	0	0	1	0	0	1	0
	Marked:	0	1	0	0	1	0	0	0	0	0
	Total:	2	3	1	1	2	1	1	0	1	1
Inflammation,	Minimal:	0	1	1	0	1	0	0	0	1	0
	Slight:	1	1	0	1	1	0	0	0	0	0
	Moderate:	1	0	0	0	1	0	1	0	0	0
	Total:	2	2	1	1	3	0	1	0	1	0
Reduction of Panniculus Carnosus	Marked:	0	0	0	0	0	0	1	0	0	0
	Total:	0	0	0	0	0	0	1	0	0	0
Scab	Minimal:	0	0	1	0	0	0	0	0	0	0
	Moderate:	1	0	0	0	0	0	0	0	0	0
	Total:	1	0	1	0	0	0	0	0	0	0
Rt Lower Flank	Number Examined:	10	1	0	10	10	10	2	3	10	10
Haemorrhage, Subcutis	Slight:	0	0	0	0	0	1	1	0	3	0
	Moderate:	1	0	0	0	0	0	1	1	0	0
	Marked:	0	0	0	0	0	0	0	0	1	0
	Total:	1	0	0	0	0	1	2	1	4	0

Histopathology - group distribution of findings for animals killed after 13 weeks of treatment

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
Rt Lower Flank - continued	Number Examined:	10	1	0	10	10	10	2	3	10	10
Inflammation	Minimal:	0	0	0	1	0	1	0	0	1	0
	Slight:	0	0	0	0	0	0	1	0	0	1
	Moderate:	1	0	0	0	0	0	1	1	2	0
	Total:	1	0	0	1	0	1	2	1	3	1
Reduction of Panniculus Carnosus	Moderate:	0	0	0	0	0	0	0	0	1	0
	Marked:	0	0	0	0	0	0	1	1	0	0
	Total:	0	0	0	0	0	0	1	1	1	0
LN Inguinal	Number Examined:	10	0	0	10	9	10	0	0	10	10
Mastocytosis		0	0	0	0	6	3	0	0	0	2
Plasmacytosis		0	0	0	0	0	1	0	0	1	0
Sinus Erythrocytosis/Erythrophagocytosis		0	0	0	0	0	0	0	0	1	0
Acc. LN Axillary	Number Examined:	10	0	0	10	10	10	0	0	10	10
Mastocytosis		0	0	0	2	6	5	0	0	1	5
Adipose tissue	Number Examined:	0	1	0	0	0	0	1	0	0	0
Fat Necrosis		0	1	0	0	0	0	0	0	0	0
Fibrosis		0	1	0	0	0	0	1	0	0	0
Haemorrhage		0	1	0	0	0	0	0	0	0	0

Histopathology - group distribution of findings for animals killed after 4 weeks of recovery

Tissue and Finding	Group/Sex: / Number:	1M 10	4M 10	5M 10	1F 10	4F 10	5F 10
Lungs + Bronchi	Number Examined:	8	6	8	9	7	4
Aggregations of Alveolar Macrophages		7	4	6	8	4	3
Haemorrhage, Alveolar		0	1	1	0	0	0
Liver	Number Examined:	0	1	0	0	0	0
Inflammation, Focal		0	1	0	0	0	0
Kidneys	Number Examined:	0	0	0	1	0	1
Pelvic Dilatation		0	0	0	0	0	1
Thymus	Number Examined:	3	1	3	4	1	3
Epithelial Hyperplasia		0	1	0	3	0	2
Haemorrhage		3	1	3	2	1	1
Thyroids	Number Examined:	0	1	0	0	0	0
Follicular Cell Hypertrophy		0	1	0	0	0	0
LN Mandibular	Number Examined:	6	2	1	0	4	1
Lymphoid Hyperplasia		3	0	0	0	1	0
Mastocytosis		0	1	0	0	0	0
Plasmacytosis		4	1	0	0	4	1
Sinus Erythrocytosis/Erythrophagocytosis		3	2	1	0	4	1

Histopathology - group distribution of findings for animals killed after 4 weeks of recovery

Tissue and Finding	Group/Sex: / Number:	1M 10	4M 10	5M 10	1F 10	4F 10	5F 10
Stomach	Number Examined:	0	1	0	0	0	0
Squamous Cyst - Limiting Ridge		0	1	0	0	0	0
Uterus	Number Examined:	-	-	-	1	3	4
Luminal Dilatation		-	-	-	0	3	4
Lt Upper Flank	Number Examined:	1	0	0	1	0	0
Inflammation	Minimal:	0	0	0	1	0	0
	Total:	0	0	0	1	0	0
Adipose tissue	Number Examined:	1	1	1	0	0	0
Fibrosis		1	0	1	0	0	0
Granulomatous Inflammation		0	0	1	0	0	0
Haemorrhage		1	0	1	0	0	0
LN Deep Cervical	Number Examined:	0	0	0	0	1	0
Sinus Erythrocytosis/Erythrophagocytosis		0	0	0	0	1	0

- **Dosing Solution Analysis**

All samples taken from formulations prepared for study use were found to be acceptable. All individual results were between -6 to -1% (for insulin 454) and -5 to +2% (for insulin aspart) of nominal concentrations.

Individual results of SIAC40 test item samples taken before and on completion of the study were between -2 to -1% (for insulin 454) and -1% for insulin aspart, suggesting acceptable shelf life of SIAC30 during the study.

In addition, the analyzed concentrations of samples that were taken from formulations prepared for study use and which were stored at 2-8 C for 7 days prior to approx -20 C storage were found to be acceptable. All individual results were between -6 to -2% (for Insulin 454) and -4 to -2% (for insulin aspart), suggesting the stability of SIAC30 formulations.

7 Genetic Toxicology

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

N/A

7.2 *In Vitro* Assays in Mammalian Cells

N/A

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

N/A

7.4 Other Genetic Toxicity Studies

N/A

8 Carcinogenicity

N/A

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

See NDA 203314 for IDeg.

9.2 Embryonic Fetal Development

See NDA 203314 for IDeg.

Note: 2 studies for IDegAsp were described here in details: preliminary and pivotal Seg II rat studies.

Study No 208333: DRF rat

SIAC30 - Preliminary embryo-fetal toxicity study in Han Wistar rats by subcutaneous administration [CRO: (b) (4)]

Study design: This study was to assess the effect of insulin 454 (70%) combined with insulin aspart (30%), SIAC 30 (batch no 412_N08702 with 99.0% impurity for insulin 454 and 98.6% purity insulin aspart), on the progress and outcome of pregnancy when administered to female rat daily from GDs 6-17 after mating, including satellite animals for TK analysis. The NPH insulin was used as a comparator to differentiate between related to insulin treatment and adverse findings related to SIAC 30. Three groups (n=8) were given SIAC30 by sc injection at doses of 20, 80, or 125 nmol/kg/day insulin 454. The control group received vehicle for SIAC30 whereas another group received 80 nmol/kg/day NPH insulin. Endpoints include the following: clinical observations, body weight, food consumption, glucose (main study animals) and TK (satellite animals) analyses, gross pathology on Day 20, and litter parameters.

Group	Treatment	Dose concentration insulin 454 (nmol/ml)#	Dose insulin 454 (nmol/kg/day)	Number of females		Animal numbers	
				Main study	Satellite study	Main study	Satellite study
1	Control	0	0	8	4	1-8	41-44
2	SIAC 30	40	20	8	4	9-16	45-48
3	SIAC 30	160	80	8	4	17-24	49-52
4	SIAC 30	250	125	8	4	25-32	53-56

Expressed in terms of insulin 454 content (SIAC 30, as supplied, contains 420 nmol/ml insulin 454 and 180 nmol/ml insulin aspart)

Group	Treatment	Dose concentration NPH insulin (nmol/ml)*	Dose NPH insulin (nmol/kg/day)	Number of females		Animal numbers	
				Main study	Satellite study	Main study	Satellite study
5	Control	160	80	8	-	33-40	-

* Expressed in terms of the test item (600 nmol/ml of NPH insulin, as supplied) diluted with diluting medium.

Finding:

- **Mortality and clinical signs:**

No deaths and no treatment-related clinical signs occurred in this study.

- **Body weight/food consumption:**

Group mean values of body weight change for SIAC-30 treated females were similar to the control during GDs 6-17. NPH-treated females had slightly lower body weight gain than the control during GDs 6-17 and GDs 11-15.

Group mean values of food consumption for SIAC-30 treated females were similar to the control during GDs 6-17. After treatment was stopped, slightly low food consumption was observed at HD females during Days 18-19. NPH-treated females had marginally lower food consumption than the control during GDs 18-19.

- **Glucose analysis:**

HD females had low mean serum glucose levels at 1, 3, and 9 hrs after dosing (3.53, 2.77, and 3.48 nmol/l respectively) on GD 17 when compared to the control (6.14, 5.73, and 5.86 nmol/l respectively).

MD/LD females had lower mean serum glucose levels at 1 and 3 hrs after dosing when compared to the control. NPH-treated also had low mean serum glucose levels at 1 and 3 hrs after dosing on GD 17.

- **Gross pathology:**

There were no treatment-related findings with females. There were no treatment-related external fetal abnormalities. However, in 1 fetus had whole body oedema in the LD group and 1 fetus had shiny skin in the HD group. In addition, 4/12 in one litter in the control group had cleft palate whereas 1 fetus in the NPH group had shiny skin, exencephaly, rudimentary lower jaw and anophthalmia.

- **Formulation analysis:**

Recovery for insulin 454 was in the range of 73-105% whereas recovery for insulin aspart was in the range of 81-106%. These results were considered valid, confirming accuracy of dose preparation.

- **TK analysis:**

Peak levels of insulin 454 were reached 3 hrs after SC administration, while the highest insulin aspart levels were seen at 1 hr post dosing. The t ½ of insulin 454 was 3-4 hrs, while t ½ of insulin aspart could not determined due to the sparse sampling regime (however considered to be eliminated within the 1st 3 hrs after dosing).

Table 10 Study No 208333 - TK Profiles of Insulin Aspart and Insulin 454 on GD 17 after Repeated Once Daily sc Administration of SIAC in Female Rats

Serum toxicokinetic parameters for insulin 454 in female rats on gestation day 17 after repeated once daily dosing of SIAC.

Group	Dose insulin 454 (nmol/kg)	C_{max} (nM)	t_{max} (h)	$AUC_{(0\text{-}24h)}$ (h*nM)	AUC (h*nM)	$AUC_{\%extra}$	$t_{½}$ (h)
1	0	NC	NC	NC	NC	NC	NC
2	20	36.8	3	155	157	1.5	3.6
3	80	162	3	765	770	0.69	3.1
4	125	186	3	1300	1320	1.1	3.5

NC – Not calculated

Plasma toxicokinetic parameters for insulin aspart in female rats on gestation day 17 after repeated once daily dosing of SIAC.

Group	Dose insulin aspart (nmol/kg)	C_{max} (nM)	t_{max} (h)	$AUC_{(0-3h)}$ (h*nM)	$AUC_{(0-9h)}$ (h*nM)	$AUC_{(0-24h)}$ (h*nM)	AUC (h*nM)	$AUC_{\%extra}$
1	0	NC	NC	NC	NC	NC	NC	NC
2	8.6	0.194	1	0.200	0.253	0.253	0.261	3.0
3	34.3	29.0	1	21.2	21.3	21.8	21.8	0.075
4	53.6	66.5	1	47.3	47.4	47.4	47.4	0.017

NC – Not calculated

- **Litter Findings:**

One MD female was not pregnant at necropsy and another MD female had a very early resorption - excluded from calculation). A total of 8, 8, 6, 8, and 8 females at 0, 20, 80, 125 nmol/kg/day SIAC 30 and 80 nmol/kg/day NPH, respectively, were assessed.

Reproductive assessment: No treatment-related effect upon pregnant outcome. Mean numbers of corpora lutea, implantations, live young and mean % of sex ratio and pre-implantation loss were similar to the control and were not affected by SIAC 30. In MD group, the mean number of early resorptions and the % post-implantation loss were higher than the control, resulting lower mean number of live young. This finding was not seen in either LD or HD group. As for NPH group, the % pre-implantation loss was slightly higher, resulting slightly low mean number of implantations and live young when compared to the control.

Placental, litter and fetal weights: In HD group, mean male and overall fetal weight were marginally lower than controls. Group mean female fetal weights were similar to the control group. In LD or MD group, male, female, and overall fetal weights were similar to control and unaffected by the treatment.

Table 11 Study No 208333 - F0/F1 Data on GD 20 after Repeated Once Daily sc Administration of SIAC in Female Rats

Litter data - group mean values 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-
1F	Mean	13.0	11.5	0.5	0.0	0.5	6.3	4.8	11.0	58.0	11.8	4.3
	SD	1.77	2.00				1.58	2.05	2.07			
	N	8	8	8	8	8	8	8	8	8	8	8
2F	Mean	13.8	12.3	0.4	0.0	0.4	6.0	5.9	11.9	50.6	11.1	3.0
	SD	1.83	1.98				2.33	2.17	2.03			
	N	8	8	8	8	8	8	8	8	8	8	8
3F	Mean	12.8	11.5	2.2	0.0	2.2	5.0	4.3	9.3	53.8	10.8	19.6
	SD	1.17	2.17				1.55	1.86	2.80			
	N	6	6	6	6	6	6	6	6	6	6	6
4F	Mean	12.8	11.4	0.6	0.0	0.6	5.3	5.5	10.8	49.4	11.1	5.9
	SD	1.49	2.07				1.67	2.07	2.31			
	N	8	8	8	8	8	8	8	8	8	8	8
5F	Mean	13.6	10.6	0.6	0.1	0.8	4.8	5.1	9.9	49.5	19.4	7.8
	SD	3.42	2.13				1.39	2.42	2.70			
	N	8	8	8	8	8	8	8	8	8	8	8

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
1F	Mean	0.52	37.93	11.00	3.53	3.29	3.44
	SD	0.042	7.795	2.070	0.174	0.192	0.169
	N	8	8	8	8	8	8
2F	Mean	0.46	39.75	11.88	3.48	3.24	3.36
	SD	0.051	6.211	2.031	0.248	0.252	0.222
	N	8	8	8	8	8	8
3F	Mean	0.50	31.99	9.33	3.48	3.37	3.44
	SD	0.029	9.500	2.805	0.164	0.154	0.128
	N	6	6	6	6	6	6
4F	Mean	0.46	35.66	10.75	3.35	3.26	3.31
	SD	0.054	8.395	2.315	0.273	0.219	0.245
	N	8	8	8	8	8	8
5F	Mean	0.51	35.41	9.88	3.73	3.46	3.58
	SD	0.063	9.796	2.696	0.068	0.165	0.136
	N	8	8	8	8	8	8

Study No 208334 (PIVOTAL): Seg II rat

Study title: Embryo-fetal toxicity study in the Han Wistar rat by subcutaneous administration

Study no.:	208334
Study report location:	Novo Nordisk, Denmark
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 19 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	412_N08702 (purity for insulin 454: 99.0% and purity for insulin aspart: 98.6%)

Key Study Findings

- Treatment of pregnant female rats with SIAC30 from GDs 6-17 at doses of 20, 80, and 125 nmol/kg/day (or 80 nmol/kg/day insulin NPH) was well tolerated.
- No treatment-related mortality and clinical signs were seen.
- There were no adverse maternal or embryo-fetal effects related to SIAC30.
- Based on this study with insulin 454 + 54 nmol/kg/day insulin aspart, NOAELs were 125 nmol/kg/day for maternal toxicity and 20 nmol/kg/day for embryo-fetal toxicity (i.e. skeletal malformations and visceral variations).

Methods

Doses:	0, 20, 80, and 125 nmol/kg/day (vs. 80 nmol/kg/day insulin NPH)
Frequency of dosing:	Once daily from GDs 6-17
Dose volume:	.5 ml/kg/day
Route of administration:	Sc injection
Formulation/Vehicle:	Phenol 1.50 mg/ml, *m*-Cresol 1.72 mg/ml, Glycerol 19.0 mg/ml, Sodium chloride 0.58 mg/ml and water for injection (b) (4) (b) (4) at pH 7.4
Species/Strain:	Han Wistar rat
Number/Sex/Group:	20/sex/group
Satellite groups:	n/a
Study design:	Based on Study No 208333 – DRF study in rats
Deviation from study protocol:	Yes

Group	Treatment	Dose concentration insulin 454 (nmol/ml)#	Dose insulin 454 (nmol/kg/day)	Number of females	Animal numbers
1	Control	0	0	20	1-20
2	SIAC 30	40	20	20	21-40
3	SIAC 30	160	80	20	41-60
4	SIAC 30	250	125	20	61-80

Expressed in terms of insulin 454 content (SIAC 30, as supplied, contains 420 nmol/ml insulin 454 and 180 nmol/ml insulin aspart)

Group	Treatment	Dose concentration NPH insulin (nmol/ml)*	Dose NPH insulin (nmol/kg/day)	Number of females	Animal numbers
5	NPH insulin	160	80	20	81-100

* Expressed in terms of the test item (600 nmol/ml of NPH insulin, as supplied) diluted with diluting medium.

Observations and Results

- **Mortality/Clinical Signs:** twice daily

No treatment-related deaths and clinical signs or changes at the injection sites seen throughout the study. One NPH-treated female showed signs of hypoglycaemia (i.e. underactive behavior, piloerection, dull eyes, and etc on Day 6 after mating approx 3 hrs after 1st dose). This female was given 5% glucose solution by oral gavage and this animal recovered.

- **Body Weight:** Days 0, 3, 6-18, and 20 after mating

In HD females, group mean values of body weight change were marginally higher than the control during GDs 6-10. However, in LD or MD females, group mean values of body weight change were similar to control. In NPH-treated females, group mean values of body weight changes were marginally higher than the control during GDs 6-8.

- **Feed Consumption:** Days 0-2, 3-5, 6-9, 10-13, 14-17 and 18-19 after mating

In HD females, group mean values of food consumption were marginally higher than the control during GDs 6-9. However, the group mean values of food consumption were lower than the control during GDs 18-19 (after the stop of treatment). In LD or MD females, group mean values of food consumption were similar to those of the control and unaffected by the treatment. In NPH-treated females, group mean values of food consumption were marginally higher than the control during GDs 6-9 and thereafter, were similar to the control and unaffected by the treatment.

- **Gross Pathology**

There were no treatment-related findings in SIAC treated females. Several females had dark areas at the injection site, attributed to the injection procedure.

- **Toxicokinetics:** n/a

- **Dosing Solution Analysis**

Recovery of insulin 454 was within the range 95-102%. Recovery of insulin aspart was in the range 87-102%. There results were considered valid, confirming accuracy of preparation.

- **Necropsy:** Day 20 after mating

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): One MD female had only 1 implantation which was an early resorption. Therefore, it was excluded from calculations. The assessment was based on 20, 20, 19, 20, and 20 pregnant females with live young on GD20 in Groups 1, 2, 3, 4, and 5, respectively.

SIAC 30 or NPH insulin had no effect on the mean numbers of corpora lutea, implantations, resorptions, live young, pre- and post-implantation loss or the mean sex ratio.

SIAC 30 or NPH insulin had no affect on mean placental and litter weights when compared to control. However, HD group had marginally (statistically significant) lower group mean male, female, and overall fetal weight than controls (same with NPH insulin treated group). MD group had marginally lower group mean male (statistically significant), female, and overall fetal weight than controls. LD group had similar mean male, female, and overall fetal weights to controls and was unaffected by SIAC30.

Table 12 Study No 208334 - C-Section Data on GD 20 after Repeated Once Daily sc Administration of SIAC in Female Rats

Litter data - group mean values on Day 20 of gestation

Group	:	1	2	3	4	5
Compound	:	Control	SIAC 30	SIAC 30	SIAC 30	NPH insulin
Dose (nmol/kg/day)	:	0	20*	80*	125*	80

Request ID: 167135

* Dose levels expressed in terms of insulin 454 content

Litter data - group mean values 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	13.3	12.3	0.7	0.0	0.7	5.9	5.7	11.6	50.9	7.2	5.7
	SD	2.12	1.89				2.00	1.95	1.93			
	N	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	12.5	11.5	0.8	0.0	0.8	5.7	5.1	10.8	52.3	7.4	6.1
	SD	1.90	1.82				1.93	1.77	1.65			
	N	20	20	20	20	20	20	20	20	20	20	20
3F	Mean	13.7	11.7	0.6	0.0	0.6	5.7	5.3	11.1	52.6	13.3	5.3
	SD	2.73	1.92				1.76	2.11	1.84			
	N	19	19	19	19	19	19	19	19	19	19	19
4F	Mean	12.9	11.6	0.8	0.1	0.8	5.3	5.6	10.8	48.0	9.4	6.4
	SD	1.59	1.31				1.89	1.79	2.26			
	N	20	20	20	20	20	20	20	20	20	20	20
5F	Mean	13.6	12.4	1.2	0.0	1.2	5.7	5.5	11.2	50.8	8.9	9.5
	SD	2.14	1.90				1.89	1.61	1.76			
	N	20	20	20	20	20	20	20	20	20	20	20

Placental, litter and fetal weights - group mean values (g) on Day 20 of gestation

Group	:	1	2	3	4	5
Compound	:	Control	SIAC 30	SIAC 30	SIAC 30	NPH insulin
Dose (nmol/kg/day)	:	0	20*	80*	125*	80

Request ID: 167136

* Dose levels expressed in terms of insulin 454 content

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:		Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	0.52	39.96	11.55	3.57	3.38	3.47
	SD	0.045	6.243	1.932	0.165	0.185	0.161
	N	20	20	20	20	20	20
2F	Mean	0.51	37.26	10.75	3.51	3.39	3.46
	SD	0.043	6.469	1.650	0.250	0.209	0.215
	N	20	20	20	20	20	20
3F	Mean	0.49	36.91	11.05	3.43*	3.28	3.35
	SD	0.055	5.599	1.840	0.185	0.199	0.188
	N	19	19	19	19	19	19
4F	Mean	0.51	35.68	10.80	3.38**	3.23*	3.31**
	SD	0.041	7.952	2.262	0.174	0.170	0.150
	N	20	20	20	20	20	20
5F	Mean	0.51	36.75	11.15	3.42*	3.18**	3.30**
	SD	0.051	5.765	1.755	0.244	0.248	0.234
	N	20	20	20	20	20	20

- **Offspring (Malformations, Variations, etc.)**

HD group had an increased incidence of fetuses/litters with short/bent/thickened humerus and bent scapula with the associated minor skeletal abnormalities in MD and/or HD group (i.e. thickened/kinked ribs, cervical verbetbral centra, and etc) compared to the concurrent control. There were also some minor visceral abnormalities (i.e. in brain, thymus lobe, and umbilical artery, and etc). NPH-insulin treated group had increased incidence of fetuses/litters with left umbilical artery compared with concurrent control. These findings seemed to be within the historical ranges (historical data provided by the applicant).

Table 13 Study No 208334 - Summary Tables of Fetal Examinations after Repeated Once Daily sc Administration of SIAC in Pregnant Female Rats (Historical Data Included)

Fetal examination, major abnormalities - group incidences

Group	1	2	3	4	5
Compound	Control	SIAC 30	SIAC 30	SIAC 30	NPH insulin
Dose (nmol/kg/day)	0	20*	80*	125*	80

* Dose levels expressed in terms of insulin 454 content

Fetal examination, major abnormalities - group incidences

	Fetuses					Litters				
Group	1	2	3	4	5	1	2	3	4	5
Number examined	231	215	210	216	223	20	20	19	20	20
Number affected	1	1	0	4	4	1	1	0	4	4
Partially split sternum	-	1	-	-	-	-	1	-	-	-
Absent cervical arches	-	-	-	-	1	-	-	-	-	1
Bent scapula	-	-	-	3[ab]	2	-	-	-	3	2
Short/bent/thickened humerus	1	-	-	2[ab]	1	1	-	-	2	1
Bent radius	-	-	-	1[b]	-	-	-	-	1	-
Diaphragmatic hernia	-	-	-	1	-	-	-	-	1	-

Superscript denotes fetuses with more than one abnormality

Fetal examination, minor skeletal abnormalities/variants - group incidences

Fetal examination, minor skeletal abnormalities/variants - group incidences

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		114	108	105	109	110	20	20	19	20	20
Skeletal abnormalities											
Cranial	additional ossified centre	-	1	-	-	-	-	1	-	-	-
	bridge of ossification/partially fused/fused maxilla to jugal	13	10	8	13	12	9	8	6	8	7
Ribs	medially thickened/kinked	3	8	-	10	4	2	5	-	6	3
Sternebrae	offset alignment	-	1	-	-	-	-	1	-	-	-
	bipartite ossified	-	-	-	1	-	-	-	-	1	-
Total affected by one or more of the above		16	20	8	22	16	10	12	6	11	9
Rib and vertebral configuration											
Cervical rib		10	5	4	9	5	7	4	4	8	5
Number with 13/14 or 14/14 short supernumerary ribs		51	48	51	54	50	14	16	16	19	17
Full supernumerary 14th rib with/without costal cartilage		3	4	3	6	1	3	2	2	3	1
20 thoracolumbar vertebrae		-	3	3	2	-	-	3	3	2	-
Offset alignment pelvic girdle		-	3	1	3	1	-	3	1	3	1

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

Fetal examination, minor skeletal abnormalities/variants - group incidences

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		114	108	105	109	110	20	20	19	20	20
Incomplete ossification/unossified											
Cranial centres		37	21	8	19	14	15	10	5	12	9
Hyoid		1	2	-	-	-	1	1	-	-	-
Vertebrae	cervical	-	-	-	-	2	-	-	-	-	2
	thoracic	-	2	-	-	1	-	2	-	-	1
	sacrocaudal	7	1	-	1	-	4	1	-	1	-
Sternebrae	5th and/or 6th	30	23	38	37	35	14	13	15	15	14
	other	5	1	8	6	9	4	1	4	6	3
	total	31	23	39	38	38	14	13	15	16	14
Metacarpals		1	-	-	-	-	1	-	-	-	-
Metatarsals		-	-	1	1	-	-	-	1	1	-
Precocious ossification											
Cervical vertebral centra (>5 ossified)		14	24	12	27	21	8	14	8	12	10
Additional observations at necropsy											
Dilated ureter		-	1	-	-	-	-	1	-	-	-
Left umbilical artery		3	4	6	1	7	3	4	4	1	6
Shiny skin		-	-	-	1	-	-	-	-	1	-

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

Fetal examination, minor visceral abnormalities - group incidences

Fetal examination, minor visceral abnormalities - group incidences

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		117	107	105	107	113	20	20	19	20	20
Number affected		26	18	29	20	34	14	13	14	12	17
Visceral abnormalities											
Brain	dilated interventricular foramen	-	-	1	1	-	-	-	1	1	-
Eye(s)	variation in lens shape	-	2	-	-	2	-	2	-	-	2
Thyroid lobe(s)	small	-	-	-	-	1	-	-	-	-	1
Thymus lobe(s)	partially undescended	1	-	1	2	-	1	-	1	1	-
Subclavian artery	arising from descending aorta	-	1	-	-	-	-	1	-	-	-
Azygos vein	dilated	-	1	-	-	-	-	1	-	-	-
Caudal vena cava	anomalous confluence left hepatic vein	-	1	-	-	-	-	1	-	-	-
Diaphragm	thin with liver protrusion(s)	6	3	3	3	5	4	3	3	3	3
Liver	folded/fissured posterior caudate lobe	-	1	1	-	-	-	1	1	-	-
Testis(es)	displaced	3	1	3	4	3	3	1	2	4	3
Umbilical artery	left	8	8	10	9	19	8	8	7	6	13
Haemorrhages	brain	5	2	4	-	5	4	2	4	-	3
	eye	1	-	-	-	-	1	-	-	-	-
	abdominal cavity	3	1	5	1	2	3	1	4	1	1
	liver lobe(s)	1	-	1	-	1	1	-	1	-	1
	subcutaneous	1	-	5	1	1	1	-	2	1	1
Additional observations at necropsy											
	shiny skin	-	-	-	-	1	-	-	-	-	1

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

Background Control data for fetal pathology

Generated outside the protocol for this study.

Study number		1		2		3		4		5	
In house/Supplier mated		IM		IM		IM		IM		IM	
Treatment - vehicle		Sterile Water		21 mg/mL sucrose, 36 mg/mL mannitol, 1.55 mg/mL histidine		21 mg/mL sucrose, 36 mg/mL mannitol, 1.55 mg/mL histidine		21 mg/mL sucrose, 36 mg/mL mannitol, 1.55 mg/mL histidine		Saline	
Treatment period - days of gestation		6 to 17		6 to 9		10 to 13		14 to 17		-45,-30,6,8,11,15	
Route		Gavage		Subcutaneous		Subcutaneous		Subcutaneous		Intramuscular	
Necropsy start date		1.08		2.08		2.08		2.08		3.08	
Major Abnormalities											
Number fetuses/litters examined#		268	22	216	20	218	20	191	19	283	22
Bent/misshapen scapula/clavicle		-	-	5[cdgh]	2	1[b]	1	1[a]	1	3[de]	3
Short/bent/thickened/misshapen long bones		-	-	4[cdgh]	1	1[b]	1	1[a]	1	2[de]	2
Skeletal Abnormalities											
Number fetuses/litters examined#		135	22	109	20	111	20	97	19	143	22
Ribs	medially thickened/kinked	8	7	8	7	19	10	9	5	12	7
Visceral Abnormalities											
Number fetuses/litters examined#		179	22	147	19	168	22	166	22	99	13
Umbilical artery	left	9	7	9	7	9	6	11	8	12	10

#: Individual fetuses/litters may occur in more than one category
IM: In house mated
Superscript denotes linked abnormalities

Study number		6		7		8		9		10	
In house/Supplier mated		IM		IM		IM		IM		IM	
Treatment - vehicle		46.7mg/mL mannitol, 14.9mg/mL sucrose in Water		Air		Sterile water		1% methylcellulose		Solutol HS15 (30%), ethanol (4.4%), Saline (65.6%)	
Treatment period - days of gestation		-14 to 17		-14 to 17		6 to 17		6 to 19		6 to 17	
Route		Subcutaneous		Inhalation		Gavage		Gavage		Subcutaneous	
Necropsy start date		6.08		8.08		11.08		1.09		2.09	
Major Abnormalities											
Number fetuses/litters examined#		248	21	223	22	259	21	261	22	238	19
Bent/misshapen scapula/clavicle		1[a]	1	2[b]	2	2[b]	2	-	-	-	-
Short/bent/thickened/misshapen long bones		1[a]	1	1[b]	1	3[b]	3	-	-	-	-
Skeletal Abnormalities											
Number fetuses/litters examined#		122	21	110	22	129	21	130	22	119	19
Ribs	medially thickened/kinked	4	3	6	6	25	13	4	3	5	3
Visceral Abnormalities											
Number fetuses/litters examined#		165	22	163	22	176	22	166	22	154	21
Umbilical artery	left	9	6	8	7	15	8	8	7	4	4

#: Individual fetuses/litters may occur in more than one category
IM: In house mated
Superscript denotes linked abnormalities

9.3 Prenatal and Postnatal Development

See NDA 203314 for IDeg.

10 Special Toxicology Studies

See NDA 203314 for IDeg with regards to local toxicity studies, impurity study, and other toxicity studies in mice.

Local Tolerance

Local tolerance studies (Study Nos. 206131, 210455, and 210297) were reviewed under NDA 203314. These studies also tested IDegAsp and are summarized briefly here.

In Study No 206131, single sc injection of IDeg at 600 or 1200 nmol/ml or IDegAsp at (b) (4) nmol/ml ("early drug development product") caused mild inflammatory reaction at the injection site in pig on Days 2 and 5 after injection. The reaction seen with IDeg or IDegAsp was comparable to that of 0.9% saline and vehicle for IDeg and less pronounced than that observed with NPH insulin.

In Study No 210455, single sc injection of IDeg at 600 or 1200 nmol/ml or IDegAsp at 600 nmol/ml ("to be marketed drug product") caused mild inflammatory reaction at the injection site in minipigs on Days 2 and 5. The reaction seen with IDeg or IDegAsp was comparable to that of vehicle for IDeg, vehicle for IDegAsp, or NPH insulin.

In Study No 210297, local reactions were mild and comparable to that of the corresponding vehicles or NPH insulin in rabbits given a single dose of insulin 600 or 1200 nmol/ml or 600 nmol/ml IDegAsp ("to be marketed drug product").

Impurity

Impurities were investigated in 2 studies: Study No 210227 (4 wk sc toxicity study, comparing aged and non-aged insulin (IDeg only; reviewed under NDA 203314) and Study 210228 (4 wk sc toxicity study, comparing aged and non-aged insulin (IDegAsp only).

Study No. NN210228: 1 mo rat [impurity – aged vs. non-aged]

1-month subcutaneous toxicity study in rats (bridging study) [by (b) (4)]

Study design: This GLP study was to compare the potential toxicity of non-aged IDegAsp (b) (4) and aged IDegAsp (b) (4) at (b) (4)% IDeg and (b) (4)% IAsp ratio ((b) (4) nmol/kg/day) administered daily by sc injection to rats for 4 week. Accelerated aging of IDegAsp was performed by placing the formulations in an oven at 37 degree for approx. 3 months. The aging of the formulation was terminated at the time when approx. (b) (4)% of the initial amount of IDegAsp remained in the formulations. The aged formulation contained impurities, corresponding to a purity of (b) (4)% for the IDeg and (b) (4)% for the IAsp after storage at 37 °C for 3 months to force

degradation whereas the non-aged formulation contained impurities, corresponding to a purity of (b) (4)% for the IDeg and (b) (4)% for the IAsp. Endpoints included clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, TK/serum glucose analyses, and organ weights, macroscopic and microscopic examinations.

Group	Compound	Compound	Dose*# (nmol/kg/day)	Dose concen-tration (nmol/ml) *	Animal Nos (Main study)		Animal Nos (Satellite)		Colour code
					Male	Female	Male	Female	
1	-	-	0 (vehicle)	0	1-10	11-20	101-108	109-116	White
2	Non-aged	(b) (4)	71.43	71.43	21-30	31-40	117-124	125-132	Blue
3	Non-aged		142.86	142.86	41-50	51-60	133-140	141-148	Green
4	Aged		71.43	71.43	61-70	71-80	149-156	157-164	Red
5	Aged		142.86	142.86	81-90	91-100	165-172	173-180	Yellow

*Material as supplied

the dose refers to the sum of insulin degludec and insulin aspart

Observations and Results

- Dose formulation analysis: The results were within the ±20% acceptance criteria.
- Clinical signs: No test article related clinical signs were observed. A few animals (2 in Group 4 had wounds at injection sites.
- Body weights: The higher body weight of the groups (of both sexes) treated with IDegAsp compared to the control was considered related to the pharmacologic effect of IDegAsp. There were no differences between treated and control groups or the between aged and non-aged IDeg groups.
- Food consumption: Statistically significantly higher food consumption were seen in some occasions in animals treated with IDegAsp when compared to the control animals. No statistically significant change was observed in the total food consumption; however, a treatment related trend was apparent. There were no differences between the 2 insulin batches were noted.
- Ophthalmoscopy: No test article related findings were observed.
- Hematology: No differences were seen between two 2 IDeg aged and non-aged batches. There were some minor changes (had been considered indicative of slight dehydration or related to local inflammation at the sc injection site) when compared to control.

Haematology changes compared to control

Parameter	Group 1 (control)		Group 2 (71.43 IDegAsp non-aged nmol/kg)		Group 3 (142.86 IDegAsp non-aged nmol/kg)		Group 4 (71.43 IDegAsp aged nmol/kg)		Group 5 (142.86 IDegAsp aged nmol/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Haematocrit	45.2	45.6	48.2 (*)	46.1	47.3	45.5	47.9 (*)	46.4	47.6	46.0
Mean cell volume	54.1	54.9	56.7 (**)	56.2	56.9 (**)	57.0	57.0 (**)	56.7	56.8 (**)	57.7 (**)
White blood cells	9.08	10.4	7.82	8.74	9.38	7.44 (*)	9.87	9.45	8.49	7.85 (*)
% Neutrophils	7.6	7.0	9.4	10.1 (*)	12.6 (***)	10.5 (**)	11.1 (**)	11.5 (***)	11.0 (**)	9.8 (*)
Neutrophils	0.71	0.73	0.71	0.88	1.18 (**)	0.79	1.12 (*)	1.15	0.95	0.77
% Lymphocytes	91.3	92.1	89.4	88.6 (*)	86.2 (***)	88.2 (*)	87.6 (**)	86.6 (***)	87.4 (**)	89.1
Lymphocytes	8.31	9.55	7.01	7.76	8.07	6.54 (**)	8.62	8.11	7.44	7.00 (*)

* means p <0.05 vs. reference group
** means p <0.01 vs. reference group
*** means p <0.001 vs. reference group

- Clinical chemistry: Several parameters in clinical chemistry were changed due to the pharmacologic effect of insulin. However, no difference between the aged and non-aged IDeg was seen.

Clinical chemistry compared to control

Parameter	Group 1 (control)		Group 2 (71.43 IDegAsp non-aged nmol/kg)		Group 3 (142.86 IDegAsp non-aged nmol/kg)		Group 4 (71.43 IDegAsp aged nmol/kg)		Group 5 (142.86 IDegAsp aged nmol/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Alkaline phosphatase	4.73	2.45	4.12	2.63	3.28 (**)	2.66	3.79	2.53	3.42 (*)	2.96
Triglyceride	1.57	1.47	1.40	1.03 (*)	0.73 (***)	0.92 (**)	0.96 (**)	1.01 (*)	0.81 (***)	0.78 (***)
Urea	9.30	9.06	10.07	12.27 (***)	11.42 (***)	12.58 (***)	11.75 (***)	11.73 (***)	11.03 (**)	11.55 (***)
Creatinine	21.5	21.0	18.3 (**)	20.1	18.8 (*)	18.7	17.9 (**)	20.4	19.0 (*)	19.0
Glucose	7.69	6.99	2.90 (***)	3.59 (***)	2.15 (***)	2.73 (***)	3.23 (***)	3.45 (***)	2.17 (***)	3.14 (***)
Inorganic phosphorus	2.38	1.99	1.88 (**)	1.62 (*)	1.97 (**)	1.74	2.29	1.76	2.10	1.94
Chloride	105.1	103.4	107.3	106.8	104.7	106.4	106.6	107.2 (*)	106.5	108.8 (**)
Protein	65.4	69.1	63.8	67.1	63.4	62.3 (*)	64.5	64.6	62.4	67.4

- Toxicokinetic: All animals dosed with either non-aged or aged IDegAsp for 4 weeks were systemically exposed to IDeg and IAsp. For IDeg, there were no large differences in the systemic exposure after dosing of either non-aged or aged IDegAsp at two does levels. The TK of IDeg in rats was gender-independent and systemic exposure increased proportionally with dose between 50 and 100 nmol/kg/day. Accumulation in system exposure to IDeg was low. For IAsp, maximum plasma concentration was seen after 1 hr which was also the 1st sampling time. Most plasma samples taken beyond this time point had plasma concentrations < LLOQ. Cmax of IAsp increased with dose and over time. There were no large differences between the IAsp Cmax after sc administration of non-aged and aged IDegAsp at 2 dose levels.

Mean toxicokinetic parameters for IDeg after once daily s.c. administration of non-aged and aged IDegAsp to male and female rats for 4 weeks.

Day	Group (formula-tion)	Sex	IDeg dose nmol/kg/day	C_{max} nM	t_{max} h	AUC_{last} h*nM	t_{last} h	AUC_{0-24h} h*nM	AUC h*nM	$\%AUC_{extra}$	Rac_{obs}
1	2 (non-aged)	F	50	136	1	562	24	562	564	0.284	NR
		M	50	97.3	3	464	9	NR[a]	489	5.04	NR
	3 (non-aged)	F	100	236	3	1100	24	1100	1100	0.222	NR
		M	100	243	3	1320	24	1320	1320	0.22	NR
	4 (aged)	F	50	80.7	3	377	9	NR[a]	398	5.33	NR
		M	50	96.1	1	465	9	NR[a]	505	8.09	NR
	5 (aged)	F	100	224	3	1050	24	1050	1060	0.231	NR
		M	100	213	3	940	24	940	945	0.467	NR
28	2 (non-aged)	F	50	151	3	801	24	801	841	4.78	1.42
		M	50	177	3	872	24	872	877	0.612	NR[a]
	3 (non-aged)	F	100	278	3	1370	24	1370	1400	1.88	1.25
		M	100	274	3	1690	24	1690	1700	0.989	1.28
	4 (aged)	F	50	111	3	697	24	697	704	0.99	NR[a]
		M	50	135	3	864	24	864	876	1.35	NR[a]
	5 (aged)	F	100	290	3	1490	24	1490	1500	0.772	1.46
		M	100	246	3	1520	24	1520	1540	1.17	1.62

F=Female, M=Male. NR=Not reported. [a] Last observable concentration on Day 1 at 9h (t_{last}) so AUC_{0-24h} not available

Mean toxicokinetic parameters for IDeg after once daily s.c. administration of non-aged and aged IDegAsp to male and female rats for 4 weeks.

Day	Group (formula-tion)	Sex	IDeg dose nmol/kg/day	C_{max} nM	t_{max} h	AUC_{last} h*nM	t_{last} h	AUC_{0-24h} h*nM	AUC h*nM	$\%AUC_{extra}$	Rac_{obs}
1	2 (non-aged)	F	50	136	1	562	24	562	564	0.284	NR
		M	50	97.3	3	464	9	NR[a]	489	5.04	NR
	3 (non-aged)	F	100	236	3	1100	24	1100	1100	0.222	NR
		M	100	243	3	1320	24	1320	1320	0.22	NR
	4 (aged)	F	50	80.7	3	377	9	NR[a]	398	5.33	NR
		M	50	96.1	1	465	9	NR[a]	505	8.09	NR
	5 (aged)	F	100	224	3	1050	24	1050	1060	0.231	NR
		M	100	213	3	940	24	940	945	0.467	NR
28	2 (non-aged)	F	50	151	3	801	24	801	841	4.78	1.42
		M	50	177	3	872	24	872	877	0.612	NR[a]
	3 (non-aged)	F	100	278	3	1370	24	1370	1400	1.88	1.25
		M	100	274	3	1690	24	1690	1700	0.989	1.28
	4 (aged)	F	50	111	3	697	24	697	704	0.99	NR[a]
		M	50	135	3	864	24	864	876	1.35	NR[a]
	5 (aged)	F	100	290	3	1490	24	1490	1500	0.772	1.46
		M	100	246	3	1520	24	1520	1540	1.17	1.62

F=Female, M=Male. NR=Not reported. [a] Last observable concentration on Day 1 at 9h (t_{last}) so AUC_{0-24h} not available

Mean toxicokinetic parameters for IAsp after once daily s.c. administration of non-

aged and aged IDegAsp to male and female rats for 4 weeks.

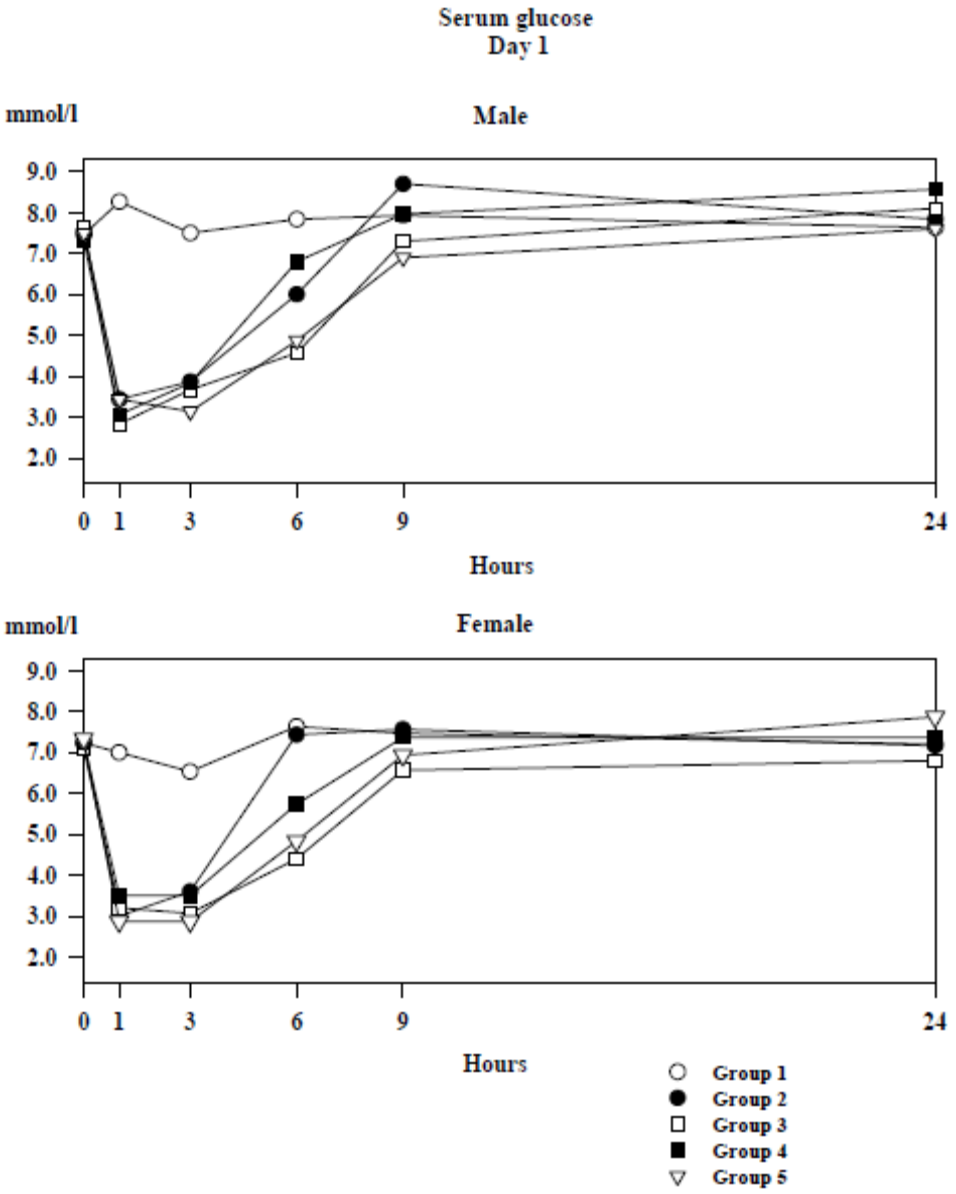
Day	Group (formulation)	IAsp dose	Sex	C_{max}	t_{max}
		nmol/kg/day		nM	h
1	2 (non-aged)	21.43	Female	2.75	1.00
			Male	4.31	1.00
	3 (non-aged)	42.86	Female	10.1	1.00
			Male	17.0	1.00
	4 (aged)	21.43	Female	2.85	1.00
			Male	3.09	1.00
	5 (aged)	42.86	Female	10.9	1.00
			Male	15.5	1.00
28	2 (non-aged)	21.43	Female	5.96	1.00
			Male	5.67	1.00
	3 (non-aged)	42.86	Female	16.5	1.00
			Male	27.3	1.00
	4 (aged)	21.43	Female	10.7	1.00
			Male	9.80	1.00
	5 (aged)	42.86	Female	18.7	1.00
			Male	22.5	1.00

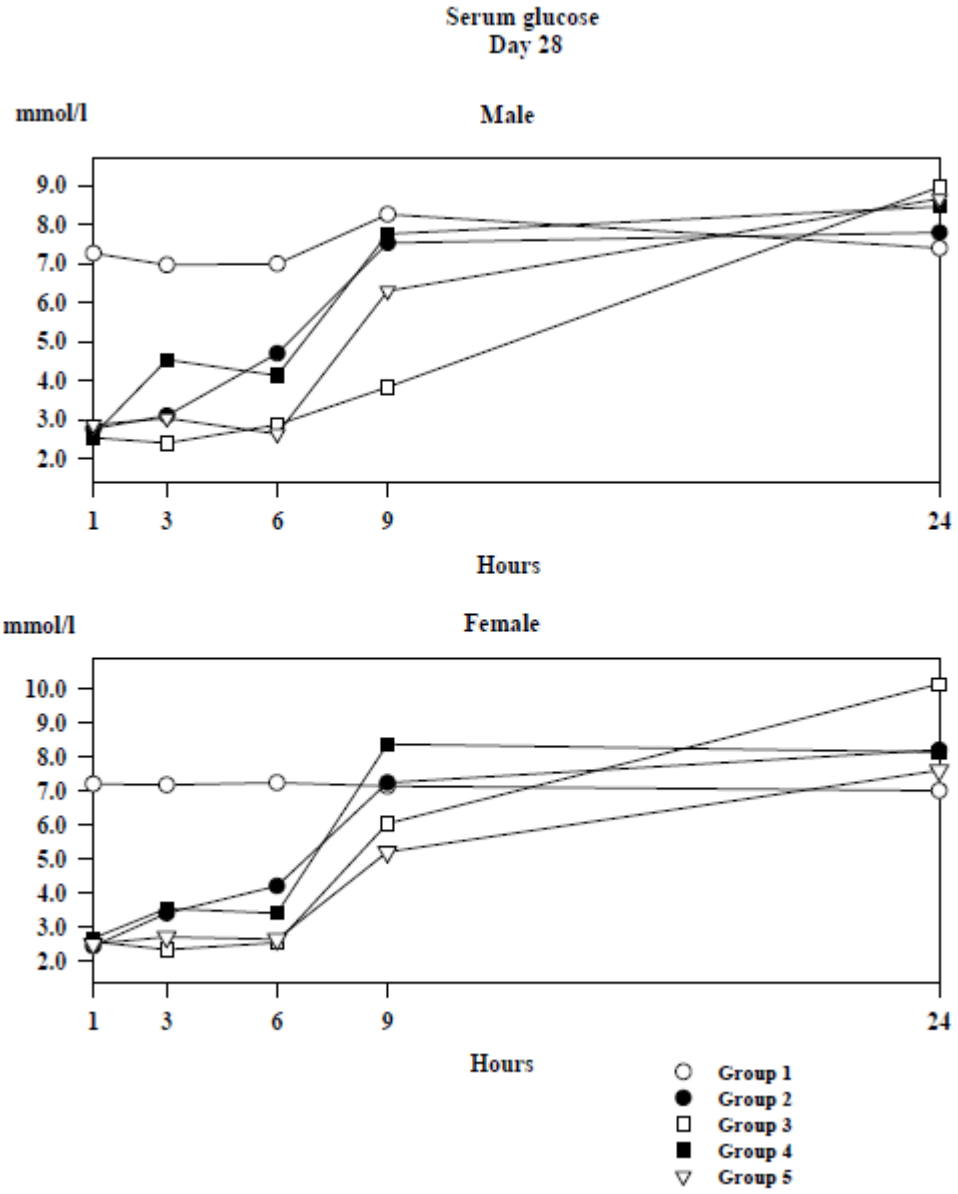
IDeg aged/non-aged ratio of $AUC_{0\text{-}24h}$ (female and males combined)

Day	IDeg dose	Mean $AUC_{0\text{-}24h}$ (females and males)		Aged/Non-aged
	nmol/kg/day	Aged IDegAsp (groups 4 and 5)	Non-aged IDegAsp (groups 2 and 3)	
1	50	NR	562	NR
28		781	837	0.93
1	100	995	1210	0.82
28		1505	1530	0.98

NR=not reported

- Serum glucose level: Day 1: a significantly lower serum glucose level was seen 1 and 3 hr after treatment. Six hrs after treatment, the levels were rising but still notably lower than normal. No differences were seen between the aged and non-aged batches. Day 28: for males, the glucose serum glucose level was significantly lower 1 hr after treatment. Due to variances in the results from the 3 hrs samples, no statistically significant was reached. However, similar to the 1 hr data, 6 hrs after treatment, the glucose level had increased, reaching to normalized level 9 hrs after treatment. Only minor differences were seen between the aged and non-aged IDegAsp. These were considered incidental.


Serum glucose
Day 1
mmol/l
Male
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0
1
3
6
9
24
Hours
mmol/l
Female
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0
1
3
6
9
24
Hours
Group 1
Group 2
Group 3
Group 4
Group 5



- Organ weights: Lower relative liver weights were seen in IDegAsp treated males (significant) and females (not significant) compared to controls. Lower absolute and relative spleen weight was seen in IDegAsp treated males (w/o histo correlates). Higher testes weight was observed in some IDeg treated groups compared to controls. In addition, higher relative heart weight was seen in Group 4 (aged IDegAsp) females compared to Group 2 (non-aged IDegAsp) females. There findings were considered incidental.

- Macroscopic findings: A red discoloration was seen at the injection sites in all groups and no differences were seen when comparing aged and non-aged IDegAsp.

Injection site reactions

Dose group	Group 1 (control)		Group 2 (71.43 IDegAsp non-aged nmol/kg)		Group 3 (142.86 IDegAsp non-aged nmol/kg)		Group 4 (71.43 IDegAsp aged nmol/kg)		Group 5 (142.86 IDegAsp aged nmol/kg)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals examined	10	10	10	10	10	10	10	10	10	10
Injection site										
Discoloration: red										
Total	2	2	6	4	3	2	3	2	3	0
Slight degree	2	2	4	4	3	1	3	1	3	-
Moderate degree	-	-	2	-	-	-	-	1	-	-
Marked degree	-	-	-	-	-	1	-	-	-	-
Sore, single										
Total	-	1	-	-	-	-	1	-	-	-

- Microscopic findings: Liver, spleen, and injection site findings were considered test-article related. The incidence of rarefaction (intracellular glycogen accumulation) of the hepatocytes had decreased in animals of both sexes in all treated groups (more pronounced in the females). No different was seen between the dose groups treated with aged or non-aged IDegAsp. The amount of extramedullary haematopoiesis in the spleen was found lower in the males in Group 3 compared to Group 1. No difference was seen in the females or in other groups in males. This decrease was considered incidental. Changes were seen in the sc injection sites of animals from all dose groups. No differences in the local reaction were observed after dosing with aged or non-aged IDegAsp. The local tissue reaction was considered to be related to the injection procedure.

Rarefaction of hepatocytes

Dose group	Group 1 (control)		Group 2 (71.43 IDegAsp non-aged nmol/kg)		Group 3 (142.86 IDegAsp non-aged nmol/kg)		Group 4 (71.43 IDegAsp aged nmol/kg)		Group 5 (142.86 IDegAsp aged nmol/kg)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals examined	10	10	10	10	10	10	10	10	10	10
Liver										
Rarefaction										
Total	10	8	6	1	5	1	6	-	9	-
Minimal	8	8	6	1	5	1	6	-	9	-
Slight	2	-	-	-	-	-	-	-	-	-

Overview of extramedullary haematopoiesis

Dose group	Group 1 (control)		Group 2 (71.43 IDegAsp non-aged nmol/kg)		Group 3 (142.86 IDegAsp non-aged nmol/kg)		Group 4 (71.43 IDegAsp aged nmol/kg)		Group 5 (142.86 IDegAsp aged nmol/kg)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals examined	10	10	10	10	10	10	10	10	10	10
Spleen										
Extramedullary haematopoiesis (EMH)										
Total	7	10	8	10	2	9	9	10	6	8
Minimal	3	2	5	1	2	6	5	2	5	3
Slight	4	6	2	5	-	1	3	4	1	3
Moderate	-	2	1	4	-	2	1	4	-	2

Details concerning injection site reactions

Dose group	Group 1		Group 2		Group 3		Group 4		Group 5	
	Vehicle Control		50/21.43 XCQ0040_N nmol/kg/day		100/42.86 XCQ0040_N nmol/kg/day		50/21.43 XCQ0040_G nmol/kg/day		100/42.86 XCQ0040_G nmol/kg/day	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals examined	10	10	10	10	10	10	10	10	10	10
Injection site										
Crust										
Total	0	3	0	0	0	0	1	0	0	1
Slight	-	2	-	-	-	-	1	-	-	1
Marked	-	1	-	-	-	-	-	-	-	-
Ulceration										
Total	0	1	0	0	0	0	1	0	0	0
Slight	-	1	-	-	-	-	-	-	-	-
Moderate	-	-	-	-	-	-	1	-	-	-
Necrosis focal sc										
Total	4	3	4	3	4	7	5	3	4	5
Minimal	-	3	1	2	-	2	2	2	2	4
Slight	2	-	3	1	2	4	2	1	1	1
Moderate	2	-	-	-	2	1	1	-	1	-
Inflam. cells focal										
Total	10	10	10	10	10	10	10	10	10	10
Minimal	3	3	2	3	3	2	3	4	2	2
Slight	3	4	4	6	3	5	5	2	6	4
Moderate	4	3	4	1	4	3	2	4	2	4

Overall Finding:

- This study showed that dosing with aged and non-aged IDegAsp at 71.42 or 142.86 nmol/kg/day for 28 days resulted in test-article related changes in body weight, food consumption, blood glucose, some clinical pathology parameters, liver weight, and liver rarefaction.

- The changes were comparable between non-aged and aged IDegAsp and were considered related to the pharmacological action of IDegAsp.
- In addition, there was no clear difference in injection site reactions between aged and non-aged IDeg.

11 Integrated Summary and Safety Evaluation

Note: The entire review mainly focuses on the co-formulation product (IDegAsp). The detailed review for insulin degludec is under NDA 203314.

Drug Product: Insulin Degludec + Insulin Aspart (IDegAsp)

The applicant submitted NDA 203313 for IDegAsp (100 U/ml strength containing 420 nmol/ml insulin degludec + 180 nmol/ml insulin aspart). This is a co-formulation of IDeg (long-acting basal insulin) and IAsp (fast-acting insulin). IDegAsp is intended for once-daily or twice-daily sc injection (in two presentations: (b) (4) pre-filled disposable PDS290 pen injector) with the main meals.

The nonclinical studies of IDeg are being reviewed under NDA 203314. The structure of IDeg allows IDeg to form soluble and stable multi-hexamers, resulting in a depot in the sc tissue after injection. A gradual separation of IDeg monomers results in a slow and continuous delivery of IDeg from the sc injection site into the circulation. On the other hand, insulin aspart is an active ingredient of the marketed products NovoRapid/NovoLog (NDA 20986). The safety and efficacy of insulin aspart is well established.

Pharmacology Studies

One in vitro study (Study No ars-23-aug-2005) was performed, where different ratios of IDeg and IAsp were tested in rat adipocytes. This study showed that the effects of IDeg and IAsp were additive and there were no synergistic or inhibitory interactions between the two.

Two in vivo studies (Study Nos: 6ulr051108-100-mar-2006 and UIR060904-0100 Feb-2007: euglycemic clamp studies in pigs) were conducted. These studies demonstrated (b) (4). One study used (b) (4) insulin molecules formulation. The study showed that a (b) (4) of IDeg:IAsp resulted in a (b) (4), when compared to those observed for biphasic insulin aspart 30 (NovoMix). Higher IDeg:IAsp ratio (i.e. 5:1 and 8:1) showed a difference in the PK profile compared to IDeg and IAsp dosed separately. However, in another study using (b) (4) molecules formulation, there was no alteration in the glucose infusion rate (GIR) or PK profiles of either IAsp or IDeg observed when compared to separate injections. These findings demonstrated that zinc played a role in the ability of IDeg (b) (4), consisting IDeg and IAsp in the formulation and upon sc injection. Based on various formulations and IDeg:IAsp ratios tested in these studies, zinc concentration and insulin ratios were optimized for later nonclinical studies.

Pharmacokinetics Studies

The PK of IDegAsp was studied in Wistar rat (Study Nos: 208289 and 208337: 4-week and 13-week repeated dose studies) and LYD pigs (Study Nos: 204383, 205053, 205220, and 205419). Studies in rats used the "to be marketed" formulation, where studies in pigs used different IDegAsp formulation (to support early clinical studies). These studies demonstrated that IDeg could be administered co-formulated with IAsp without changing the individual PK profiles of the two insulin molecules.

Table 14 PK Profiles of IDeg and IAsp Administered (Alone vs. In Co-formulation)

Dose-normalized (1 nmol/kg) mean pharmacokinetic of insulin degludec and insulin aspart administered alone of in a co-formulation (IDegAsp)

Species	Administration	Insulin degludec			Insulin aspart		
	Single-dose	C_{max} (nmol/L)	$AUC_{(0-24h)}$ (h×nmol/L)	$t_{½}$ (h)	C_{max} (nmol/L)	$AUC_{(0-24h)}$ (h×nmol/L)	$t_{½}$ (h)
Rat	IDegAsp[a]	2.25	12.1	3.2	0.354	0.308	0.3
	insulin degludec[b]	2.46	11.9	3.1			
	insulin aspart[c]				0.497	0.352	0.4
Pig	IDegAsp[d]	0.98	9.63	7.0	0.578	0.694	0.6
	insulin degludec[e]	1.67	12.3	5.5			
	insulin aspart[f]				0.624	0.693	0.5

a – Study No.: 208289, 208337
b – Study No.: 206315, 206539
c – Data obtained from [16]
d – Study No.: 204383, 205053, 205220, 205419
e – Study No.: 204342
f – Study No.: 204383, 205053, 205220, 205419

Repeat-dose Toxicity Studies (for IDegAsp Studies Only)

The **4 week DRF study** (Study No 208289) in rats showed that the sc administration of 2 different fixed combinations of insulin 454/insulin aspart (SIAC 30 (B) and SIAC 45 (B)) resulted in an expected decrease in plasma glucose, with consequential signs (i.e. hypoglycemia) that were similar for both combinations. For SIAC 30(B), hypoglycemia-related signs occurred mostly in HD males (1 male premature sacrifice). The MD level of SIAC 30 (B) was well tolerated. For SIAC 45(B), hypoglycemia-related signs were seen in 1 MD male (premature sacrifice). Besides this one death, even the HD level of SIAC 45 (B) was well tolerated. Therefore, this study concluded that MD (75 mg/kg/day) of SIAC 30 (B) and HD (100 mg/kg/day) of SIAC 45(B) were suitable for their pivotal study. The SIAC40 was used in their pivotal toxicology studies (i.e. 13 week tox study and Seg I/II studies).

The majority of other study findings seen in this 4-week toxicity study (i.e. increased food consumption and body weight gains) were considered secondary to the glucose lowering effect of these test articles. Similarity, the low plasma triglyceride concentrations seen in treated groups were considered an effect on fatty acid metabolism, secondary to the low plasma glucose levels. There were also effects on protein metabolism since there were some changes in α- globulin in SIAC 45(B) animals. Hematological changes (i.e. slightly low lymphocyte counts in HD males or HD females, slightly low neutrophil and eosinophil counts in females, with either test articles) were seen. However, these were not indicative of adverse findings since limited histopathological exam was conducted. Clinical chemistry changes (i.e. increased phosphorus with both combinations and low sodium and chloride with SIAC 30(B)) were seen. These could reflect changes in water balance. In addition, there were decreases in liver weights in both sexes at all doses which were likely as a result of depletion of glucose stores (glycogen) but not confirmed with histopathological exam. Reduced spleen weight was seen in HD males with SIAC 45(B). However, this was not indicative of any adverse effect since limited histopathological exam was done. Furthermore, there was a small increase of axillary lymph node weights at HD males, suggesting a minimal response following repeated sc injection but was not considered to be an adverse finding.

The **13-week pivotal study** (Study No 208337) in rats showed that the sc administration of a fixed combination up 75 nmol/kg/day insulin 454 and up to 32 nmol/kg/day insulin aspart, SIAC 30, resulted in an expected decrease in plasma glucose, with consequential signs (i.e. hypoglycemia). There was transient underactivity seen in MD and HD animals in Week 4 or 5 (also seen in NPH treated animals). Three animals had hypoglycemia shock (1 HD female in Week 13, 1 satellite MD female, 1 NPH male), resulted in one death (1 satellite MD female). The NOAEL was established at 36 nmol/kg/day (25 nmol/kg/day insulin 454 + 11 nmol/kg/day insulin aspart).

The majority of other study findings seen in this 13-week toxicity study (i.e. increased food consumption and body weight gains, especially in males) were considered secondary to the glucose lowering effect of SIAC 30. Similarity, the low plasma triglyceride concentrations seen in treated groups were considered an effect on fatty acid metabolism, secondary to the low plasma glucose levels. There were also effects on protein metabolism as indicated by the reduced plasma urea levels in males and low protein levels in females (a consequence of reduced albumin and α- globulin), as well as urea levels. Hematological changes (i.e. slightly low lymphocyte and neutrophil counts in females at all doses and slightly low basophil an dmonocyte counts in MD/HD females) were seen. However, these were not indicative of adverse findings. Clinical chemistry changes (i.e. low plasma calcium in females) were seen, which likely had been secondary to the lower plasma albumin levels. In addition, there were slight decreases in liver weights in MD/HD females which were likely as a result of depletion of glucose stores (glycogen). There were also low urinary volumes and high specific gravity at all doses and slight higher plasma phosphorus levels in HD animals, reflecting minor changes in water balance with no indicative of renal toxicity.

Systemic exposure to IDeg and IAsp was confirmed in animals dosed with IDegAsp. Antibodies towards IDeg and IAsp were detected in approx 1/3 of the animals. However, decreased blood glucose levels and/or decreased Deg/IAsp exposure seen in antibody-positive animals suggested that the formed antibodies did not have neutralizing effects.

Table 15 Summary Table of Pivotal Repeat Dose Studies in Rats (IDeg or IDegAsp)

Study No.	205239	206315	206539	208337
Species/strain	Wistar rats	Wistar rats	Sprague-Dawley rats	Wistar rats
Test article	Insulin degludec	Insulin degludec	Insulin degludec	IDegAsp[c]
Duration (weeks)	4	26	52	13
Comparator	None	NPH insulin	NPH insulin	NPH insulin
Route of administration	s.c	s.c	s.c	s.c.
Animals/sex/group	Main study: 4 groups: 10 males and 10 females Satellite study: 4 groups: 9 males and 9 females	Main study: 4 groups: 20 males and 20 females Satellite study: 4 groups: 12 males and 12 females Recovery study: 2 groups: 10 males and 10 females Comparator group: 20 males and 20 females Comparator satellite group: 12 males and 12 females	Main study: 3 groups: 40 males and 40 females and one group of 50 males and 50 females (high-dose) Comparator group: 50 males and 50 females	Main study: 4 groups: 10 males and 10 females Satellite study: 4 groups: 6 males and 6 females (vehicle), 14 males and 14 females (IDegAsp) Recovery study: 2 groups: 10 males and 10 females Comparator group: 10 males and 10 females Comparator recovery: 10 males and 10 females
Dose levels (nmol/kg/day)	0, 25, 150, 250	0, 20, 50, 125 and 80/50[a] NPH insulin	0, 20, 65/50/40[b], 100/80/60[b] and 65/50/40[b] NPH insulin	0, 36, 71, 107 and 75 NPH insulin
Conclusion	NOEL: <25 nmol/kg/day NOAEL: 250 nmol/kg/day	NOEL: <20 nmol/kg/day NOAEL: 125 nmol/kg/day	NOEL: <20 nmol/kg/day NOAEL: 60 nmol/kg/day	NOEL: <36 nmol/kg/day NOAEL: 107 nmol/kg/day

a - Dose-level reduced study day 130 due to hypoglycaemia and hypoglycaemia-related mortality

b - Dose-levels reduced study day 76 and again study day 225 due to hypoglycaemia and hypoglycaemia-related mortality

c - Insulin degludec co-formulated with insulin aspart in a ratio of 70:30

Table 16 Summary Table of Antibodies against IDeg or IAsp from Pivotal Repeat Dose Studies for IDeg (in Rats and Dogs) and IDegAsp (in Rats)

Number of animals with antibodies against insulin degludec, insulin aspart or NPH insulin

Species	Rat				Dog	
Test article	Insulin degludec			IDegAsp	Insulin degludec	
Study duration in weeks (study identification)	4 (205239)	26 (206315)	52 (206539)	13 (208337)	4 (205238)	26 (206314)
Insulin degludec antibody positive animals / total number of animals[a]	7 / 54	7 / 51	1 / 213	46 / 143	0 / 18	0 / 23
Insulin aspart antibody positive animals / total number of animals[a]	-	-	-	44 / 143	-	-
Insulin degludec antibody positive animals / total number of animals[a] - after 4 weeks recovery	-	2 / 16	-	5 / 20	-	0 / 4
Insulin aspart antibody positive animals / total number of animals[a] - after 4 weeks recovery	-	-	-	4 / 20	-	-
NPH insulin antibody positive animals / total number of animals[a]	-	9 / 14	1 / 79	12 / 20	-	6 / 6

-: Not applicable
a –- Only insulin degludec, IDegAsp or NPH insulin dosed animals included

Reproductive and Developmental Toxicity (Seg II) Studies

The **Seg II RF study** in rats (Study No 208333) showed that SIAC30 at 20, 80, or 125 nmol/kg/day (expressed in term of insulin 454) from GDs 6-17 was well-tolerated with no deaths and no treatment-related clinical signs. There was a dose-proportional systemic exposure to insulin 454 and insulin aspart in satellite female rats receiving SIAC30. SIAC30 had no effect upon body weight or food consumption during GDs 6-17. After treatment period, MD or HD females had marginally reduced body weight gain and HD females had slightly low food consumption. SIAC 30 was also associated with a lowering of serum glucose levels on Day 17 after mating.

In addition, there was no effect of SIAC 30 upon pregnancy outcome or embryo-fetal survival. No external findings in fetuses were seen. However, in HD group, there were marginally lower group mean male and overall fetal weights than the control. Based on these study findings, SIAC30 at 20, 80, or 125 nmol/kg/day (expressed in term of insulin 454) would be appropriate for their pivotal study.

The **Seg II pivotal study** in rats (Study No 208334) showed that SIAC30 at 20, 80, or 125 nmol/kg/day (expressed in term of insulin 454) or NPH insulin at 80 nmol/kg/day

from GDs 6-17 was well-tolerated with no deaths, no treatment-related clinical signs. One NPH-insulin treated female did have hypoglycaemia-related signs (i.e. underactive, piloerection, hunched posture, and etc). SIAC30 did not affect body weight or food consumption during the treatment period. However HD group had marginally low food consumption on GDs 18-19 (after the treatment period). There was no effect upon pregnancy outcome or embryo-fetal survival with SIAC30 or NPH insulin. Mean placental weights were similar to control across all groups. HD group had marginally (5%) but statistically significantly lower male, female, and total fetal weights than controls whereas MD group had marginally but statistically significantly lower male fetal weights than controls. Due to small magnitude and there were no effects on embryo-fetal survival and fetal morphological development, the SIAC30 did not present an adverse effect on embryo-fetal development. In addition, NPH-insulin treated group had similar mean placental weights to controls, and had marginally lower mean litter weight, mean male, female and overall fetal weights than controls. Lastly, HD group had an increased incidence (still within historical range) of litters with short/bent/thickened humerus and bent scapula with the associated minor skeletal abnormality (i.e. medially thickened/kinked ribs) and NPH insulin-treated group had an increased incidence (still within historical control) of litters with left umbilical artery, compared to their concurrent controls. These were not considered to be SIAC30 or NPH insulin related adverse effects. Based on theses study findings, the NOAEL of SIAC 30 for maternal and embryo fetal toxicity was 125 nmol/kg/day insulin 454 + 54 nmol/kg/day insulin aspart.

Table 17 Summary Tables of Seg II Studies in Rats (for IDegAsp or IDeg)

Study ID	208333	208334
Type of study	Preliminary embryo-foetal toxicity	Embryo-foetal toxicity
Species/strain	Wistar rats	Wistar rats
Test article	IDegAsp	IDegAsp
Comparator	NPH insulin	NPH insulin
Route of administration	s.c	s.c
Animals/sex/group	4 groups: 8 females Comparator group: 8 females	4 groups: 20 females Comparator group: 20 females
Dose levels (nmol/kg/day)	IDegAsp: 0, 29, 114, 179 NPH insulin: 80	IDegAsp: 0, 29, 114, 179 NPH insulin: 80
Dosing period	Females: day 6 to 17 after mating	Females: day 6 to 17 after mating
Result/conclusion	Treatment of pregnant female rats with IDegAsp up to 179 nmol/kg/day was well tolerated with no deaths and no treatment-related clinical signs. At 179 nmol/kg/day IDegAsp, mean male and overall foetal weights were marginally lower than in the control group. However, overall IDegAsp had no effect on embryo-foetal survival and development.	Treatment of pregnant female rats with IDegAsp up to179 nmol/kg/day had no adverse effect on embryo-foetal survival or growth. NOAEL for maternal and embryo-foetal toxicity in the Wistar rat was179 nmol/kg/day IDegAsp.

Study No.	206075	206076
Type of study	Preliminary combined fertility and embryo-foetal toxicity	Combined fertility and embryo-foetal toxicity
Species/strain	Wistar rats	Wistar rats
Test article	Insulin degludec	Insulin degludec
Comparator	NPH insulin	NPH insulin
Route of administration	s.c	s.c
Animals/sex/group	4 groups: 8 males and 8 females Comparator group: 8 males and 8 females	4 groups: 22 males and 22 females Comparator group: 22 males and 22 females
Dose levels (nmol/kg/day)	Insulin degludec: 0, 25, 100, 150 NPH insulin: 100	Insulin degludec: 0, 20, 80, 125 NPH insulin: 80
Dosing period	Males: 2 weeks prior to mating until termination of majority of females Females: 2 weeks prior to mating until day 17 after mating	Males: 4 weeks prior to mating until termination of majority of females Females: 2 weeks prior to mating until day 17 after mating
Result/conclusion	Low mortality occurred in the females treated at 100 and 150 nmol/kg/day insulin degludec and 100 nmol/kg/day NPH insulin, most likely caused by hypoglycaemia. Insulin degludec had no effect on fertility, mating performance and embryo-foetal development. For the main study the doses were reduced.	Treatment of rats with insulin degludec up to 125 nmol/kg/day had no effect on mating performance fertility, and embryo-foetal survival or growth. In the groups treated with 80 and 125 nmol/kg/day insulin degludec and 80 nmol/kg/day NPH insulin and to a lesser extent also in the 20 nmol/kg/day insulin degludec group, there were increased incidences of minor skeletal abnormalities, which were below the incidences in the NPH insulin comparator group and/or within background control data range. NOAEL for fertility and embryo-foetal development in the Wistar rat was 125 nmol/kg/day.

Local Tolerance Studies

Three studies (Study Nos 206131, 210455, and 210297) were conduced in pigs or rabbits. These studies were reviewed under NDA 203314 for IDeg. However, IDegAsp (in "early development" or "to be marketed" drug product) were tested also. Similar to IDeg, IDegAsp caused mild inflammation at the injection site.

Table 18 Summary Tables of Local Tolerance Studies in Pigs and Rabbits (for IDegAsp or IDeg)

Study No.	206131	210455	210297
Species/strain	Pig/non-pigmented SPF	Minipigs/Göttingen SPF	Rabbit/New Zealand White
Test article	Insulin degludec early development drug product (600 and 1200 nmol/mL) IDegAsp early development drug product ((b) (4) nmol/mL: (b) (4) nmol/mL insulin degludec co-formulated with (b) (4) nmol/mL insulin aspart) Vehicle for insulin degludec	Insulin degludec "to be marketed" drug product (600 and 1200 nmol/mL) IDegAsp "to be marketed" drug product (600 nmol/mL: 420 nmol/mL insulin degludec co-formulated with 180 nmol/mL insulin aspart) Vehicle for insulin degludec and IDegAsp	Insulin degludec "to be marketed" drug product (600 and 1200 nmol/mL) IDegAsp "to be marketed" drug product (600 nmol/mL: 420 nmol/mL insulin degludec co-formulated with 180 nmol/mL insulin aspart) Vehicle for insulin degludec and IDegAsp
Comparator	NPH insulin (600 nmol/mL) 0.9% saline	NPH insulin (600 nmol/mL) 0.9% saline	NPH insulin (600 nmol/mL) Vehicle for NPH insulin
Dose route	s.c	s.c	i.m., i.v., i.a.
No of animals	6 females	20 females	48 females
Dosing	2 or 5 days before sacrifice, 100 µl/injection site of each test article or comparator was injected	2 or 5 days before sacrifice, 100 µl/injection site of each test article, vehicle or comparator was injected. In addition, for insulin degludec and vehicle (1200 nmol/mL) 50 µl/injection site was injected	4 days before sacrifice, 100 µl/injection site of insulin degludec 600 nmol/mL or IDegAsp 600 nmol/mL, vehicle or comparator was injected. For insulin degludec (1200 nmol/mL) and vehicle 50 µl/injection site was injected

Study No.	206131	210455	210297
Species/strain	Pig/non-pigmented SPF	Minipigs/Göttingen SPF	Rabbit/New Zealand White
Results	Single subcutaneous injection of insulin degludec or IDegAsp caused mild inflammatory reaction at the injection site on day 2 and 5 after injection. The reaction was comparable to that of 0.9% saline and vehicle for insulin degludec and less pronounced than that observed after injection of NPH insulin.	Single subcutaneous injection of insulin degludec or IDegAsp caused mild inflammatory reaction at the injection site 2 and 5 days after injection. At day 2, the reaction was comparable to that of vehicle for insulin degludec, vehicle for IDegAsp and NPH insulin. At day 5, the reaction was comparable to that of 0.9% saline, vehicle for insulin degludec, vehicle for IDegAsp and NPH insulin.	Single i.v., i.m. or i.a. injection of insulin degludec or IDegAsp caused haemorrhage/bruising, swelling and a mild inflammatory reaction comparable to that of NPH insulin or vehicles.

Impurity

The impurities of IDegAsp were evaluated in a 4 week sc toxicity study (Study No 210228) comparing aged and non-aged IDegAsp. Aged and non-aged IDegAsp containing different levels of impurities administered daily by sc injection to rats for 4 weeks at 71 and 143 nmol/kg/day resulted in changes of body weight, body weight gain, food consumption, blood glucose levels, clinical pathology parameters, decreased liver weight and liver glycogen depletion. These effects were related to the pharmacological effect of IDegAsp. Minor differences between the aged and non-aged IDegAsp were seen in body weight and body weight gain, hematology, clinical chemistry, and organ weights. There differences were considered incidental (mainly seen in one sex or going in opposite directions).

Six impurities (3 from IDeg and 3 from IAsp) were identified in the IDegAsp drug product. The impurities and HMWP have been tested in nonclinical studies at same concentration or slightly above the proposed in used (including shelf-life) limits. The animal exposure was 33-116x the human exposure for these impurities and HMWP. Therefore, the proposed in-use limits were acceptable.

Table 19 Summary Table of Calculated Exposure Ratios for Impurities for IDegAsp

Calculation of exposure ratios for impurities and related substances for IDegAsp

Impurity	Proposed limits (%)[a]	Tested non-clinical studies[b] (%)	Study number[b]	NOAEL (nmol/kg)	Clinical dose[c] (nmol/kg)	Impurity NOAEL (nmol/kg)	Impurity clinical dose (nmol/kg)	Non-clinical tested vs proposed limits (fold)	Animal to human exposure ratio (fold)
	(A)	(B)	(C)	(D)	(E)	(F) = B×D/100	(G) = A×E/100	(H) = B/A	(I) = F/G
(b) (4)		(b) (4)	210228	179[d]	6.48				(b) (4)
			210227	150	4.536				
			210227	150	4.536				
			210227	150	4.536				
			210228	54	1.944				
			210228	54	1.944				
			210228	54	1.944				

a – Proposed in-use limits (including shelf-life)
b – See table 2.6.7.4.A and 2.6.7.4.B
c – Mean dose (1.08 U/kg) in most insulin requiring therapeutic confirmatory trial (NN5401-3592) ~ 6.48 nmol/kg IDegAsp (4.536 nmol/kg IDeg + 1.944 nmol/kg IAsp)
d – IDegAsp dose

Overall Review of IDegAsp and Safety Margin

The nonclinical program of both IDeg alone and IDeg+IAsp co-formulation showed no unexpected safety concerns for chronic sc administration of IDeg or IDegAsp in human. Toxicity findings observed in animals were mostly associated with hypoglycemia which was an exaggerated pharmacology of insulin. With IDeg, the nonclinical studies have

demonstrated that IDeg did not change its metabolic efficacy and safety profiles compared to human insulin. With IDegAsp, the nonclinical studies (in assessing repeated dose toxicity, reproductive toxicity, and local tolerance) showed that co-formulation of IDeg and IAsp did not affect the safety and efficacy of the individual components and no unexpected safety concerns for IDegAsp were identified. Based on AUCs obtained from the 13-week toxicity studies, there were exposure multiples of 2.9 with IDeg and of 1.7 with IAsp to those of in humans.

Table 20 Safety Margins

Toxicity (Species)	NOAEL (nmol/kg/day)	$AUC_{0\text{-}24h}$ at Wk13 (h*nmol/L)	Safety Margin (Based on AUC)***
13 week tox* (rat)	36 IDeg: 25 IAsp: 11 107 IDeg: 75 IAsp: 32 (sponsor's NOAEL)	 493.5 3.25 1209.5 28*	 **2.9x** **1.7x** 7.0x 14.4x
Seg II repro tox** (rat)	**Maternal:** 179 IDeg: 125 IAsp: 54 **Fetal Dev:** 28.6 IDeg: 20 IAsp: 8.6	 1300 47.4 155 0.25	 **7.6x** **24x** **0.9x** **0.13x**

* Exposure extrapolated from Study No 208337: 13 week toxicity rat study
** Exposure extrapolated from Study No 208333: preliminary study in pregnant rats
*** $AUC_{0\text{-}24h}$ in human at clinical dose of 6.48 nmol/kg IDegAsp (or 1.08 U/kg)

- For **IDeg**: 172 h*nmol/L based on two clinical studies: NN1250-1993 (IDeg exposure in steady state) and NN5401-3592 (clinical exposure)
- For **IAsp**: 1.95 h*nmol/L based on two clinical studies: NN5401-3539 (IAsp exposure in steady state) and NN5401-3592 (clinical exposure)

12 Appendix/Attachments

n/a

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MIYUN M TSAI-TURTON
06/05/2012

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 203314
Supporting document/s:	0000
Applicant's letter date:	Sept 29th 2011
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Product:	Insulin Degludec (Tresiba™)
Indication:	T1DM and T2DM
Applicant:	Novo Nordisk Inc.
Review Division:	DMEP
Reviewer:	Miyun Tsai-Turton, PhD, MS
Supervisor/Team Leader:	Karen Davis Bruno, PhD
Division Director:	Mark Parks, MD
Project Manager:	Rachel Hartford

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1 Executive Summary

1.1 Introduction

This NDA 203314 is for insulin degludec (IDeg), a long acting soluble insulin analogue. It was first submitted in Sept 2007 under IND 76496. The applicant is filing for approval of this drug product in two strengths: U-100 and U-200, containing 600 nmol/ml and 1200 nmol/ml drug substance respectively. This insulin degludec is intended for once-daily sc administration at any time of the day, independent of meals, in diabetic patients. During development of this drug product, several names have been used: insulin degludec (IDeg), insulin 454, SIBA, and NN1250.

1.2 Brief Discussion of Nonclinical Findings

Since IDeg is designated to alter absorption rate, the nonclinical development strategy focused on evaluating the long PK/PD effects of IDeg without affecting efficacy and safety compared to human insulin.

- PK/PD profile

A series of biological in vitro studies were done to demonstrate that IDeg is a specific agonist to the human insulin receptor and the MOA is identical to that of human insulin and other insulin analogues. In vivo studies showed the blood glucose lowering effect of IDeg in rats and pigs and the slow absorption resulting in the long and stable PK/PD profiles in pigs. In addition, a series of safety pharmacology studies were conducted to assess its potential effects on CNS, CV, and respiratory in rats and/or dogs.

- Tox profile

The toxicity of IDeg was evaluated after sc single dose administration in rats and dogs and after sc repeat-dose administration in rats (up to 52 weeks) and dogs (up to 26 weeks). Hypoglycaemia and hypoglycaemia-related adverse effects were results of the exaggerated pharmacological effect of insulin. In addition, genotoxicity and standard carcinogenicity studies were not performed with IDeg. The applicant justified their rationale with published literature/reference for insulin. The 52-week repeat-dose tox study in rats used NHP insulin as comparator to assess mammary tumor potential. Reproductive and developmental toxicity of IDeg were assessed in rats and rabbits. The adverse effects were considered related to pharmacological effects of insulin. Local tolerance studies showed that local tissue reaction (at injection site) was mild and comparable to that of vehicle or NPH insulin.

Moreover, product related impurities (i.e. (b) (4) were identified. All impurities were related to IDeg. Other impurities (i.e. leachables including (b) (4)) from the container-closure system) are being determined for (b) (4) months shelf-life and 8 weeks in-use periods of the 100 U/ml IDeg.

Excipients of IDeg (i.e. phenol and m-cresol (b) (4) and glycerol (b) (4)) were frequently used excipients which were present in similar of higher concentrations in currently marketed insulin products for sc administration such as insulin aspart.

- Safety Margin

The animal/human exposure ratios were calculated based on NOAELs in the animal studies of longest duration (as AUC and Cmax at steady state) and human exposure at the mean clinical dose (0.75 U/kg) in the confirmatory clinical trial using the highest insulin dose. Based on repeat dose toxicity studies, there were 5.2x and 1.3x exposure multiple in rats and dogs respectively.

1.3 Recommendations

1.3.1 Approvability

APPROVAL

1.3.2 Additional Non Clinical Recommendations

NO

1.3.3 Labeling

(b) (4)

(b) (4)

[redacted]

SUGGESTED BY DMEP

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no well-controlled clinical studies of the use of insulin degludec in pregnant women. Patients should be advised to discuss with their health care provider if they intend to or if they become pregnant. Because animal reproduction studies are not always predictive of human response, insulin degludec should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is essential for patients with diabetes or a history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in these patients.

Subcutaneous reproduction and teratology studies have been performed with insulin degludec and human insulin (NPH) as a comparator in rats and rabbits. In these studies, insulin was given to female rats before mating throughout pregnancy until weaning, and to rabbits during organogenesis. The effect of insulin degludec was

consistent with those observed with human insulin as both caused pre- and post-implantation loses and visceral/skeletal abnormalities in rats at an insulin degludec dose of 21 U/kg/day (approximately 5 times the human subcutaneous dose of 0.75 U/kg/day, (b) (4)) and in rabbits at a dose of 3 U/kg/day (approximately 10 times the human subcutaneous dose of 0.75 U/kg/day (b) (4)). The effects are probably secondary to maternal hypoglycemia.

8.3 Nursing mothers
It is unknown whether insulin degludec is excreted in human milk. Because many drugs, including human insulin, are excreted in human milk, caution should be exercised when insulin degludec is administered to a nursing mother. Patients with diabetes who are lactating may require adjustments in insulin dose, meal plan, or both.

In rats, insulin degludec was secreted in milk. The concentration in milk was (b) (4) (b) (4) plasma.

13 NONCLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of insulin degludec. In a 52-week (b) (4) study including human insulin (NPH insulin) as comparator (6.7 U/kg/day), Sprague-Dawley rats were dosed subcutaneously with insulin degludec at 3.3, 6.7, and 10 U/kg/day, resulting in 5 times the human exposure (AUC) when compared to a human subcutaneous dose of 0.75 U/kg/day. Human insulin was dosed at 6.7 U/kg/day. No treatment-related increases in incidences of hyperplasia, benign or malignant tumors were recorded in female mammary glands from rats dosed with insulin degludec and no treatment related changes in the female mammary gland cell proliferation were found using BrdU incorporation. Further no treatment related changes in the occurrence of hyperplastic or neoplastic lesions were seen in (b) (4) animals (b) (4) dosed with insulin degludec when compared to vehicle or human insulin.

Genotoxicity testing of insulin degludec was not performed.

In a combined fertility and embryo-fetal study in male and female rats, treatment with insulin degludec up to 21 U/kg/day (approximately 5 times the human subcutaneous dose of 0.75 U/kg/day, based on U/body surface area) prior to mating and in female rats during gestation had no effect on mating performance and fertility.

2 Drug Information

2.1 Drug

CAS Registry 844439-96-9

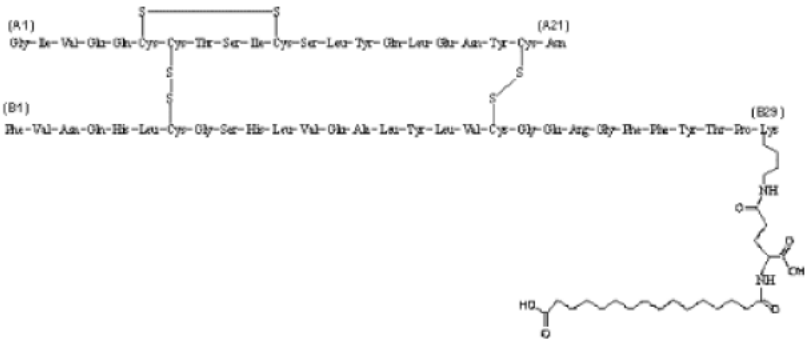
Generic Name NN1250 soluble insulin basal analog (SIBA)

Code Name Insulin 454

Chemical Name LysB29(Nε-hexadecandioyl-γ-Glu) des(B30) human insulin

Molecular Formula/Molecular Weight $C_{274}H_{411}N_{65}O_{81}S_6$ / ~610 (b) (4)

Structure or Biochemical Description



Pharmacologic Class long acting insulin analogue for diabetes

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 76496 (IDeg), IND 73198 (NN5401, SIAC, IDegAsp), NDA 203313 (IDegAsp)

2.3 Drug Formulation

The compositions of drug product 100 U/ml (600 nmol/ml insulin degludec) and 200 U/ml (1200 nmol/ml insulin degludec) are listed in the table below. For 100 U/ml, it is intended to market (b) (4) pre-filled disposable PDS290-pen injector. For 200 U/ml, it is intended to market in a pre-filled disposable PDS290 pen-injector.

Table 1 Drug Product Formulations.

Composition of insulin degludec 100 U/ml

Name of components	Quantity per ml		Function	Reference to standards
	Insulin degludec 100 U/ml	Insulin degludec 200 U/ml		
Active substance				
Insulin degludec	600 nmol	1200 nmol	Drug substance	Novo Nordisk
Excipients				
Phenol[1]	1.50 mg	1.50 mg	(b) (4)	Ph Eur ,USP, JP
Metacresol[1]	1.72 mg	1.72 mg		Ph Eur, USP
Glycerol	19.6 mg	19.6 mg		Ph Eur ,USP, JP
Zinc	32.7 µg	71.9 µg		Ph Eur ,USP, JPE[2]
Hydrochloric acid[3]	q.s.	q.s.	pH adjustment	Ph Eur ,USP, JP
Sodium hydroxide[3]	q.s.	q.s.	pH adjustment	Ph Eur ,USP, JP
Water for injections	(b) (4)			Ph Eur ,USP, JP
(b) (4)				

[3] To reach pH 7.6

2.4 Comments on Novel Excipients

None of the excipients were classified as novel excipients.

2.5 Comments on Impurities/Degradants of Concern

No issue was raised with regards to drug product impurities.

Three groups of impurities were identified (b) (4) impurities. The HMWP was also listed. All impurities were qualified in the nonclinical studies during the IDeg development.

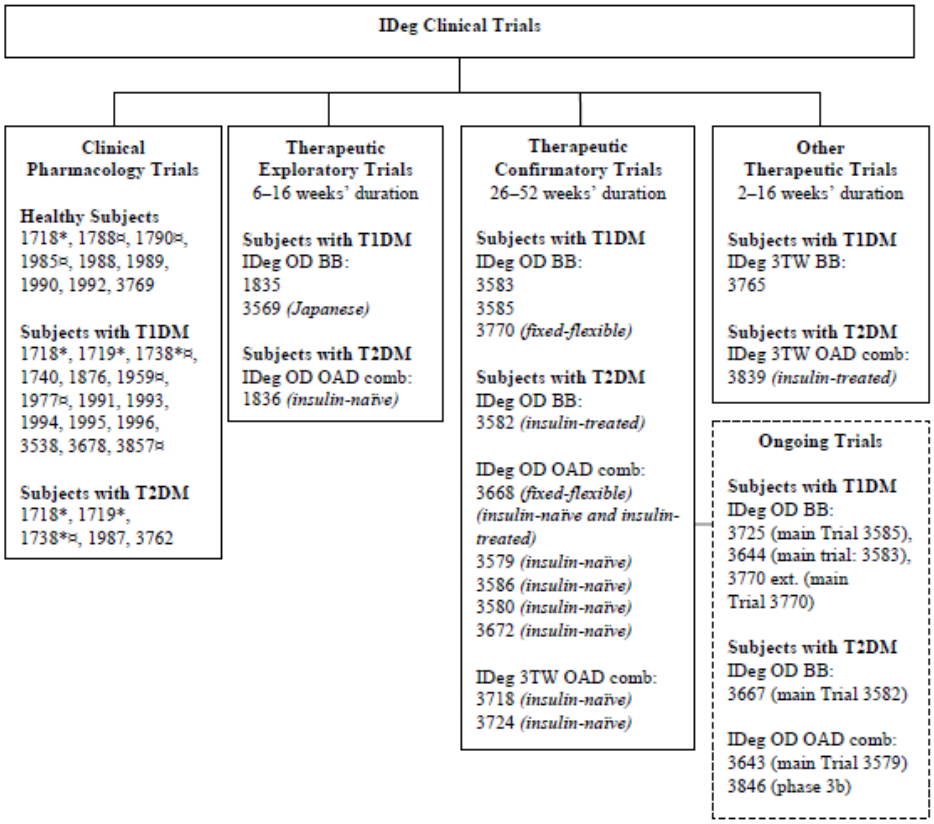
Table 2 Drug Product Impurities.

Degradation products identified in degraded insulin degludec drug product

Type of related substances and impurities	Classification	Identity/Content
Insulin degludec related substances and impurities analysed with RP-HPLC method A6020a	(b) (4)	(b) (4)
Impurities analysed with GPC method A6022a	HMWP (High Molecular Weight Proteins)	

2.6 Proposed Clinical Population and Dosing Regimen

This is intended for once-daily sc administration at any time of the day, independent of meals, in diabetic patients. The clinical trials for IDeg are listed below.



2.7 Regulatory Background

Timeline:

- Sept 2007 – IND submission
- Feb 2009 – EOP2 meeting
- June 2011 – pre-NDA meeting
- Sept 2011 – NDA submission

3 Studies Submitte

3.1 Studies Reviewed

PHARMACOLOGY

- Primary PD studies: clamp studies, mitogenicity, binding affinities of IR and IGF-1R, glycogen synthesis, lipolysis

- Secondary PD study
- Safety pharm studies: CV, neurobehavioral, respiratory, QT prolongation, ECG

PHARMACOKINETICS
- Absorption studies: iv/sc routes in pigs/dogs/rats
- Distribution studies: iv/sc route in rats
- Metabolism studies: in vitro and in vivo (iv/sc routes in rats/dogs)
- Excretion studies: sc route in rats

TOXICOLOGY
- Single dose tox study in rats
- Repeat dose tox studies in rats/dogs/rabbits
- Repro/dev tox studies in rats/rabbits
- Other tox studies: local tox studies in pigs/rabbits/minipigs, 28 days (impurities) tox study in rats, and up to 13 weeks repeat dose tox studies in mice

3.2 Studies Not Reviewed

n/a

3.3 Previous Reviews Referenced

Some PD/PK/Tox studies were reviewed under IND 76496 and the reviews can be found in DARRTS (2007 Nov Review #1 and 2008 August Review #2).

4 Pharmacology

4.1 Primary Pharmacology

Study No. 6UIR050902-100 Mar-2006: clamp pig study

Action profile of insulin 454 (0.6 mM and 1.2 mM) or Glargine (0.6 mM). A euglycaemic clamp study in pigs [by Novo Nordisk A/S, Denmark]

Study design: This study was to characterize two different strengths of insulin 454, 600 µM and 1200 µM, (vs. 600 µM glargine) in euglycaemic clamp studies in health pigs (n=8). Eight LYD pigs participated each on three days of experiment with an interval of 1 week.

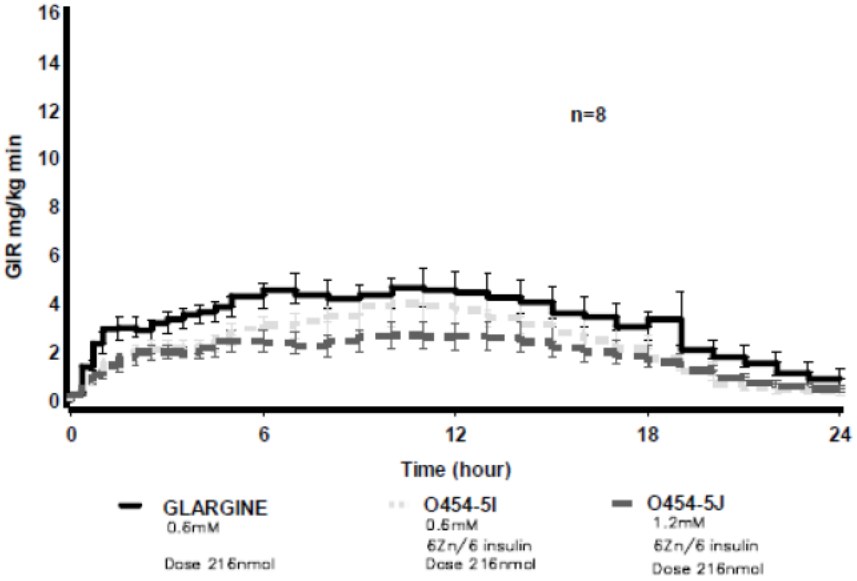
Insulin doses tested and number of rats included at each dose level

Infusions dose (pmol/min/kg)	insulin 454	HI	insulin 454	HI
15	5	5		
30	5	5		
45				5
90			4	5
180			4	

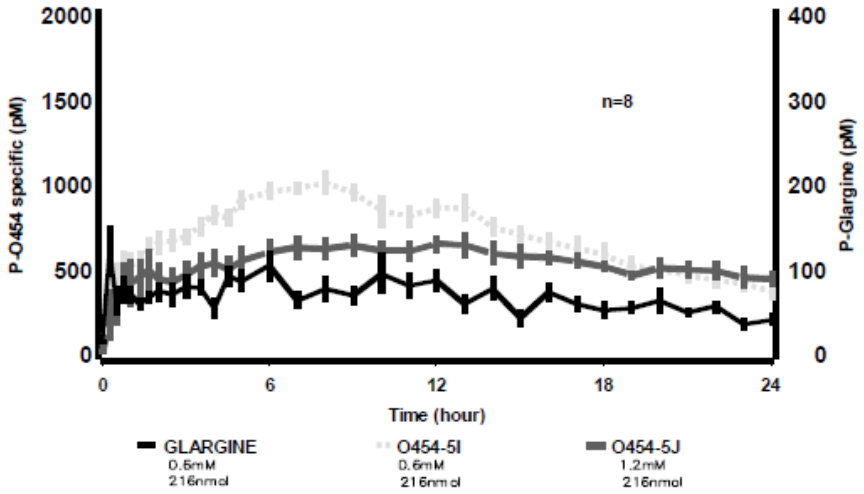
Findings: The glucose utilization 0-24 h and profiles of the infusion rats used to maintain euglycaemia were almost similar for the 2 insulin 454 preparations. There was a tendency that the high-strength 454 preparation was absorbed more slowly, and in the study period of 0-24 hr, gave rise to slightly lower glucose utilization (2722 for 1.2 mM vs. 3397 mg/kg for 0.6 mM). The plasma 454 insulin profiles also confirmed the dynamic profiles with a lower plasma insulin area for high-strength 454 preparation (12774 pMxh for 1.2 mM vs. 16795 pMxh for 0.6 mM).

Increasing the concentration of the insulin 454 preparation from 600 to 1200 µM resulted in a slightly longer protraction as well as a "flattening" of the action profile due to a similar flattening of the PK profile (lower Cmax).

Action profile of O454 in two different concentrations and Glargine
A euglycaemic glucose clamp in pigs



Insulin profile of (b) (4) O454 in two different concentrations and Glargine
A euglycaemic clamp in pigs



Study No. 205402: in vitro mitogenicity in L6-hIR cells

In vitro mitogenicity study of NNC-0100-0000-0454 in the cell line L6-hIR, a rat skeletal muscle cell line stably transfected with the gene encoding the human insulin receptor [by Novo Nordisk A/S, Denmark]

Study design: This study was to study the effect of the insulin 454 analogue (batch 13289/087) on 3H-thymidine incorporation into newly synthesized DNA was compared to that of non-modified native human insulin using rat L6 myoblasts overexpressing the human insulin receptor (L6-hIR cells). Findings: Insulin 454 had a lower mitogenic potency than non-modified human insulin. Even though the L6hIR system had a relatively high variability as expected for this kind of in vitro biological assay, the insulin 454 analogue was found to be 5X less potent than non-modified human insulin.

Comparison of ED_{50} values of insulin 454 and native (non-modified) human insulin, and data for the mitogenic potencies of insulin 454 determined in the L6-hIR cell line

Assay ID	ED_{50}, Human Insulin [nM]	ED_{50}, insulin 454 [nM]	Realtive mitogenic potency of insulin 454 ($ED_{50, HI}$ / $ED_{50, insulin\ 454}$)
1	0,10	5,91	1,7 %
2	0,07	5,93	1,2 %
3	0,04	1,987	2,0 %
4	0,12	2,64	4,5 %
5	1,29	6,18	20,9 %
6	1,31	8,24	15,9 %
7	1,43	4,29	33,0 %
8	1,69	4,73	36,0 %
9	6,07	11,89	51,0 %
Mean	1,35	5,80	18,5 % ***
Standard deviation	1,9	3,0	18,2

*** Significantly different from the 100 % potency of human insulin ($p < 0.0001$)

Relative mitogenic potency: Numbers below 100% indicate a mitogenic potency smaller than that found for human insulin and numbers above 100% indicate potencies higher than that found for human insulin.

Study No. 205403: in vitro mitogenicity in HMEC system (0.1% HSA)

In vitro mitogenicity study of insulin analogue 454 in primary human mammary epithelial cells [by Novo Nordisk A/S, Denmark]

Study design: This study was to study the effect of the insulin 454 analogue (batch 13289/087) on simulation of 3H-thymidine incorporation into newly synthesized DNA was compared to that of non-modified native human insulin using the primary human mammary epithelial cell (HMEC) system. Findings: In the absence of human serum albumin in the medium, the relative mitogenic potency of insulin 454 was found to be 6.3%. In the presence of 0.1% human serum albumin in the medium, the relative mitogenic potency of insulin 454 was found to be 3.2%. All in all, the mitogenic potency of insulin 454 was found to be lower than the mitogenic potency of non-modified human insulin.

Relative mitogenic potency of Insulin 454 in Study 1 and Study 2

Study ID	Sample ID	HSA in assay medium	ED_{50}, IGF-1 [nM]	ED_{50}, X-10 [nM]	ED_{50}, human insulin [nM]	ED_{50}, insulin 454 [nM]	Relative mitogenic potency of insulin 454 (ED_{50}, HI/ $ED_{50,\ insulin\ 454}$)
Study 1A	Plate A	No	2,861	ND	13,37	230,8	5,8%
	Plate B	No	3,191	ND	17,43	319,0	5,5 %
Study 2	Plate F	No	2,299	ND	47,13	703,2	6,7 %
	Plate G	No	ND	2,565	32,89	455,1	7,2 %
Mean of Study 1A and Study 2							6,3 %
Standard deviation							0,8
[a]P-value							[a]P<0.0001
Study 1B	Plate C	Yes, 0.1 %	3,840	ND	23,45	1221	1,9 %
	Plate D	Yes, 0.1 %	6,904	ND	52,41	1158	4,5 %
Mean of Study 1B							3,2 %

[a]Mean relative mitogenic potency of insulin 454 was significantly lower than the 100% potency of human insulin by one-sample t-test.

Study No. 205404: in vitro mitogenicity in synchronized L6-hIR cells

In vitro mitogenicity study of NNC-0100-0000-0454 in synchronized L6-hIR cells [by Novo Nordisk A/S, Denmark]

Study design: This study was to study the effect of the insulin 454 analogue (batch 13289/087) was compared to that of non-modified native human insulin using rat L6 myoblasts overexpressing the human insulin receptor (L6-hIR cells). Synchronized cells had higher fold response rats and lower inter-experimental variability. Findings: Insulin 454 had a lower mitogenic potency than non-modified human insulin (9.2% mitogenic potency compared to the 100% mitogenic potency of human insulin).

Mitogenic potency of insulin 454.

Best fit values				Human Insulin		Insulin 454		
Assay ID #	Bottom [cpm]	Top [cpm]	fold induction	ED50 nM	R value	ED50 nM	R value	potency (%)
1	326	1521	4,7	0,424	0,9719	2,655	0,9722	16
2	247	1225	5,0	0,129	0,9764	2,475	0,9610	5,2
3	222	810	3,6	0,054	0,9254	0,500	0,9654	11
4	627	1894	3,0	0,011	0,7925	0,242	0,9528	4,5
Average potency								9,2***

Potency numbers below 100% indicate a mitogenic potency smaller than that found for human insulin.

*** Using one-sample t-test (GraphPad Prism soft ware) for statistical analysis, it was shown that the average potency of O454 was significantly different from the 100 % potency of human insulin ($p<0.0001$). SD was 5.4.

Study No. 206632: in vitro mitogenicity in HMEC system (0-0.5% HSA)

In vitro mitogenicity of insulin 0100-0000-0454 in HMEC cells using 0 to 0.5% HSA [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the modulatory effect of human serum albumin (HAS) on the mitogenic effect of insulin 454 in HMEC cells. Insulin 454 binds to HAS, and the mitogenic effect of insulin 454 was therefore expected to be inhibited by HAS added to the cell culture medium. Findings: In the absence of HAS, insulin 454 exhibited a relative mitogenic potency of 5.9%. HAS significantly reduced the relative mitogenic potency of insulin 454 compared to native human insulin (in agreement with the HAS-binding nature of insulin).

EC50 values, fold response and relative mitogenic potency of insulin 454

Study	HSA conc. (wt%, g/100 ml)	Fold response	EC50 , nM				[a]Mitogenic potency of insulin 454 relative to human insulin, %
			human insulin	X-10	IGF-1	Insulin 454	
Study 1	0 %	5.7	16.47	5.80	0.46	170.6	9.7
	0.1 %	5.4	13.34	99.7	1.23	nd	nd
	0.2 %	3.2	23.57	1.93	0.22	nd	nd
	0.3 %	2.3	12.52	7.25	0.48	419	3.0
	0.5 %	na	nd	nd	nd	nd	nd
Study 2	0 %	3.2	5.02	0.19	0.08	164.2	3.1
	0.1 %	2.8	8.06	0.34	0.14	1009	0.8
	0.2 %	2.5	12.56	3.26	0.42	1112	1.1
	0.3 %	na	nd	nd	nd	nd	nd
	0.5 %	na	nd	nd	nd	nd	nd
Study 3	0 %	3.9	15.58	2.046	0.401	288.2	5.4
	0.1 %	3.3	19.28	3.542	0.321	915.0	2.1
	0.2 %	2.9	28.81	3.641	0.479	1418	2.0
	0.3 %	2.4	63.54	4.063	0.550	1342	4.7
	0.5 %	2.6	62.87	12.54	0.963	30890	0.2
Study 4	0 %	3.9	27.38	2.512	0.2640	522.0	5.2
	0.1 %	3.6	139.5	5.116	0.5444	19311	0.7
	0.2 %	2.3	47.95	1.664	0.3445	3034	1.6
	0.3 %	2.1	47.32	3.453	1.150	3622	1.3
	0.5 %	1.9	9.509	2.141	1.077	2727	0.4

[a]As determined by the GraphPrism software, irrespective of goodness of fit for dose response curves.

Na, not applicable.

Nd, not determined, poor quality of dose-response curves.

Study No. 206633: in vitro mitogenicity in synchronized L6-hIR cells (0-2.5%HSA)

In vitro mitogenicity of insulin 0100-0000-0454 in sunchronized L6-hIR cells using HAS doses in the range 0-2.5 wt% [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the modulatory effect of human serum albumin (HAS) on the mitogenic effect of insulin 454 in L6-hIR cells. Insulin 454 binds to HAS, and the mitogenic effect of insulin 454 was therefore expected to be inhibited by HAS added to the cell culture medium. Findings: In the absence of HAS, insulin 454 exhibited a relative mitogenic potency of 7.5% compared to human insulin. HAS significantly reduced the relative mitogenic potency of insulin 454 compared to native human insulin (in agreement with the HAS-binding nature of insulin).

Mean of the relative mitogenic potency of insulin 454

HSA concentration	Mean potency of insulin 454 relative to human insulin, %	P value*
0 %	7.5	NA
0.1 %	9.5	0.0318
0.2 %	7.4	0.8888
0.3 %	7.7	0.9028
0.5 %	5.2	0.0552
1.0 %	2.4	0.1280
2.5 %	0.8	0.0095*

* One sample t test of the relative potencies of insulin 454 generated in GraphPad Prism

* Significantly different from the potency of insulin 454 at 0% HSA, $P<0.05$, one-sided t-test.

Study No. 206707: in vitro mitogenicity in synchronized L6-hIR cells

In vitro mitogenicity of insulin 0100-0000-0454 in sunchronized L6-hIR cells [by Novo Nordisk A/S, Denmark]

Study design: This GLP study was to assess the mitogenic potential of the insulin 454, compared to three reference compounds – human insulin, humanAspB10 (X-10), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was found to be 13.1%, compared to that of human insulin (100%). Insulin 454 was found to have a 7.6X lower mitogenic potency than human insulin.

***In vitro* mitogenicity assay results**

Human insulin			*X-10*			*IGF-1*			*Insulin 454*		
EC_{50} [nM]	R^2	Mitogenic Potency	EC_{50} [nM]	R^2	Mitogenic potency*	EC_{50} [nM]	R^2	Mitogenic potency*	EC_{50} [nM]	R^2	Mitogenic potency*
3.02	0.983	100	1.23	0.970	246	118.5	0.990	2.5	23.13	0.981	13.1

*Relative mitogenic potency expressed as percentage of potency of human insulin (100 %).
The maximal fold response to human insulin in this experiment was 57.

Note: X10 (human AspB10) had much higher mitogenic potency than rh insulin. That was the reason we have asked for 12 month rodent assessment for mammary proliferation for all insulin analogues. In Hansen et al paper, mitogenicity of AspB10 was not due to induced IGF1R binding

affinity but to slower dissociation at insulin R ***[Reference: Hansen BF, Danielsen GM, Drejer K, Sorensen AR, Wiberg FC, Klein HH, and Lundemose AG. (1996). Sustained signaling from the insulin receptor after stimulation with insulin analogues exhibiting increased mitogenic potency. Biochem J 315 (pt 1):271-279.]***

Study No. 207059: in vitro mitogenicity in synchornized L6-hIR cells (0-2.5% HSA)

In vitro mitogenicity of insulin 0100-0000-0454 in sunchronized L6-hIR cells: study comprising 5 experiments with 0 to 2.5 wt % HSA [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in L6-hIR cells. Insulin 454 binds to human serum albumin. The mitogenic effect of insulin 454 was expected to be inhibited by HSA added to the cell culture medium. Three reference compounds, HI, IGF-1, and X-10, were used in this study. Findings: In the absence of HSA, the relative mitogenic potency of insulin 454 was 3.57%. The mitogenic potency was 28X lower than mitogenic potency on human insulin. The ranking of mitogenic potencies (X-10 more mitogenic than insulin, insulin more mitogenic than IGF-1) was as expected for the L6-hIR cell system.

Effect of HSA on relative mitogenic potency of test and reference compounds, individual results from experiments 1, 2, 3, 4 and 6

HSA concentration in the 96 well plates	IGF-1		X-10		Insulin 454	
	[a]Relative mitogenic potency	[b]Relative mitogenic potency, % of no HSA	[a]Relative mitogenic potency	[b]Relative mitogenic potency, % of no HSA	[a]Relative mitogenic potency	[b]Relative mitogenic potency, % of no HSA
0 wt %	2.92 0.08 0.05 0.34 1.39	100 100 100 100 100	518.58 870.58 544.70 319.46 200.77	100 100 100 100 100	8.11 0.85 0.56 1.32 7.02	100 100 100 100 100
Mean Relative mitogenic potency	0.96	-	490.82	-	3.57	-
0.1 wt%	3.10 0.11 0.06 0.57 1.03	106.16 137.50 120.00 167.65 74.10	464.30 777.91 479.17 256.34 227.85	89.53 89.36 87.97 80.24 113.49	7.00 0.91 0.61 2.20 5.13	86.31 107.06 108.93 166.67 73.08
Mean Relative mitogenic potency	0.97	-	441.11	-	3.17	-
0.2 wt%	3.67 0.12 0.08 0.65 1.55	125.68 150.00 160.00 191.18 111.51	415.53 732.62 423.24 281.03 267.19	80.13 84.15 77.70 87.97 133.08	7.29 0.74 0.44 2.29 5.37	89.89 87.06 78.57 173.48 76.50
Mean Relative mitogenic potency	1.21	-	423.92	-	3.23	-
0.3 wt%	4.28 0.09 0.07 0.56 1.90	146.58 112.50 140.00 164.71 136.69	498.96 462.21 278.13 163.11 224.50	96.22 53.09 51.06 51.06 111.82	7.54 0.46 0.33 1.96 5.39	92.97 54.12 58.93 148.48 76.78
Mean Relative mitogenic potency	1.38	-	325.38	-	3.14	-
0.5 wt%	2.88 0.09 0.08	98.63 112.50 160.00	403.17 453.35 333.18	77.74 52.07 61.17	3.63 0.29 0.25	44.76 34.12 44.64

	0.66 1.66	194.12 119.42	232.49 260.18	72.78 129.59	1.62 3.93	122.73 55.98
Mean Relative mitogenic potency	1.07	-	336.47	-	1.94	-
1 wt%	1.92 0.07 0.05 0.42 1.30	65.75 87.50 100.00 123.53 93.53	315.16 422.39 225.88 207.05 262.85	60.77 48.52 41.47 64.81 130.92	1.66 0.16 0.14 0.60 2.04	20.47 18.82 25.00 45.45 29.06
Mean Relative mitogenic potency	0.75	-	286.67	-	0.92	-
2.5 wt%	3.67 0.07 0.06 0.32 1.06	125.68 87.50 120.00 94.12 76.26	510.31 421.07 249.56 126.10 262.78	98.41 48.37 45.82 39.47 130.89	1.10 0.05 0.04 0.35 1.22	13.56 5.88 7.14 26.52 17.38
Mean Relative mitogenic potency	1.04	-	313.96	-	0.55	-

[a]Relative mitogenic potency values (Mitogenic potency of HI defined as 100%). [b]EC_{50} values expressed relative to EC_{50} values at 0 % HSA (defined as 100 %). The EC_{50} values of the test and reference compounds were calculated as described in Appendix D. In each cell, numbers from top to bottom indicate results from experiments 1, 2, 3, 4 and 6.

Study No. 207087: in vitro mitogenicity in HMEC system (0-2.5% HSA)

In vitro mitogenicity of insulin 0100-0000-0454 in primary human mammary epithelial cells (HMEC): study comprising 5 experiments with 0 to 2.5 wt % HSA [by Novo Nordisk A/S, Denmark]

Study design: This GLP study was to examine the mitogenic potential of the insulin 454 in HMEC cells. Insulin 454 binds to human serum albumin. The mitogenic effect of insulin 454 was expected to be inhibited by HSA added to the cell culture medium. Two reference compounds, IGF-1, and X-10, were used in this study. Findings: In the absence of HSA, the relative mitogenic potency of insulin 454 was 3.57%. The ranking of mitogenic potencies (IGF-1 and X-10 more mitogenic than insulin) was as expected for the L6-hIR cell system.

Effect of HSA on the relative mitogenic potency of insulin 454 in HMEC, pooled data

HSA [wt%, g/100 ml]	$EC_{50, HI}$ [nM]	$EC_{50, insulin\ 454}$ [nM]	*Relative mitogenic potency [%]	§95% Con Lim Lower - 95% Con Lim Upper
Not added	12.754	387.61	3.29	2.45 - 4.42
0.1	18.247	760.518	2.4	1.54 - 3.74
0.2	27.7157	997.249	2.78	1.67 - 4.62
0.3	19.1251	914.155	2.09	1.18 - 3.7
0.5	15.627	2008.21	0.78	0.28 - 2.14

* Relative mitogenic potency expressed as percentage of the potency of human insulin (100 %). Relative mitogenic potency was calculated as the ratio between the estimated EC_{50} values (EC_{50}, human insulin/EC_{50}, insulin 454). The EC_{50} values of the test and reference compounds were calculated as described in Appendix E. §Upper and lower 95 % confidence intervals for relative potency.

Effect of HSA on the relative mitogenic potency of X-10 in HMEC, pooled data

HSA [wt%, g/100 ml]	$EC_{50, HI}$ [nM]	$EC_{50, X-10}$ [nM]	*Relative mitogenic potency [%]	§95% Con Lim Lower - 95% Con Lim Upper
Not added	20.45035	0.252334	8103.38	5906.76 - 11116.9
0.1	38.93914	0.701173	5555.61	3517.29 - 8775.17
0.2	47.13425	0.522568	9020.53	5188.64 - 15682.3
0.3	52.66758	0.501576	10499.94	5310.3 - 20761.3
0.5	27.93834	0.550461	5075.66	1898.46 -13570.1

* Relative mitogenic potency expressed as percentage of the potency of human insulin (100 %). Relative mitogenic potency was calculated as the ratio between the estimated EC_{50} values (EC_{50}, human insulin/EC_{50}, X-10). The EC_{50} values of the test and reference compounds were calculated as described in Appendix E. §Upper and lower 95 % confidence intervals for relative potency.

Effect of HSA on the relative mitogenic potency of IGF-1 in HMEC, pooled data

HSA [wt%, g/100 ml]	$EC_{50, HI}$ [nM]	$EC_{50, IGF-1}$ [nM]	*Relative mitogenic potency [%]	§95% Con Lim Lower - 95% Con Lim Upper
Not added	20.47081	0.402122	5089.46	3596.48-7202.22
0.1	34.70903	0.566091	6131.62	3626.42-10367.5
0.2	ND	ND	ND	ND
0.3	64.00748	0.548263	11676.1	5248.44-25975.5
0.5	ND	ND	ND	ND

* Relative mitogenic potency expressed as percentage of the potency of human insulin (100 %). Relative mitogenic potency was calculated as the ratio between the estimated EC_{50} values (EC_{50}, human insulin/EC_{50}, IGF-1). The EC_{50} values of the test and reference compounds were calculated as described in Appendix E. ND, not done due to variability on the data for IGF-1. §Upper and lower 95 % confidence intervals for relative potency.

HSA [wt%, g/100 ml]	$EC_{50, HI}$ [nM]	$EC_{50, IGF-1}$ [nM]	*Relative mitogenic potency [%]	§95% Con Lim Lower - 95% Con Lim Upper
Not added	20.47081	0.402122	5089.46	3596.48-7202.22
0.1	34.70903	0.566091	6131.62	3626.42-10367.5
0.2	ND	ND	ND	ND
0.3	64.00748	0.548263	11676.1	5248.44-25975.5
0.5	ND	ND	ND	ND

* Relative mitogenic potency expressed as percentage of the potency of human insulin (100 %). Relative mitogenic potency was calculated as the ratio between the estimated EC_{50} values (EC_{50}, human insulin/EC_{50}, IGF-1). The EC_{50} values of the test and reference compounds were calculated as described in Appendix E. ND, not done due to variability on the data for IGF-1. §Upper and lower 95 % confidence intervals for relative potency.

Study No. 208134: in vitro mitogenicity in COLO-205 cells (0-2.5% HSA)

In vitro mitogenicity of insulin 0100-0000-0454 in COLO-205 human colon adenocarcinoma cells: study comprising 5 experiments with 0 to 2.5 wt % HSA [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in COLO-205 cells. Insulin 454 binds to human serum albumin. The mitogenic effect of insulin 454 was expected to be inhibited by HSA added to the cell culture medium. Three reference compounds, HI, IGF-1, and X-10, were used in this study. Findings: In the absence of HSA, the relative mitogenic potency of insulin 454 was 44.3% compared to human insulin. The mitogenic potency was 2.26X lower than mitogenic potency on human insulin. The ranking of mitogenic potencies (IGF-1 and X-10 more mitogenic than HI) was as expected for the COLO-205 cell system.

Effect of HSA on relative mitogenic potency of test and reference compounds, individual results from experiments 2, 3, 4, 5 and 6

HSA [wt%, g/100 ml]	IGF-1		X-10		Insulin 454	
	[a]Relative Mitogenic Potency [%]	[b]Relative Mitogenic Potency, % of no HSA [%]	[a]Relative Mitogenic Potency [%]	[b]Relative Mitogenic Potency, % of no HSA [%]	[a]Relative Mitogenic Potency [%]	[b]Relative Mitogenic Potency, % of no HSA [%]
0	737.19	100	349.59	100	52.87	100
	456.89	100	1235.43	100	36	100
	700.73	100	391.48	100	45.86	100
	410.84	100	524.36	100	33.16	100
	793.71	100	1011.34	100	53.6	100
Mean Relative Potency	**619.872**	**100**	**702.44**	**100**	**44.298**	**100**
0.1	771.03	104.5904041	342.58	97.9947939	40.84	77.24607528
	529.45	115.8812843	1062.77	86.02429923	35.45	98.47222222
	593.66	84.72022034	325.25	83.08214979	49.2	107.2830353
	669.78	163.0269691	436.89	83.31871234	38.33	115.5910736
	673.11	84.80553351	635.47	62.83445725	38.15	71.17537313
Mean Relative Potency	**647.406**	**110.60488**	**560.592**	**82.6508825**	**40.394**	**93.95355591**
0.2	772.6	104.803375	335.04	95.83798	36.7	69.41554757
	578.88	126.700081	583.3	47.21433	26.01	72.25
	626.65	89.42816777	349.24	89.21018	31.88	69.51591801
	884.6	215.3149645	331.4	63.20085	29.91	90.19903498
	462.38	58.25553414	828.38	81.90915	20.82	38.84328358
Mean Relative Potency	**665.022**	**118.90042**	**485.472**	**75.4745**	**29.064**	**68.04475683**
0.3	1050.62	142.5168545	337.56	96.55882605	36.04	68.16720257
	365.22	79.93608965	786.83	63.68875614	13.04	36.22222222
	466.6	66.5877014	368.98	94.25257995	21.58	47.05625818
	708.75	172.5124136	476.56	90.88412541	29.16	87.93727382
	354.56	44.67122753	753.13	74.4685269	12.95	24.16044776
Mean Relative Potency	**589.15**	**101.2448573**	**544.612**	**83.97056289**	**22.554**	**52.70868091**
0.5	1353.13	183.5524085	480.04	137.3151406	22.65	42.84093058
	277.45	60.72577645	635.11	51.40801179	9.06	25.16666667
	288.9	41.22843321	308.81	78.88270154	10.23	22.30702137
	566.97	138.0026288	494.63	94.33023114	17.58	53.01568154
	382.24	48.15864736	828.79	81.94969051	8.06	15.03731343
Mean Relative Potency	**573.738**	**94.33357885**	**549.476**	**88.77715511**	**13.516**	**31.67352272**
1.0	301.81	40.9406	504.13	144.20607	5.21	9.85435975
	214.15	46.87124	726.92	58.83943242	5	13.88888889

HSA [wt%, g/100 ml]	IGF-1		X-10		Insulin 454	
	357.97	51.0853	514.76	131.490753	5.8	12.64718709
	314.84	76.63324	476.91	90.95087345	6.42	19.36067551
	359.83	45.3352	656.29	64.89311211	3.53	6.585820896
Mean Relative Potency	**309.72**	**52.17311**	**575.802**	**98.0760482**	**5.192**	**12.46738643**
	205.45	27.86934	269.69	77.14466	4.65	8.795157935
	194.79	42.63389	586.76	47.49439	2.46	6.833333333
2.5	216.11	30.84069	370.29	94.58721	1.85	4.034016572
	102.7	24.99757	303.3	57.84194	1.89	5.699638118
	334.64	42.16149	694.71	68.69203	1.97	3.675373134
Mean Relative Potency	**210.738**	**33.7006**	**444.95**	**69.15205**	**2.564**	**5.807503819**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound. [b]EC_{50} values expressed relative to EC_{50} values at 0 % HSA (defined as 100 %).The EC_{50} values of the test and reference compounds were calculated as described in Appendix C. In each cell, numbers from top to bottom indicate results from experiment 2, 3, 4, 5 and 6.

Study No. 208181: in vitro mitogenicity in MCF-7 cells (0-2% HSA)

In vitro mitogenicity of insulin 0100-0000-0454 in MCF-7 human mammary adenocarcinoma cells: study comprising 5 experiments with 0 to 2 wt % HSA [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in MCF-1 cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Insulin 454 binds to human serum albumin. The mitogenic effect of insulin 454 was expected to be inhibited by HSA added to the cell culture medium. Findings: In the absence of HSA, the relative mitogenic potency of insulin 454 was 54% compared to human insulin. The mitogenic potency was 1.85X lower than mitogenic potency on human insulin. The ranking of mitogenic potencies (IGF-1 and X-10 more mitogenic than HI) was as expected for the MCF-7 cell system. However, HAS exhibited an unexpected inhibitory effect on the mitogenicity of HI as well as X-10 and IGF-1 in MCF-1 cells. Therefore, the MCF-7 cell system should be considered invalid for testing insulin analogues under the presence of HSA.

Effect of HSA on the relative mitogenic potency of insulin 454 in MCF-7 cells

		HI	Insulin 454			
HSA [wt%, g/100 ml]	HSA [µM]	EC50 [nM]	EC50 [nM]	*Relative Mitogenic Potency [%]	Mean Relative Mitogen Potency [%]	STDEV
Not added	0	3.31	5.97	55.43	54.12	13.23
		10.30	18.75	54.91		
		8.36	11.19	74.71		
		3.68	9.21	39.98		
		3.31	7.26	45.55		
0.1	15	3.97	6.22	63.84	89.95	41.34
		16.84	14.89	113.07		
		15.49	10.27	150.82		
		7.38	14.73	50.09		
		9.83	13.67	71.92		
0.2	30	13.69	14.84	92.27	112.54	44.02
		30.02	19.61	153.1		
		25.46	15.75	161.67		
		33.58	34.02	98.74		
		14.44	25.36	56.94		
0.3	45	32.01	27.58	116.03	79.01	41.62
		35.95	31.34	114.67		

		HI	Insulin 454			
		9.37	17.43	53.75		
		45.70	50.30	90.81		
		6.95	35.16	19.77		
0.5	75	5.97	20.86	28.63	32.63	18.14
		16.66	37.79	44.1		
		6.77	24.53	27.6		
		46.48	84.10	55.29		
		2.34	31.12	7.52		
1.0	150	2.34	48.72	4.8	7.43	2.77
		3.51	41.97	8.36		
		3.41	47.61	7.17		
		7.20	61.81	11.65		
		4.18	80.56	5.19		
2.0	300	1.61	59.80	2.69	2.90	1.02
		2.23	103.13	2.16		
		3.52	87.88	4		
		5.74	147.53	3.89		
		2.80	160.93	1.74		

* Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, insulin 454. The EC_{50} values of the test and reference compounds were calculated as described in Appendix B. A relative mitogenic potency of 54.12 % (mean relative mitogenic potency of insulin 454 at 0 % HSA) corresponds to a 1.85-fold lower mitogenic effect than HI. HSA molarity was calculated based on a molecular weight of 67 kDa. In each cell, numbers from top to bottom indicate results from experiments 1, 2, 3, 4 and 5.

Effect of HSA on relative mitogenic potency of test and reference compounds, individual results from experiments 1, 2, 3, 4 and 5.

HSA [wt%, g/100 ml]	IGF-1		X-10		Insulin 454	
	[a]Relative Mitogenic Potency [%]	[b]Relative Mitogenic Potency, % of no HSA [%]	[a]Relative Mitogenic Potency [%]	[b]Relative Mitogenic Potency, % of no HSA [%]	[a]Relative Mitogenic Potency [%]	[b]Relative Mitogenic Potency, % of no HSA [%]
0	1503.19	100	421.48	100	55.43	100
	1523.14	100	359.98	100	54.91	100
	2270.17	100	694.82	100	74.71	100
	1187.88	100	446.57	100	39.98	100
	973.21	100	296.59	100	45.55	100
Mean Relative Potency	1491.52	100	443.89	100	54.12	100
0.1	960.33	63.89	244.71	58.06	63.84	115.17
	2454.99	161.18	414.09	115.03	113.07	205.92
	3688.84	162.49	530.79	76.39	150.82	201.87
	1629.96	137.22	316.71	70.92	50.09	125.29
	2033.45	208.9	300.65	101.37	71.92	157.89
Mean Relative	2153.51	146.74	361.39	84.35	89.95	161.23

HSA [wt%, g/100 ml]	IGF-1		X-10		Insulin 454	
Potency						
	1786.64	118.86	169.19	40.14	92.27	166.46
	2942.36	193.18	238.77	66.33	153.1	278.82
0.2	7607.1	335.09	778.15	111.99	161.67	216.40
	3718.84	313.07	202.29	45.30	98.74	246.97
	2820.37	289.80	393.15	132.56	56.94	125.01
Mean Relative Potency	3775.06	250.00	356.31	79.26	112.54	206.73
	2691.37	179.04	209.4	49.68	116.03	209.33
	3636.02	238.72	273.77	76.05	114.67	208.83
0.3	1902.1	83.79	574.38	82.67	53.75	71.94
	4951.83	416.86	269.18	60.28	90.81	227.14
	1325.9	136.24	314.1	105.90	19.77	43.40
Mean Relative Potency	2901.44	210.93	328.17	74.92	79.01	152.13
	660.52	43.94	160.79	38.15	28.63	51.65
	1885.8	123.81	383.85	106.63	44.1	80.31
0.5	738.17	32.52	564.64	81.26	27.6	36.94
	2475.29	208.38	293.62	65.75	55.29	138.29
	462.18	47.49	372.93	125.74	7.52	16.51
Mean Relative Potency	1244.39	91.23	355.17	83.51	32.63	64.74
	137.48	9.15	119.47	28.35	4.8	8.66
	339.23	22.27	252.53	70.15	8.36	15.22
1.0	400.85	17.66	512.72	73.79	7.17	9.60
	506.73	42.66	258.98	57.99	11.65	29.14
	540.41	55.53	296.75	100.05	5.19	11.39
Mean Relative Potency	384.94	29.45	288.09	66.07	7.43	14.80
	126.05	8.39	124.7	29.59	2.69	4.85
	199.75	13.11	335.49	93.20	2.16	3.93
2.0	365.31	16.09	324.51	46.70	4	5.35
	421.36	35.47	283.75	63.54	3.89	9.73
	237.9	24.44	129.37	43.62	1.74	3.82
Mean Relative Potency	270.07	19.50	239.56	55.33	2.90	5.53

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound. [b]Relative Mitogenic Potencies expressed relative to Relative Mitogenic Potencies at 0 % HSA (defined as 100 %).The EC_{50} values of the test and reference compounds were calculated as described in Appendix B. In each cell, numbers from top to bottom indicate results from experiment 1, 2, 3, 4 and 5.

Study No. 209374: in vitro mitogenicity in L6-hIR cells (5 expts)

In vitro mitogenicity of insulin 0100-0000-0454 in L6-hIR cells: study comprising 5 experiments [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in L6-hIR cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was 13.5% compared to human insulin. The ranking of mitogenic potencies (X-10 more

mitogenic than HI and IGF-1 less mitogenic than HI) was as expected for the L6-hIR cell system.

Relative mitogenic EC50 values and potencies of human insulin, insulin 454, X-10 and IGF-1

Exp. no.	IGF-1		X-10		Human insulin		Insulin 454	
	EC_{50}, nM	[a]Relative Mitogenic Potency [%]	[b]EC_{50}, nM	[a]Relative Mitogenic Potency, % [%]	[c]EC_{50}, nM	[a]Relative Mitogenic Potency [%]	EC_{50}, nM	[a]Relative Mitogenic Potency [%]
1	12.871	7.08	0.5036	285.6	1.0050	100	23.855	4.21
2	35.766	1.93	0.2108	472.0	0.7068	100	6.3662	11.1
3	21.306	3.52	0.2211	591.0	0.6880	100	7.1064	9.68
4	78.189	1.74	0.3110	487.3	0.8843	100	6.7666	13.1
5	13.846	2.82	0.2932	333.4	0.6370	100	2.1771	29.3
Mean	**32.396**	**3.418**	**0.3079**	**433.9**	**0.7842**	**100**	**9.2542**	**13.5**
STDEV	**27.188**	**2.168**	**0.1178**	**123.6**	**0.1547**	**0**	**8.4025**	**9.44**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound.

[b]The EC_{50} values of the test and reference compounds were calculated as described in Appendix C.

[c]Listed EC_{50} value for human insulin is the value calculated pairwise with insulin 454.

Study No. 209375: in vitro mitogenicity in HMEC system (3 expts)

In vitro mitogenicity of insulin 0100-0000-0454 in HMEC cells: study comprising 3 experiments [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in HMEC cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was 12.5% compared to human insulin. The ranking of mitogenic potencies (IGF-1 more mitogenic than X-10 and X-10 more mitogenic than HI) was as expected for the HMEC cell system.

Relative mitogenic EC50 values and potencies of human insulin, insulin 454, X-10 and IGF-1

Exp. no.	IGF-1		X-10		Human insulin		Insulin 454	
	EC_{50}, nM	[a]Relative Mitogenic Potency [%]	[b]EC_{50}, nM	[a]Relative Mitogenic Potency, % [%]	[c]EC_{50}, nM	[a]Relative Mitogenic Potency [%]	EC_{50}, nM	[a]Relative Mitogenic Potency [%]
1	0.2837	11227	2.4278	2102.3	14.999	100	170.72	8.79
4	0.5975	2503.2	3.4418	589.21	39.885	100	175.91	22.67
6	0.3420	2479.0	0.8914	1587.4	8.5079	100	141.46	6.02
Mean	**0.4077**	**5403.1**	**2.2537**	**1426.3**	**21.131**	**100**	**162.70**	**12.49**
STDEV	**0.1669**	**5043.7**	**1.2841**	**769.30**	**16.563**	**0**	**18.574**	**8.921**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound.

[b]The EC_{50} values of the test and reference compounds were calculated as described in Appendix C.

[c]Listed EC_{50} value for human insulin is the value calculated pairwise with insulin 454.

Study No. 209376: in vitro mitogenicity in COLO-205 cells (5 expts)

In vitro mitogenicity of insulin 0100-0000-0454 in COLO-205 human colon adenocarcinoma cells: study comprising 5 experiments [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in HMEC cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was 4.72% compared to human insulin. The ranking of mitogenic potencies (X-10 and more mitogenic than HI) was as expected for the COLO-205 cell system.

Relative mitogenic EC50 values and potencies of human insulin, insulin 454, X-10 and IGF-1

Exp. no.	IGF-1		X-10		Human insulin		Insulin 454	
	EC_{50}, nM	[a]Relative Mitogenic Potency [%]	[b]EC_{50}, nM	[a]Relative Mitogenic Potency, % [%]	[c]EC_{50}, nM	[a]Relative Mitogenic Potency [%]	EC_{50}, nM	[a]Relative Mitogenic Potency [%]
1	0.6887	1891.5	1.6837	830.59	9.6408	100	124.34	7.76
2	0.3631	413.06	0.4575	410.37	1.2561	100	52.932	2.37
3	0.4602	381.69	0.4705	407.75	1.7057	100	56.940	3.00
4	0.3332	1584.7	0.6921	1035.6	2.9683	100	57.111	5.20
5	0.0863	1919.7	0.5016	525.69	1.6112	100	30.114	5.35
Mean	**0.3863**	**1238.1**	**0.7611**	**642.00**	**3.4364**	**100**	**64.287**	**4.74**
STDEV	**0.2181**	**778.74**	**0.5244**	**279.53**	**3.5282**		**35.386**	**2.14**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound.

[b] The EC_{50} values of the test and reference compounds were calculated as described in Appendix C.

[c]Listed EC_{50} value for human insulin is the value calculated pairwise with insulin 454.

Study No. 209377: in vitro mitogenicity in MCF-7 cells (5 expts)

In vitro mitogenicity of insulin 0100-0000-0454 in MCF-7 human breast adenocarcinoma cells: study comprising 5 experiments [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in MCF-7 cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was 14.2% compared to human insulin. The ranking of mitogenic potencies (X-10 and IGF-1 more mitogenic HI) was as expected for the MCF-7 cell system.

Relative mitogenic EC50 values and potencies of human insulin, insulin 454, X-10 and IGF-1

Exp. no.	IGF-1		X-10		Human insulin		Insulin 454	
	EC_{50}, nM	[a]Relative Mitogenic Potency [%]	[b]EC_{50}, nM	[a]Relative Mitogenic Potency, % [%]	[c]EC_{50}, nM	[a]Relative Mitogenic Potency [%]	EC_{50}, nM	[a]Relative Mitogenic Potency [%]
3	0.9704	2232.2	4.0431	424.62	21.737	100	119.34	18.2
4	0.4347	2200.9	3.0926	322.14	10.085	100	106.70	9.45
5	0.7619	1649.2	3.5716	301.15	14.969	100	115.93	12.9
6	0.4781	4688.3	4.1913	422.50	15.410	100	88.058	17.5
8	0.2882	2679.0	1.6905	328.25	7.4559	100	58.207	12.8
Mean	**0.5867**	**2689.9 2689.92**	**3.3178**	**359.73**	**13.931**	**100**	**97.647**	**14.17**
STDEV	**0.2747**	**1175.3 1175.307**	**1.0065**	**59.132**	**5.4981**	**0**	**25.171**	**3.643**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound.

[b] The EC_{50} values of the test and reference compounds were calculated as described in Appendix C.

[c]Listed EC_{50} value for human insulin is the value calculated pairwise with insulin 454.

Study No. 209379: in vitro mitogenicity in HMEC system (3 expts)

In vitro mitogenicity of insulin 0100-0000-0454 in HMEC cells: study comprising 3 experiments [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in HMEC cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was 7.27% compared to human insulin. The ranking of mitogenic potencies (IGF-1 more mitogenic than X-10 and X-10 more mitogenic than HI) was as expected for the HMEC cell system.

Relative mitogenic EC_{50} values and potencies of human insulin, insulin 454, X-10 and IGF-1

Exp. no.	IGF-1		X-10		Human insulin		Insulin 454	
	EC_{50}, nM	[a]Relative Mitogenic Potency [%]	[b]EC_{50}, nM	[a]Relative Mitogenic Potency, % [%]	[c]EC_{50}, nM	[a]Relative Mitogenic Potency [%]	EC_{50}, nM	[a]Relative Mitogenic Potency [%]
1	1.8757	1038.3	2.5370	991.01	17.444	100	133.09	13.1
4	0.5461	4275.0	3.2576	1508.7	19.531	100	340.36	5.74
6	0.2466	2778.1	1.2649	1101.6	8.9085	100	299.17	2.98
Mean	**0.8895**	**2697.1**	**2.3532**	**1200.4**	**15.294**	**100**	**257.54**	**7.27**
STDEV	**0.8671**	**1619.9**	**1.0090**	**272.63**	**5.628306**	**0**	**109.73**	**5.23**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound.

[b]The EC_{50} values of the test and reference compounds were calculated as described in Appendix C.

[c]Listed EC_{50} value for human insulin is the value calculated pairwise with insulin 454.

Study No. 209380: in vitro mitogenicity in COLO-205 cells (5 expts)

In vitro mitogenicity of insulin 0100-0000-0454 in COLO-205 human colon adenocarcinoma cells: study comprising 5 experiments [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in COLO-205 cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was 6.86% compared to human insulin. The ranking of mitogenic potencies (X-10 and IGF-1 more mitogenic than HI) was as expected for the COLO-205 cell system.

Relative mitogenic EC50 values and potencies of human insulin, insulin 454, X-10 and IGF-1

Exp. no.	IGF-1		X-10		Human insulin		Insulin 454	
	EC_{50}, nM	[a]Relative Mitogenic Potency [%]	[b]EC_{50}, nM	[a]Relative Mitogenic Potency, % [%]	[c]EC_{50}, nM	[a]Relative Mitogenic Potency [%]	EC_{50}, nM	[a]Relative Mitogenic Potency [%]
1	0.9822	632.16	1.2436	435.50	7.6829	100	61.190	12.55
2	0.5283	688.95	1.0931	520.83	3.6730	100	111.05	3.31
4	0.6928	1833.4	1.4434	1008.1	11.427	100	166.50	6.87
5	0.7929	2180.9	1.4405	1284.8	12.782	100	235.10	5.44
6	0.9851*	7681.3*	1.1388	964.51	11.728	100	190.57	6.15
Mean	**0.7491**	**1333.9**	**1.2719**	**842.75**	**9.4586**	**100**	**152.88**	**6.86**
STDEV	**0.1899**	**790.63**	**0.1646**	**356.04**	**3.76484**	**0**	**68.050**	**3.44**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound.

[b] The EC_{50} values of the test and reference compounds were calculated as described in Appendix C.

[c]Listed EC_{50} value for human insulin is the value calculated pairwise with insulin 454.

*This value is considered non-valid due to bad curve fitting, see Appendix C, Figure 5, and is omitted from the calculated Mean and STDEV.

Study No. 209381: in vitro mitogenicity in MCF-7 cells (3 expts)

In vitro mitogenicity of insulin 0100-0000-0454 in MCF-7 human breast adenocarcinoma cells: study comprising 3 experiments [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in COLO-205 cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: The relative mitogenic potency of insulin 454 was 10.12 % compared to human insulin. The ranking of mitogenic potencies (X-10 and IGF-1 more mitogenic than HI) was as expected for the MCF-7 cell system.

Relative mitogenic EC50 values and potencies of human insulin, insulin 454, X-10 and IGF-1

Exp. no.	IGF-1		X-10		Human insulin		Insulin 454	
	EC_{50}, nM	[a]Relative Mitogenic Potency [%]	[b]EC_{50}, nM	[a]Relative Mitogenic Potency, % [%]	[c]EC_{50}, nM	[a]Relative Mitogenic Potency [%]	EC_{50}, nM	[a]Relative Mitogenic Potency [%]
5	1.3648	901.42	2.700	649.89	24.582	100	189.24	12.99
6	1.5746	525.24	4.100	285.61	15.150	100	181.64	8.340
7	0.2444	3322.2	1.130	725.46	5.4249	100	60.039	9.030
Mean	**1.0613**	**1583.0**	**2.643**	**553.65**	**15.052**	**100**	**143.64**	**10.12**
STDEV	**0.7152**	**1517.9**	**1.486**	**235.19**	**9.5787**	**0**	**72.500**	**2.509**

[a]Relative mitogenic potency expressed as percentage of the potency of non modified human insulin (100 %). The relative mitogenic potency was calculated as: EC_{50}, HI/EC_{50}, test compound.

[b] The EC_{50} values of the test and reference compounds were calculated as described in Appendix C.

[c]Listed EC_{50} value for human insulin is the value calculated pairwise with insulin 454.

Study No. 210073: in vitro mitogenicity in COLO-205 cells

In vitro mitogenicity of insulin 0100-0000-0454 in COLO-205 human colon adenocarcinoma cell line [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in COLO-205 cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: Based on EC50, the relative mitogenic potency of insulin 454 was 6.1% compared to human insulin. Based on EC20 and EC80 ratio, the potency was within a range from 5.0 to 7.5%.

EC_{20}, EC_{50} and EC_{80} ratios for NNC 0100-0000-0454

Experiment	EC_{20} ratio	EC_{50} ratio	EC_{80} ratio
1	0.0695875	0.0698692	0.0701521
2	0.0396139	0.0449191	0.0509348
3	0.0234635	0.0385879	0.0634613
5	0.0345843	0.0448927	0.0582738
6	0.1462766	0.1617698	0.1789039
Geometrical mean	0.050	0.061	0.075

Study No. 210107: in vitro mitogenicity in MCF-7 cells

In vitro mitogenicity of insulin 0100-0000-0454 in MCF-7 human mammary adenocarcinoma cell line [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the mitogenic potential of the insulin 454 in MCF-7 cells, compared to three reference compound, HI (human insulin), X10 (human AspB10 insulin), and IGF-1. Findings: Based on EC50, the relative mitogenic potency of insulin 454 was 6.8% compared to human insulin. Based on EC20 and EC80 ratio, the potency was within a range from 3.7 to 12.5%.

EC_{20}, EC_{50} and EC_{80} ratios for NNC 0100-0000-0454

Experiment	EC_{20} ratio	EC_{50} ratio	EC_{80} ratio
1	0.0340601	0.0516859	0.0784328
2	0.0303922	0.0698528	0.1605484
4	0.0489566	0.0859832	0.1510134
6	0.0700475	0.1010650	0.1458172
9	0.0202661	0.0472514	0.1101693
Geometrical mean	0.037	0.068	0.125

The relative mitogenic potencies were calculated as the ratio between EC_{50} for HI and EC_{50} for the compound (EC_{50} HI/ EC_{50} compound).

Study No. ARS 15-Feb-2005: lipogenesis in rat adipocytes

Lipogenic effect of 0100-0000-0454 in primary rat adipocytes [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the biological potency of 0100-0000-0454 relative to human insulin, and to assess whether 0100-0000-0454 was a full agonist using primary adipocytes from rats. The assay measured the conversion of 3H-labelled glucose into extractable lipid, a process that is dependent on the degree of insulin stimulation. Findings: The estimated potency of 0100-0000-0545 was 0.55% at 1% human serum, and decreased to 0.45% with 2% serum and 0.3% with 5% serum. The insulin 0100-0000-0545 functioned as a full agonist.

Combined potency estimates

% HSA	Potency (% of human insulin)	95% con. lim		W
1	0.55	0.58	0.52	6136
2	0.45	0.51	0.39	1240
5	0.30	0.34	0.26	1365

Study No. BFH 20-Aug-2005: glycogen synthesis in L6-hIR cells

Glycogen synthesis in L6-hIR cells [by Novo Nordisk A/S, Denmark]

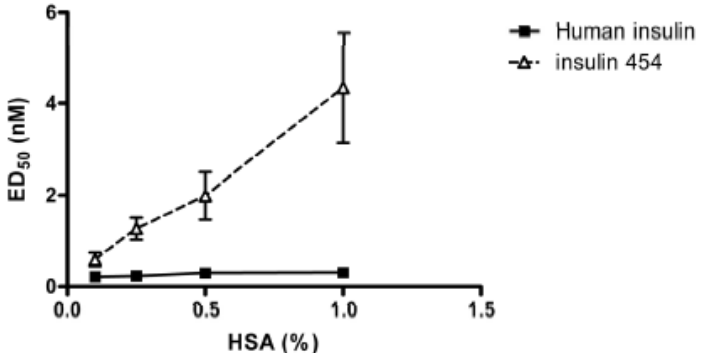
Study design: This study was to determine the effect of insulin 454 on stimulation of 14C-glucose incorporation into newly synthesized glycogen, compared to human insulin, using L6-hIR cells. The effect of albumin on the estimated ED50 values was also investigated. Increased glycogen synthesis is a classical effect of insulin stimulation and could be used to determine the metabolic potency of insulin analogues. Findings: Similar to human insulin, insulin 454 stimulated the glycogen synthesis in L6-hIR cells, but with a relative potency of 11.5%. The estimated relative potency depended on the HSA concentration, since the estimated EC50 for insulin 454 increased with increasing HSA levels.

Calculation of combined metabolic potential of insulin 454 relative to human insulin (100 %) determined in L6-hIR cell line

Assay no.	Potency %	W	Log strenght	m * W	Chi-Square
1	29.46	11.03	1.4693	16.2176198	1.82965576
2	13.78	57.29	1.1395	65.2878985	0.34273156
3	10.85	49.53	1.0356	51.2970538	0.03493628
4	9.57	108.2	0.981	106.230743	0.71324572
5	12.32	41.29	1.0907	45.040182	0.03364163

No. assays:	5	W =	267.44949	284.073498	2.95421095
P (chi-sq):	56.552%	homogeneous combination			
Combined potency		11.5	%		
95% confidence intervals:		8.8		15.2	%

The effect of the HSA concentration on the estimated ED_{50} values for insulin and insulin 454



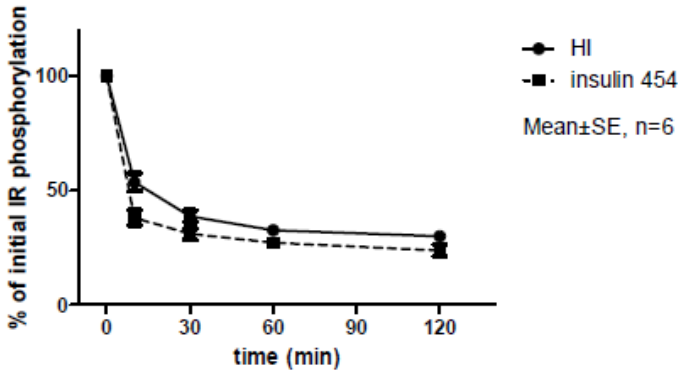
Note: ↑ HAS → ↑ EC50 → ↓ potency for mitogenicity for insulin 454 relative to rh-insulin, probably due to insulin binding to HAS.

Study No. BFH 23-Feb-2009: phosphorylation of IR in CHO-hIR cells

Decline of response in CHO-hIR cells [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the phosphorylation of the insulin receptor after stimulation with insulin and insulin 454 (batch 14794/060 and 17672/004) in CHO-hIR cells. There is a correlation between increased mitogenic potential and prolonged signaling from the receptor. Therefore, it is undesired for insulin 454 to have prolonged signaling potential from the insulin receptor upon stimulation. Findings: Insulin 454 had the same effect as human insulin in that there was no evidence for prolonged signaling after stimulation with insulin 454, since the signal as determined by the IR phosphorylation rate, declined as for native insulin.

Phosphorylation after stimulation with insulin or insulin 454. Results are presented as % of the initial phosphorylation (at t=0) as Mean±SE, n=6.

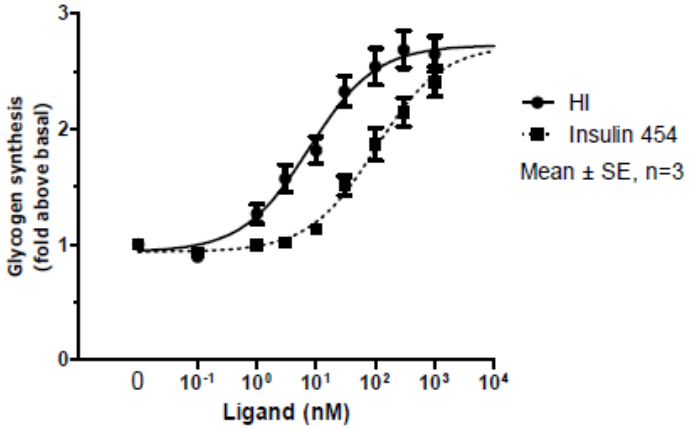


Study No. BFH 23-Feb-2009 GLST MCF7: glycogen synthesis in MCF-7 cells

Glycogen synthesis in MCF-7 cells [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the effect of insulin 454 on stimulation of 14C-glucose incorporation into newly synthesized glycogen, compared to human insulin, using MCF-7 cells, over 3 hr period. Increased glycogen synthesis is a classical effect of insulin stimulation and could be used to determine the metabolic potency of insulin analogues. Findings: The estimated potency was 7.7% assuming parallel dose-response curves. Insulin 454 stimulated glycogen synthesis in MCF-7 cells in a dose-dependent fashion similar to human insulin. .

Log-dose response curves for ^{14}C-glucose incorporation into glycogen induced by human insulin and insulin 454 determined in MCF-7 cells. Results are presented as Mean±SE, n=3, each perfomed in duplicates

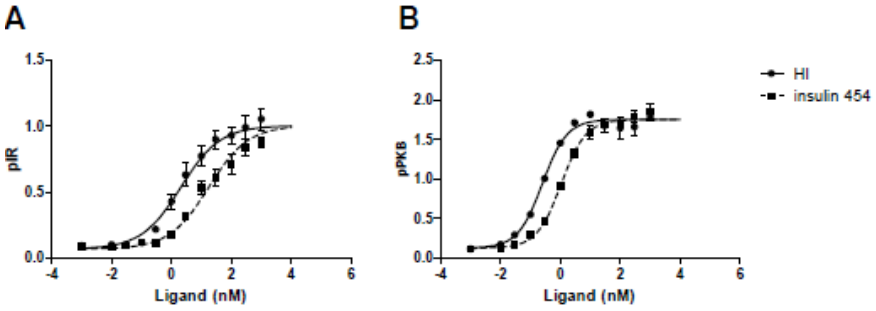


Study No. BFH 23-Feb-2009 Phos DRC: phosphorylation in L6-hIR cells

IR and PKB Phosphorylation in L6-hIR cells [by Novo Nordisk A/S, Denmark]

Study design: This study was to compare the ability of insulin and insulin 454 to stimulate two elements in insulin signaling – auto-phosphorylation of IR and further phosphorylation of downstream kinases (i.e. Protein Kinase B, PKB). Findings: Insulin 454 and insulin stimulated phosphorylation of insulin receptor and PKB in a dose dependent manner. This study demonstrated that insulin 454 stimulated these signaling cascades as native human insulin but with a lower potency.

Log-dose response curves for phosphorylation of the insulin receptor(A) and PKB (B) induced by human insulin and insulin 454 determined in L6-hIR cells (results are presented as Mean ± SE, n=3)



Study No. CES15460-041 Jan-2007: binding kinetics for IR in BHK cells

Insulin receptor binding kinetics [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the association and dissociation rate constants for binding of the insulin 454 to the insulin receptor, and to compared with those of human insulin, using baby hamster kidney cells (BHK) expressing hIR-A. Findings: The observed rate constants (K_{obs}) for the association of ^{125}I-human insulin and ^{125}I-insulin 454 were 0.0239 and 0.0222 min^{-1}. The dissociation of both insulins had a fast (K_{off-2}) and a slow (K_{off-1}) component, reflecting the 2 binding sites for insulin on the receptor. The dissociation rats for each of these components varied slightly. The observed association rate constants contained components of both association and dissociation. As the dissociation rates for human insulin and insulin 454 did not differ, the observed association rates would be equally affected by dissociation. All in all, this study showed that the binding kinetics of insulin 454 for the human insulin receptor isoform A was similar to that of human insulin.

Apparent rate constants for association and dissociation of ^{125}I-human insulin and ^{125}I-insulin 454

	^{125}I-HI			^{125}I-insulin 454			p$
	Mean	SEM	n	Mean	SEM	n	
k_{obs} (min^{-1})	0.0239	0.0024	4	0.0222	0.0026	4	>>0.05
k_{off-1} (min^{-1})	0.0181	0.0081	4	0.0139	0.0081	4	>>0.05
k_{off-2} (min^{-1})	0.1232	0.0557	4	0.1055	0.0255	4	>>0.05
Fraction$_2$ (%)	56.2	19.1	4	76.0	13.5	4	>>0.05

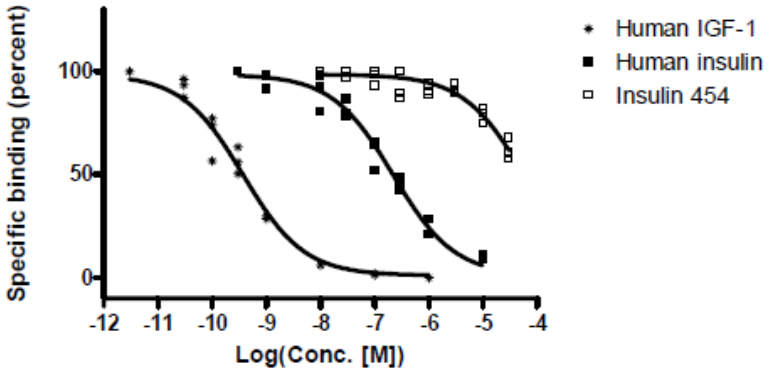
$ Student's t-test

Study No. CES02-Feb-2006: binding affinity for hIGF-1R in BHK cells

Binding affinity for membrane-associated human insulin-like growth factor-1 receptor (hIGF-1R) [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the affinities of human insulin and insulin 454 for membrane-associated human IGF-1 receptors, using plasma membranes purified from baby hamster kidney (BHK) cells, expressing IGF-1 receptor (labeled with ^{125}I). Findings: Binding of ^{125}I-IGF-1 to its receptors was displaced with human IGF, human insulin, and insulin 454, with the IC_{50} was estimated to 0.43, 212, and 51,700 nm, respectively. Therefore, in the presence of 0.1% human serum albumin, the apparent affinity of insulin 454 relative to human insulin for human IGF-1R was 0.4%.

Displacement by hIGF-1, human insulin and insulin 454 of ^{125}I-hIGF-1 binding to membrane-associated human IGF-1 receptors



Note: **Insulin 454 has lower affinity for IGF1R than human insulin, suggesting ↓ mitogenicity as IGF1 is implicated in this effect.**

Results summary table

	hIGF-1	HI	Insulin 454
Log(IC_{50}) ± SD (n)	-9.37 ± 0.16 (3)	-6.67 ± 0.10 (3)	-4.29 ± 0.16 (3)
IC_{50} (nM) [95% CI]	0.43 [0.18 – 1.0]	212 [123 – 372]	51,700 [20,000 – 126,000]

Study No. CES 10-Jun-2005: binding affinity of IR in BHK cells

Binding affinities for recombinant insulin receptor isoforms of human, porcine, and rat origin [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the affinities of human insulin and insulin 454 for membrane-associated human IGF-1 receptors, using plasma membranes purified from baby hamster kidney (BHK) cells, expressing IGF-1 receptor (labeled with ^{125}I). Findings: Human insulin and insulin 454 had slightly higher affinity for the short isoform of insulin receptors of human, porcine, and rat origin. The affinities of insulin

454 relative to human insulin were not significantly different between short and long isoforms of the insulin receptor for all three species. In addition, the interspecies differences in affinity of human insulin and insulin 454 were small between human and pig, however, the rat insulin receptor exhibited lower affinities for human insulin as well as insulin 454. The relative affinities between human insulin and insulin 454 were not changed. Moreover, increasing concentrations of human serum albumin attenuated the apparent receptor affinity caused by reversible albumin binding of insulin 454.

Summary results table of human insulin receptor isoforms

	Human insulin		Insulin 454		Affinity ratio
Source	IC50 (nM) [95% CI]	pIC50 ± SD (n)	IC50 (nM) [95% CI]	pIC50 ± SD (n)	% of HI [95% CI]
Human IR-A	0.31 [0.21-0.48]	9.50 ± 0.07 (3)	7.5 [5.6-10]	8.13 ± 0.05 (3)	4.2 [2.5-7.0]
Human IR-B	0.40 [0.33-0.49]	9.40 ± 0.04 (3)	13 [11-15]	7.90 ± 0.02 (3)	3.2 [2.5-4.0]

Summary results table of porcine insulin receptor isoforms

	Human insulin		Insulin 454		Affinity ratio
Source	IC50 (nM) [95% CI]	pIC50 ± SD (n)	IC50 (nM) [95% CI]	pIC50 ± SD (n)	% of HI [95% CI]
Porcine IR-A	0.32 [0.28-0.35]	9.50 ± 0.02 (3)	7.5 [3.3-17]	8.12 ± 0.14 (3)	4.2 [1.8-9.6]
Porcine IR-B	0.39 [0.20-0.75]	9.41 ± 0.12 (3)	8.6 [4.5-16]	8.07 ± 0.11 (3)	4.5 [1.8-11]

Summary results table of rat insulin receptor isoforms

	Human insulin		Insulin 454		Affinity ratio
Source	IC50 (nM) [95% CI]	pIC50 ± SD (n)	IC50 (nM) [95% CI]	pIC50 ± SD (n)	% of HI [95% CI]
Rat IR-A	0.68 [048-097]	9.17 ± 0.06 (3)	30 [28-31]	7.53 ± 0.01 (3)	2.3 [1.6-3.3]
Rat IR-B	0.65 [0.25-1.7]	9.19 ± 0.17 (3)	22 [15-31]	7.67 ± 0.07 (3)	3.0 [1.1-8.5]

Effects on relative binding affinities of insulin 454 for human, porcine and rat insulin receptor isoforms of increasing concentrations of human serum albumin (HSA)

Receptor		0.1% HSA	0.25% HSA	0.5% HSA	1% HSA
		AR (% of HI)	AR (% of HI)	AR (% of HI)	AR (% of HI)
hIR-A	Mean ± SD	5.2 ± 0.7	2.8 ± 0.8	1.5 ± 0.5	1 ± 0.2
hIR-B	Mean ± SD	4.8 ± 0.4	2.3 ± 0.1	1.3 ± 0.1	0.8 ± 0
pIR-A	Mean ± SD	6.3 ± 1.1	2.8 ± 0.4	1.3 ± 0.2	1.2 ± 0.3
pIR-B	Mean ± SD	5.3 ± 0.6	2.5 ± 0.4	0.9 ± 0.1	0.9 ± 0.1
rIR-A	Mean ± SD	3.4 ± 1.1	1.5 ± 0.6	0.9 ± 0.3	0.6 ± 0.2
rIR-B	Mean ± SD	6 ± 0.3	2.7 ± 0.2	1.5 ± 0.5	0.7 ± 0.4

Study No. CES 21-Jan-2005: binding affinity for IR in rat/pig/dog livers

Binding affinities for porcine, rat, and canine liver insulin receptors [by Novo Nordisk A/S, Denmark]

Study design: This study was to estimate the affinities of human insulin and insulin 454, using plasma membrane obtained form livers of pig, rat, and dog (labeled with ^{125}I). Findings: Binding of ^{125}I-insulin to porcine liver insulin receptors was displaced with human insulin and insulin 454, with IC50 estimated to be 0.46 and 13 nM, respectively. This resulted in an affinity of 3.6% for insulin 454 relative to human insulin. Binding of ^{125}I-insulin to rat liver insulin receptors was displaced with human insulin and insulin 454, with IC50 estimated to be 3.6 and 146 nM, respectively. This resulted in an affinity of 3.3% for insulin 454 relative to human insulin. Binding of ^{125}I-insulin to canine liver insulin receptors was displaced with human insulin and insulin 454, with IC50 estimated to be 3.2 and 45 nM, respectively. This resulted in an affinity of 7.0% for insulin 454 relative to human insulin. This study revealed that the porcine insulin receptor affinity for insulin 454 relative to human insulin was not significantly different from rat or canine insulin receptor affinity, whereas rat vs. canine affinity ratios differed 2 fold. All in all, the apparent affinity of insulin 454 relative to human insulin is reduced due to albumin binding. In the presence of 0.1% human serum albumin, the apparent affinity of insulin 454 was found to be 3.5% for porcine, 3.3% for rat, and 7.0% for canine liver insulin receptors.

Summary result table

	Human Insulin		Insulin 454		Affinity ratio
Source	IC_{50} [95% CI] (nM)	pIC_{50} ± SD (n)	IC_{50} [95% CI] (nM)	pIC_{50} ± SD (n)	AR [95% CI] (% of HI)
Porcine liver	0.46 [0.24–0.86]	9.34 ± 0.17 (4)	13 [7.6–21]	7.90 ± 0.14 (4)	3.6* [2.3–3.8]
Rat liver	3.6 [1.7–7.8]	8.45 ± 0.14 (3)	146 [57–375]	6.84 ± 0.17 (3)	3.3*$ [1.3–4.8]
Canine liver	3.2 [1.8–5.4]	8.50 ± 0.18 (5)	45 [29–70]	7.34 ± 0.15 (5)	7.0*$ [4.2–11]

* $p > 0.05$ for porcine vs. rat and for porcine vs. canine
$ $p < 0.05$ for rat vs. canine

Study No. CES 28-Sep-2004: binding affinity for IR in human liver

Binding affinities for porcine, rat, and canine liver insulin receptors [by Novo Nordisk A/S, Denmark]

Study design: This study was to estimate the affinities of human insulin and insulin 454, using plasma membrane obtained form human liver (labeled with ^{125}I). Findings: Binding of ^{125}I-insulin to human liver insulin receptors was displaced with human insulin and insulin 454, with IC50 estimated to be 0.77 and 22 nM, respectively. This resulted in an affinity of 3.5% for insulin 454 relative to human insulin. All in all, in the presence of 0.1% human serum albumin, the apparent affinity of insulin 454 was found to be 3.5% for human liver insulin receptors.

Summary result table

	Human insulin		NNC 0100-0000-0454		Affinity ratio
Source	IC_{50} [95% CI] (nM)	pIC_{50} ± SD (n)	IC_{50} [95% CI] (nM)	pIC_{50} ± SD (n)	AR [95% CI] (% of HI)
Human liver	0.77 [0.41–1.4]	9.12 ± 0.17 (4)	22 [14–36]	7.66 ± 0.13 (4)	3.5 [1.5–8.2]

Study No. CES 101122: binding affinity for IGF-1R in BHK cells

Binding affinities for rat and canine IGF-1 receptors [by Novo Nordisk A/S, Denmark]

Study design: This study was to estimate the affinities of human insulin and insulin 454, using plasma membrane obtained from baby hamster kidney (BHK) cells, expressing the rat and canine IGF-1 receptors and rat insulin receptor (labeled with ^{125}I). Findings: Binding of ^{125}I-human insulin or ^{125}I-IGF-1 to membrane-associated recombinant rat or canine IGF-1 receptors and rat insulin receptor isoforms was displaced with human IGF-1, human insulin and insulin 454. This study showed that relative to human insulin, the affinity of insulin 454 for the rat and canine IGF-1 receptors was 1.2% and 0.7%, respectively. This was well below the relative affinities of insulin 454 for the insulin receptors.

IC_{50} values for binding of insulin 454, human insulin and human IGF-I to rat insulin and IGF-I receptors and canine IGF-I receptors (mean and 95% confidence intervals of three experiments)

	IC_{50} (nM) [95% CI]			
Compound	**rIR-A**	**rIR-B**	**rIGF1-R**	**cIGF1-R**
Human insulin	0.43 [0.15 - 1.3]	0.53 [0.25 - 1.1]	230 [93 - 570]	170 [71 - 420]
Insulin 454	11 [7.7 - 15]	7.8 [4.9 - 12]	19,000 [8,200 - 42,000]	26,000 [13,000 - 52,000]
Human IGF-I	-	-	0.64 [0.25 - 1.6]	0.42 [0.12 - 1.5]

Relative affinities of insulin 454 for rat insulin and IGF-I receptors and canine IGF-I receptors (human insulin = 100%)

	Relative affinity (%)			
	rIR-A	**rIR-B**	**rIGF-1R**	**cIGF1-R**
Mean	4.0	6.8	1.2	0.66
95% CI	1.9 - 8.3	4.0 - 11	1.1 - 1.4	0.48 - 0.90
n	3	3	3	3

Study No. CLB3 31-Aug-2005: clamp study in normal and insulin-resistant rats

Effect of insulin 454 on glucose metabolism under steady state clamp conditions in normal Spraque Dawley and insulin resistant Zucker rats [by Novo Nordisk A/S, Denmark]

Study design: This study was 1) to estimate the potency of insulin 454 relative to human insulin on whole body glucose disposal and 2) to investigate potential preferential effects on target tissues (liver, muscle, and fat tissues), under iv steady state clamp conditions in normal and obese/insulin resistant rats. Insulin 454 and HI were infused by IV administration for 5 hrs until steady state in Spraque Dawley (SD) and Zucker Obese (ZO) rats. The mean glucose infusion rate (GIR) to maintain euglycemia during the last hour of the clamp was taken as measure of efficacy.

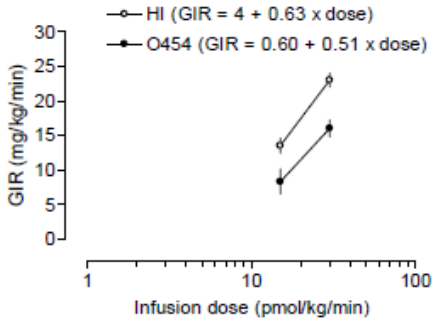
Insulin doses tested and number of rats included at each dose level

	Sprague Dawley rats (n)		Zucker obese rats (n)	
Infusions dose (pmol/min/kg)	**insulin 454**	**HI**	**insulin 454**	**HI**
15	5	5		
30	5	5		
45				5
90			4	5
180			4	

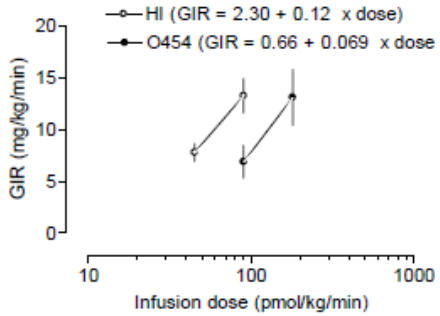
Findings: In Spraque Dawley (SD) rats, the potency of insulin 454 was 65% or reduced by 1.5X relative to HI. In Zucker Obese (ZO) rats, the potency of insulin 454 was 47% or reduced by 2.1X relative to HI. Similarly reduced potencies of insulin 454 relative to

HI were obtained on tracer-determined (^{3}H-glucose) rates of glucose uptake and production in SD and ZO rats. In addition, no preferential effect of insulin 454 compared to HI was seen on tracer-determined (^{14}C-2-deoxy-glucose) rates of glucose uptake in various skeletal muscles and adipose tissues.

Potency of insulin 454 in SD rats estimated by linear regression



Potency of insulin 454 in ZO rats estimated by linear regression



Tracer-determined (^{3}H-glucose) R_d and endogenous R_a in the basal state and during clamp in Sprague Dawley rats

	Infusion dose (pmol/kg/min)	Basal Ra (mg/kg/min)	Basal R_d (mg/kg/min)	Clamp R_a (mg/kg/min)	Clamp R_d (mg/kg/min)	ΔR_a (mg/kg/min)	ΔR_d (mg/kg/min)
HI	15	6.3±0.5	6.4±0.4	1.4±0.5	14.7±1.1	-4.9±0.7	8.3±1.5
	30	5.9±0.7	5.9±0.7	0.8±1.4	23.7±1.3	-4.7±0.9	18.0±1.3
Insulin 454	15	7.1±0.6	7.2±0.6	4.4±0.8	12.8±1.1	-2.7±0.3	5.6±1.6
	30	6.3±0.7	6.4±0.7	2.2±0.7	18.2±1.3	-4.1±0.5	11.8±1.5

Data are presented as mean±SE (n=5 per group)

Tracer-determined (^{3}H-glucose) R_d and endogenous R_a in the basal state and during clamp in Zucker obese rats

	Infusion dose (pmol/kg/min)	Basal Ra (mg/kg/min)	Basal R_d (mg/kg/min)	Clamp R_a (mg/kg/min)	Clamp R_d (mg/kg/min)	ΔR_a (mg/kg/min)	ΔR_d (mg/kg/min)
HI	45	5.0±0.3	4.9±0.5	3.4±0.3	11.0±0.7	-1.6±0.2	6.1±0.7
	90	5.1±0.6	5.1±0.5	2.6±0.5	15.3±1.1	-2.5±0.4	10.1±1.5
454	90	4.5±0.5	4.3±0.4	3.5±0.6	11.7±1.1	-1.1±1.0	7.4±1.5
	180	5.1±0.4	5.2±0.4	2.3±0.6	14.9±2.3	-2.8±1.0	9.7±2.2

Data are presented as mean±SE (n=4-5 per group)

Tracer-determined (^{14}C-2-deoxy-glucose) tissue specific glucose uptake during clamp in Sprague Dawley rats

	Infusion dose (pmol/kg/min)	Soleus R'g (µmol/kg/min)	W. Gastr. R'g (µmol/kg/min)	R. Gastr. R'g (µmol/kg/min)	Epid. fat R'g (µmol/kg/min)	Sc. fat R'g (µmol/kg/min)
HI	15	57±12	156±19	328±34	6±2	44±16
	30	96 ±9	188±25	359±77	7±1	57±13
454	15	62±15	73±24	261±62	6±1	41±9
	30	93±24	151±21	361±35	7±2	42.6

Data are presented as mean±SE (n=5 per group)

Tracer-determined (^{14}C-2-deoxy-glucose) tissue specific glucose uptake during clamp in Zucker obese rats

	Infusion dose (pmol/kg/min)	Soleus R'g (µmol/kg/min)	W. Gastr. R'g (µmol/kg/min)	R. Gastr. R'g (µmol/kg/min)	Epid. fat R'g (µmol/kg/min)	Sc. fat R'g (µmol/kg/min)
HI	45	29±5	90±13	250±32	10±1	16±1
	90	46±17	155±31	323±55	13±1	14±1
454	90	27±7	134±28	234±38	11±2	12±1
	180	51±23	319±33	316±55	10±1	17±2

Data are presented as mean±SE (n=4-5 per group)

Study No. ENis 27-Jul-2005: glycogen synthesis in primary hepatocytes

Insulin 454 stimulation of glycogen synthesis and inhibition of PEPCK gene expression in primary rat hepatocytes: potency and effect of albumin [by Novo Nordisk A/S, Denmark]

Study design: This study was to examine the biological effect of the insulin 454 in liver cells, using cultured primary rat hepatocytes and to determine the metabolic potency of the insulin 454 relative to that of human insulin by measuring glycogen accumulation. In addition, inhibition of PEPCK (phosphoenolpyruvate carboxykinase) mRNA expression was also measured as an indicator of the inhibition of glucose synthesis in the liver. Insulin is known to mediate inhibition of PEPCK gene expression rather rapidly, representing a more acute determination of insulin action in the liver compared to glycogen synthesis.

Findings: Insulin 454 stimulated glycogen storage and inhibited PEPCK mRNA expression as human insulin, suggesting that insulin 454 is a IR agonist. The relative potency of insulin 454 in stimulating glycogen accumulation was 21.3% of human insulin in the absence of albumin. The apparent potency decreased with increasing concentrations of albumin, suggesting that high albumin binding capacity of insulin 454.

The relative potency of insulin 454 in inhibiting PEPCK mRNA expression in rat hepatocytes was 13.4 in the presence of 0.1% HSA.

EC_{50} values of insulin 454 in stimulating glycogen storage in primary rat hepatocytes and the effect of albumin on the relative potency of insulin 454 compared to human insulin

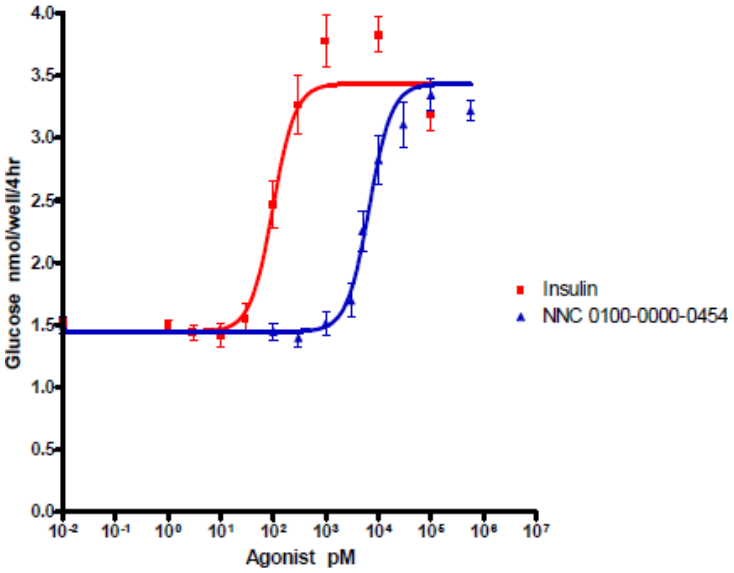
Albumin (%)	Potency (%)	insulin 454 ED50 (nM)	H. Insulin ED_{50} (nM)
None added	21.3 ±4.4	2.6±0.3	0.50±0.08
0.1%	10.1±4.5	8.0±1.3	0.67±0.18
0.5%	4.5±1.2	13.5±4.2	0.55±0.14
1%	3.3±1.6	92.4±61	0.62±0.12

Study No. HanT 30-Aug-2005: lipolysis in human adipocytes

Potency of insulin 454 in promoting glucose uptake and inhibiting isoproterenol stimulated lipolysis and net fatty acid mobilization in human SGBS adipocytes [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the potency of insulin 454 with regard to the metabolic effects in human SGBS adipocytes by using glucose utilization, inhibition of isoproterenol-stimulated lyposis (glycerol release), and net factty acid mobilization assays. Findings: Insulin 454, like human insulin, stimulated glucose uptake and utilization and potently inhibited catecholamine-stimulated lipolysis and net fatty acid mobilization. The potency of insulin 454 compared to human insulin in the presence of 1% human albumin is 1.5% for stimulating glucose utilization, 1.8% for inhibition of lipolysis, and 1.7% for inhibition of net fatty acid mobilization.

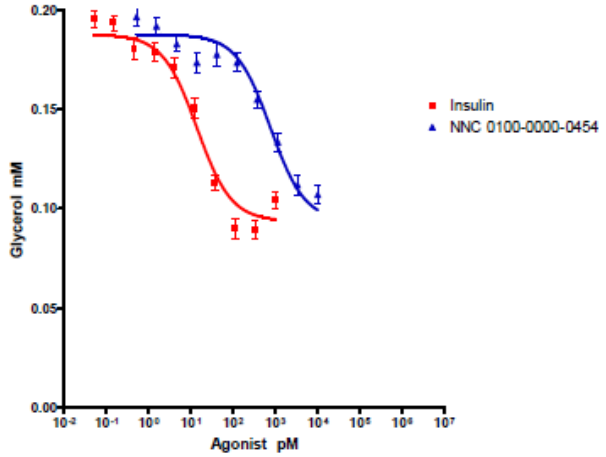
Activation of glucose utilisation in human adipocytes by NNC 0100-0000-0454 and Human Insulin



Activation of glucose utilisation in human SGBS adipocytes

	Potency %	95 % confidence interval (N=18)
NNC 0100-0000-0454	1,47	1,07 – 2,01
	EC_{50} pM	
Human insulin	98	75 - 128
NNC 0100-0000-0454	6683	4870 -9171

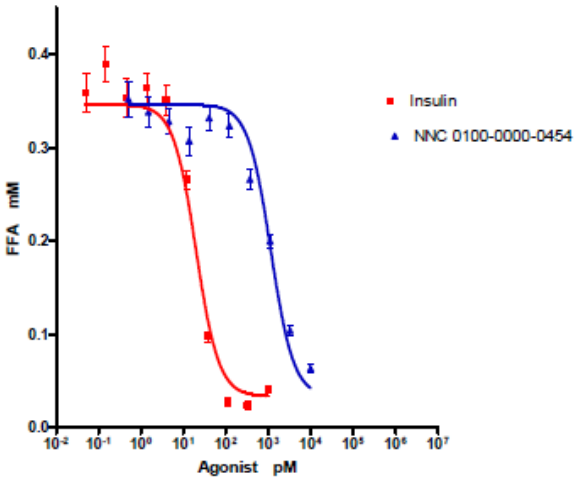
Inhibition of isoproterenol stimulated lipolysis (glycerol release) by NNC 0100-0000-0454 and Human Insulin



Inhibition of lipolysis (glycerol release) in human SGBS adipocytes

	Potency %	95 % confidence interval (N=24)
NNC 0100-0000-0454	1,81	1,27 – 2,58
	IC_{50} pM	
Human insulin	14	10 - 19
NNC 0100-0000-0454	760	534 – 1083

Inhibition of isoproterenol stimulated fatty acid mobilisation by NNC 0100-0000-0454 and Human Insulin



Inhibition of net fatty acid mobilisation from human SGBS adipocytes

	Potency %	95 % confidence interval (N=24)
NNC 0100-0000-0454	1,73	1,35 – 2,22
	IC_{50} pM	
Human insulin	20	16 - 25
NNC 0100-0000-0454	1164	909 - 1491

Study No. JRDA 21-Sept-2005: glycogen synthesis in human/rat skeletal muscle cells

Effect of insulin 454 on glycogen synthesis in human and primary rat skeletal muscle cells: potency and effect o albumin [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the potency of insulin 454 with regard to the glycogen synthesis in human skeletal muscle cells (XM5) and primary rat muscle cells. Findings: In 1st set of experiment, the estimated potencies relative to human insulin ranged 2.7-5.2% in human and from 1/1 to 10.1% in rat skeletal muscle cells with 0.1% human serum albumin in the medium. There was no difference for maximal effect between human insulin and insulin 454. In 2nd set of experiments, the reduced potency was observed when the albumin concentration increased.

The effect of insulin 454 relative to human insulin in human skeletal muscle cells

AssayID	Batch	Potency (% of human insulin)	95% con. lim.		W
4385	NNC 0100-0000-0454-4A	5.15	3.56	7.44	150
4386	NNC 0100-0000-0454-4A	2.81	1.40	5.67	41
4387	NNC 0100-0000-0454-4A	2.73	0.71	10.60	11
Combined potency		**4.39**	**3.20**	**6.03**	

The potency of insulin 454 in % of human insulin is given for three independent experiments as well as a combined below. In Appendix A, the raw data from the individual assay are given.

The effect of insulin 454 relative to human insulin in primary rat skeletal muscle cells

AssayID	Batch	Potency (% of human insulin)	95% con. lim.		W
4382	0100-0000-0454-4A	1.12	0.53	2.90	22
4383	0100-0000-0454-4A	10.13	3.74	27.49	20
4384	0100-0000-0454-4A	4.24	2.58	6.97	83
Combined potency		**3.86**	**2.58**	**5.77**	

The potency of insulin 454 in % of human insulin is given for three independent experiments as well as a combined below. In Appendix A, the raw data from the individual assay are given.

The effect of albumin on potency and ED50 of Insulin 454 as compared to human insulin measured as glycogen synthesis in human muscle cells

Albumin in %	Potency in % of human insulin (95 % conf. lim.)	ED50 Insulin 454 (95 % conf. lim.)	ED50 of human insulin (95 % conf. lim.)
0.1	5.5 (3.9-7.8)	50 (36-71)	793 (646-1291)
0.5	0.7 (0.4-1.0)	26 (17-39)	3479 (2258-5360)

The combined biological potency of insulin 454 in % of human insulin as well as ED50 values for four independent experiments are given. In Appendix A, the raw data from the individual assay are given.

Note: ED50 for glycogen synthesis in muscle cells increases for human insulin when HAS concentration increases but decreases for insulin 454, suggesting ↓ affinity for insulin for ↑ affinity for insulin 454.

Study No. LSc 16-Feb-2005: binding affinity for IR/IGF-1R in BHK cells

Binding affinity of NNC 0100-0000-0454 to human insulin receptor (A and B) an dIGF1 receptor [by Novo Nordisk A/S, Denmark]

Study design: This study was to determine the affinity of insulin 454 for the 2 isoforms of the human insulin and for the human IGF-1 receptor in transfected BHK cells.
Findings: The affinity of insulin 454 for both isoforms of the insulin receptor was similar and that that relative affinity for the IGF-1 receptor was lower. .The affinity of insulin 454 for IRA was 13% whereas the affinity of insulin 454 for IRB was 15% compared to that of human insulin. The affinity of insulin 454 for the human IGF-1 receptor was 2% compared to that of human insulin and 0.05% compared to that of IGF-1 itself.

Affinity of insulin 454 for human insulin and IGF-1 receptors

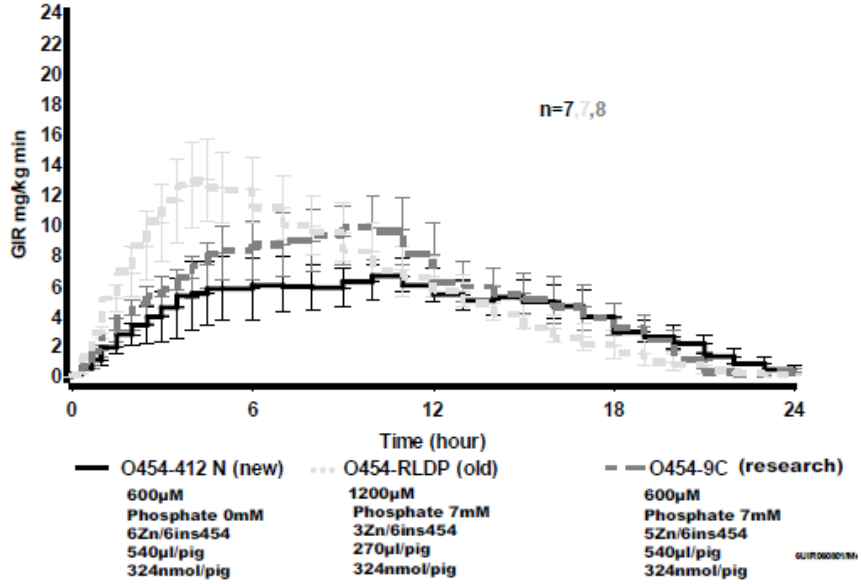
	Kd HI, pM	Kd IGF-1, pM	Kd Insulin 454, pM	Affinity of Insulin 454 relative to HI	Affinity of Insulin 454 relative to IGF1
HIR A	19±4		150±20	13%	
HIR B	9±3		60±21	15%	
HIGF1R	2100±600	50±11	102000±62000	2.0%	0.05%

Study No. UIR060801-100 Feb-2007: clamp study in pigs with clinical batches

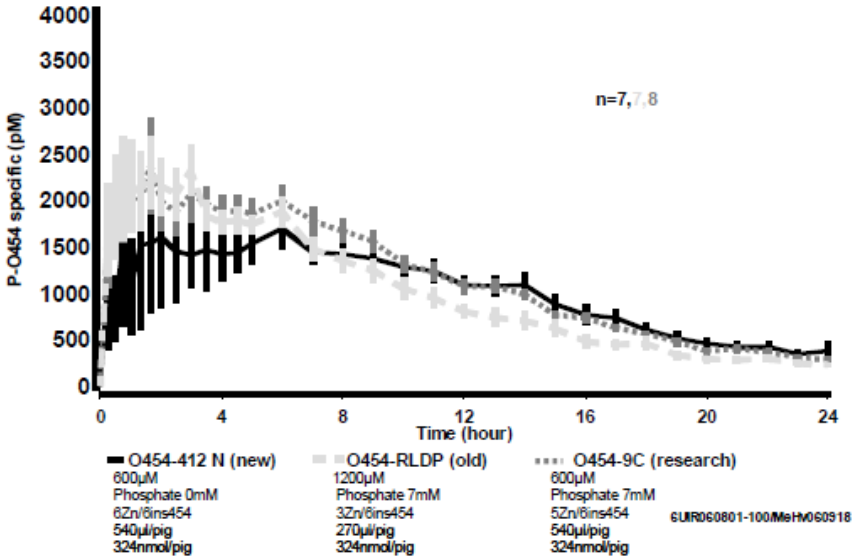
Action profile of two clinical batches of insulin 454; (0.6 mM, 6 Zn/6 insulin 454 vs. 1.2 mM, 3 Zn/6 insulin 454). A Euglycaemic clamp study in pigs [by Novo Nordisk A/S, Denmark]

Study design: This study was to characterize 2 different formulations of insulin 454 used in clinical trials. Seven pigs received the two clinical preparations in random order, whereas 4 pigs received a research insulin 454 preparation twice (via sc injection). Plasma samples were collected for glucose and insulin 454 determinations. Findings: The glucose utilization and profile of the infusion rats used to maintain euglycaemia were different for the 2 clinical preparations. The action profile of the 6 Zn/0.6 mM preparation was flatter and less peaked than the 3 Zn/1.2 mM preparation. The action profile of the research preparation was found to lie between those of the 2 clinical preparations.

Glucose infusion rate after sc administration of two clinical preparations and a research preparation of insulin 454



Plasma insulin 454 profile after sc administration of two clinical preparations and a research preparation

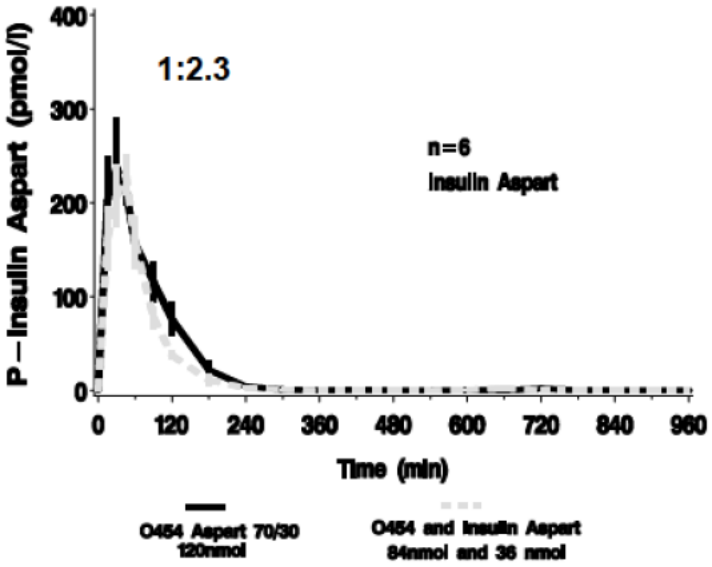


Study No. UIR 09-Sep-2005: clamp study in pigs with insulin 454 and insulin 454 + insulin aspart

Effect profile of insulin 454 and insulin 454/insulin aspart combinations. A Euglycaemic clamp study in pigs [by Novo Nordisk A/S, Denmark]

Study design: The study was to characterize an equipotent once daily basal insulin 454 and in addition with insulin aspart without blunting the individual activity profiles. Thirty pigs participated in 5 separated experiments. They were treated with either insulin 454 (3 Zn/6 insulin 454) of the insulin454+aspart. Blood samples were collected and analyzed for insulin 454 and insulin aspart. Finding: In pigs receiving insulin 454, the time to peak activity (i.e. max. glucose infusion rate GIR) occurred at about 6 hr and the duration of action was 18 hrs. Insulin combined in different ratios with aspart showed that decreasing concentrations of insulin 454 resulted in less blunting of insulin aspart. At a ratio of 1:2 of aspart/insulin 454 (30/70 combination), blunting of the aspart profile was insignificant while at ratios of 1:5 or 1:8, blunting was observed.

PK profile of Insulin Aspart administered separately or in a 1:2 combination with insulin 454 (3 Zn)



4.2 Secondary Pharmacology

Study No. mdsps 1058777: pharma binding activity report

(b) (4) services pharmacology data report on compound NOV (b) (4), NNC 0100-0000-0454 for Novo Nordisk A/S [by Novo Nordisk A/S, Denmark]

Study design: This study was to evaluate the activity of compound NOV (b) (4) by using radioligand binding assays. Biochemical assay results were presented as the percent inhibition of specific binding or activity. No significant responses were noted in the primary assays listed below.

EXPERIMENTAL RESULTS - BIOCHEMICAL ASSAYS

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC_{50}	K_i	n_H	R
						% -100 -50 0 50 100				
200510	Adenosine A_1	133671	hum	2	1 µM	12				
200610	Adenosine A_{2A}	133649	hum	2	1 µM	-15				
200720	Adenosine A_3	133650	hum	2	1 µM	-5				
203100	Adrenergic α_{1A}	133786	rat	2	1 µM	-6				
203200	Adrenergic α_{1B}	133787	rat	2	1 µM	1				
203400	Adrenergic α_{2D}	133788	hum	2	1 µM	2				
203620	Adrenergic α_{2A}	133673	hum	2	1 µM	-12				
204010	Adrenergic β_1	133705	hum	2	1 µM	-3				
204110	Adrenergic β_2	133706	hum	2	1 µM	10				
212510	Bradykinin B_1	133677	hum	2	1 µM	-1				
212610	Bradykinin B_2	133678	hum	2	1 µM	-3				
214510	Calcium Channel L-Type, Benzothiazepine	133811	rat	2	1 µM	10				
214600	Calcium Channel L-Type, Dihydropyridine	133771	rat	2	1 µM	0				
216000	Calcium Channel N-Type	133773	rat	2	1 µM	-2				
219500	Dopamine D_1	133679	hum	2	1 µM	-1				
219700	Dopamine D_{2L}	133681	hum	2	1 µM	1				
219800	Dopamine D_3	133682	hum	2	1 µM	1				
219900	Dopamine $D_{4.2}$	133683	hum	2	1 µM	-8				
224010	Endothelin ET_A	133629	hum	2	1 µM	5				
224110	Endothelin ET_B	133630	hum	2	1 µM	-6				
225500	Epidermal Growth Factor (EGF)	133876	hum	2	1 µM	4				
226010	Estrogen ERα	133631	hum	2	1 µM	5				
226500	$GABA_A$, Agonist Site	133686	rat	2	1 µM	6				
226600	$GABA_A$, Benzodiazepine, Central	133627	rat	2	1 µM	1				
228600	$GABA_{B1A}$	133687	hum	2	1 µM	-6				
232010	Glucocorticoid	133774	hum	2	1 µM	12				
232700	Glutamate, Kainate	133711	rat	2	1 µM	5				
232810	Glutamate, NMDA, Agonism	133634	rat	2	1 µM	24				
232910	Glutamate, NMDA, Glycine	133689	rat	2	1 µM	9				
233000	Glutamate, NMDA, Phencyclidine	133690	rat	2	1 µM	-12				

* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)
R=Additional Comments
ham=hamster; hum=human

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC_{50}	K_i	n_H	R
						% -100 -50 0 50 100				
239610	Histamine H_1	133637	hum	2	1 µM	-1				
239710	Histamine H_2	133638	hum	2	1 µM	-2				
239810	Histamine H_3	133970	hum	2	1 µM	-6				
241000	Imidazoline I_2, Central	133775	rat	2	1 µM	18				
243510	Interleukin IL-1	133802	mouse	2	1 µM	5				
250460	Leukotriene, Cysteinyl $CysLT_1$	133712	hum	2	1 µM	4				
251600	Melatonin MT_1	133640	hum	2	1 µM	-2				
252600	Muscarinic M_1	133789	hum	2	1 µM	-9				
252700	Muscarinic M_2	133790	hum	2	1 µM	11				
252800	Muscarinic M_3	133791	hum	2	1 µM	-1				
257010	Neuropeptide Y Y_1	133642	hum	2	1 µM	-7				
257110	Neuropeptide Y Y_2	133713	hum	2	1 µM	-3				
258590	Nicotinic Acetylcholine	133765	hum	2	1 µM	-8				
258700	Nicotinic Acetylcholine, Bungarotoxin-Sensitive, Neuromuscular	133767	hum	2	1 µM	-2				
260110	Opiate δ (OP1, DOP)	133779	hum	2	1 µM	0				
260210	Opiate κ (OP2, KOP)	133780	hum	2	1 µM	12				
260410	Opiate µ (OP3, MOP)	133781	hum	2	1 µM	-10				
264500	Phorbol Ester	133692	mouse	2	1 µM	5				
265010	Platelet Activating Factor (PAF)	133693	hum	2	1 µM	3				
265600	Potassium Channel [K_{ATP}]	133769	ham	2	1 µM	-2				
265900	Potassium Channel HERG	133739	hum	2	1 µM	-2				
268410	Prostanoid EP_4	133758	hum	2	1 µM	-26				
268700	Purinergic P_{2X}	133714	rabbit	2	1 µM	0				
268810	Purinergic P_{2Y}	133715	rat	2	1 µM	-2				
270000	Rolipram	133702	rat	2	1 µM	6				
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	133695	hum	2	1 µM	14				
271910	Serotonin (5-Hydroxytryptamine) 5-HT_3	133699	hum	2	1 µM	5				
278110	Sigma σ_1	133776	hum	2	1 µM	-9				
278200	Sigma σ_2	133777	rat	2	1 µM	-2				
279510	Sodium Channel, Site 2	133701	rat	2	1 µM	-1				

* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)
R=Additional Comments
ham=hamster; hum=human

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC_{50}	K_i	n_H	R
						% -100 -50 0 50 100				
255510	Tachykinin NK_1	133691	hum	2	1 µM	7				
285010	Testosterone	133717	rat	2	1 µM	3				
285900	Thyroid Hormone	134511	rat	2	1 µM	34				
220320	Transporter, Dopamine (DAT)	133806	hum	2	1 µM	1				
226400	Transporter, GABA	133710	rat	2	1 µM	2				
204410	Transporter, Norepinephrine (NET)	133805	hum	2	1 µM	-8				
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	133700	hum	2	1 µM	-2				

4.3 Safety Pharmacology

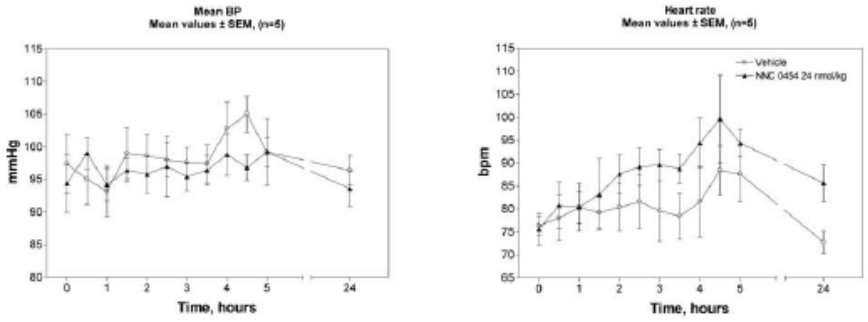
Study No. 204275: CV effect in dogs

Effect on cardiovascular parameters recorded by telemetry in conscious Beagle dogs [by Novo Nordisk A/S, Denmark] non-GLP

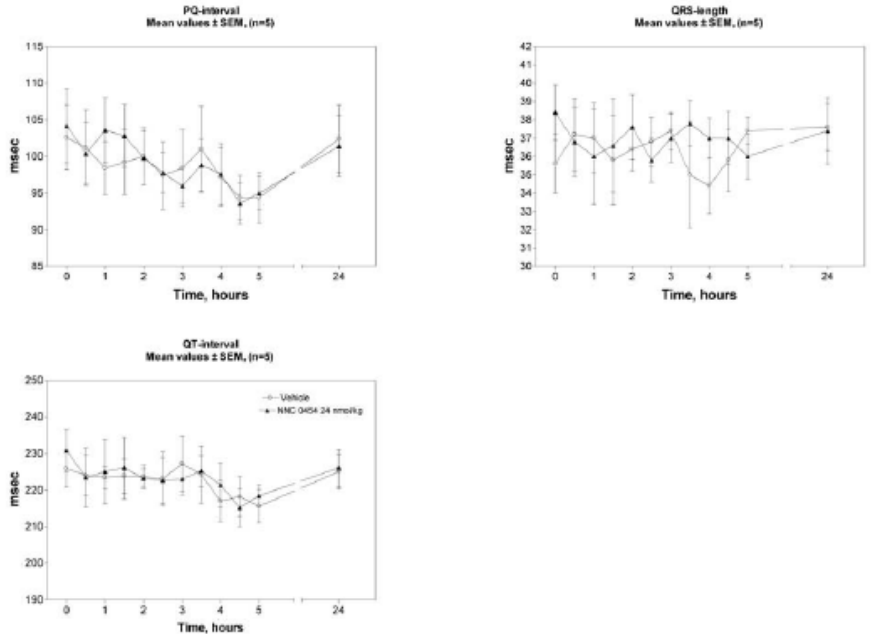
Study design: This telemetry non-GLP study was to evaluate the effect of sc administration of insulin 454 on CV parameters in the conscious 5 Beagle dogs (24 nmol/kg insulin or vehicle). Each dog served as its own control and received vehicle and insulin with min. 3 days wash-out period in between. The following parameters were analyzed: systolic, diastolic, mean arterial blood pressure, heart rate, and ECG time intervals (PQ-, QRS-, and QT intervals). Heart rate (bpm) was obtained from the ECG (signal counting beats with the 30s data acquisition period).

Findings: Insulin 454 had no significant effects on systolic, diastolic, or mean blood pressure up to 24 hrs after dosing in dogs. Heart rate tended to increase (but not significant) following dosing of insulin 454, probably as a consequence of a significant fall in blood glucose. There was no change in the ECG following insulin or vehicle control. Blood glucose measured before and after treatment (24 hr) showed significant decrease following insulin 454. All in all, insulin 454 given 24 nmol/kg sc to conscious Telemetered dogs had no significant effects on CV parameters, blood pressure, heart rate or ECG up to 24 hrs after dosing, despite a significant fall in blood glucose, 24 hrs after dosing in the insulin 454 treated dogs.

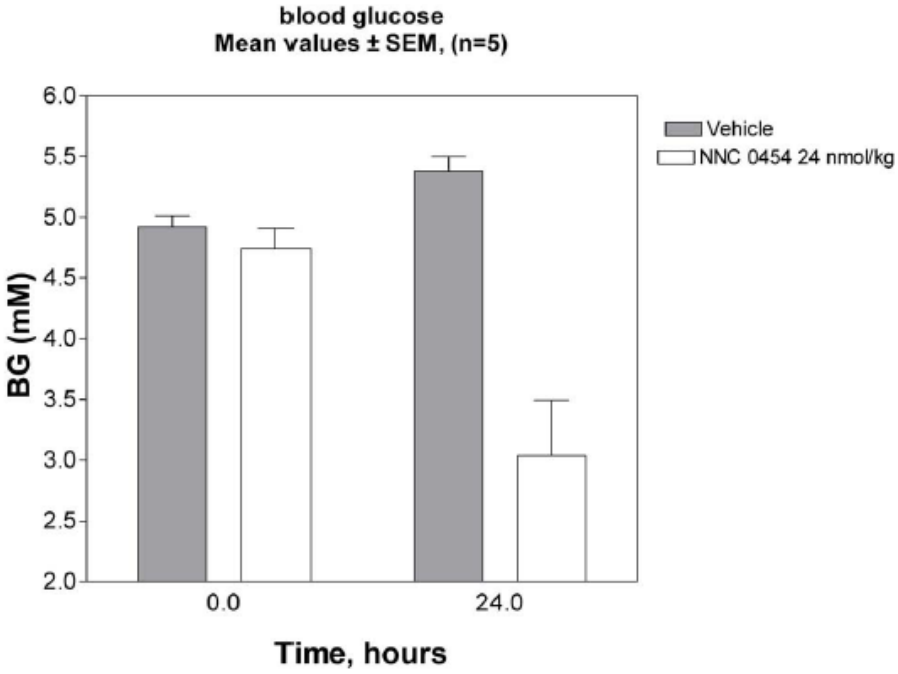
Effect of NNC 0100-0000-0454, 24 nmol/kg s.c. on mean blood pressure and heart rate. Pre-dose values are indicated at t=0.



Effect of NNC 0100-0000-0454, 24 nmol/kg on ECG time intervals (PQ-, QRS- and QTR-intervals). Pre-dose values are indicated at t=0.



Effect of NNC 01000-0000-0454, 24 nmol/kg (open bars) and vehicle (closed bars) on blood glucose prior to dosing and 24 hours after dosing.



Study No. 205157: CNS effect in rats (Irwin test)

Effect in the Irwin test in rats [by (b) (4)] GLP

Study design: This GLP study was to assess the acute effects of sc administration of insulin 454 (batch 412_N05169) at doses of 3, 30, and 300 nmol/kg on the gross behavioral and physiological state in male rats. Chlorpromazine hydrochloride was used as a reference. There were 5 groups with 6 rats per group. Animals were administered with test article, reference, or vehicle and then observed at 2, 4, 6, and 24 hr post-dose, when parameters in the Irwin Test were systematically evaluated for each rat. Endpoints included body weights, clinical signs, and tolerability.

A	Vehicle for NNC 0100-0000-0454	5 mL/kg
C	NNC 0100-0000-0454	3 nmol/kg
D	NNC 0100-0000-0454	30 nmol/kg
B	NNC 0100-0000-0454	300 nmol/kg
E	Chlorpromazine	5 mg/kg

Findings: There were findings such as passivity and/or vocalization, were observed in rats treated with insulin 454. However, no significant changes were observed during the 24 hr post dosing in rats administered insulin 454 at 3, 30, or 300 nmol/kg.

Study No. 205158: respiratory effect in rats

Effects on respiration rate, tidal volume, and minute volume in rats [by (b) (4)] GLP

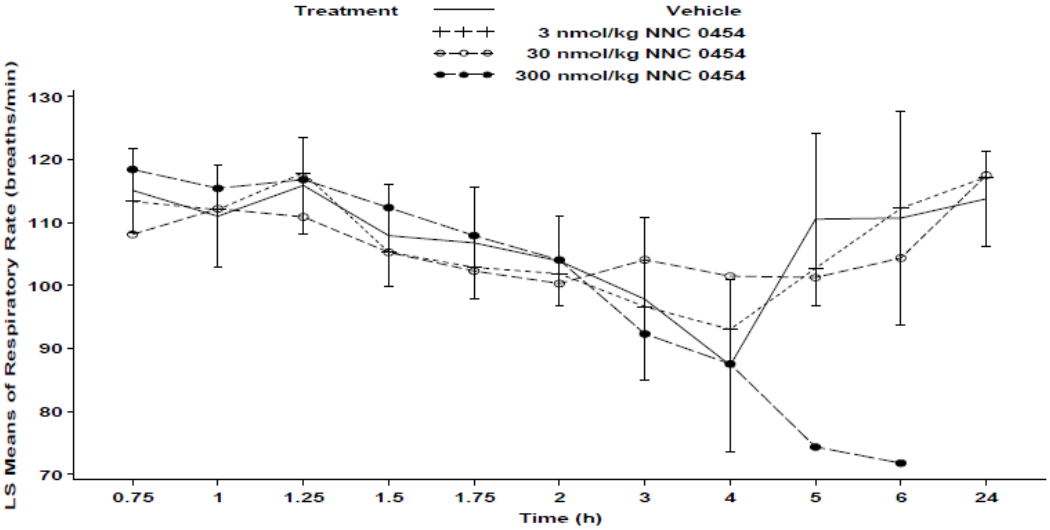
Study design: This GLP study was to assess the acute effects of subcutaneously administered insulin 454 (batch 412_N05169), at doses of 3, 30, and 300 nmol/kg on respiration rate, tidal volume, and minute volume in rats. There were 4 treatment groups with 8 rats per group. Endpoints included body weights, clinical signs, respiration rate, tidal volume, and minute volume.

B	Vehicle for NNC 0454	5 mL/kg
C	NNC 0454	3 nmol/kg
D	NNC 0454	30 nmol/kg
A	NNC 0454	300 nmol/kg

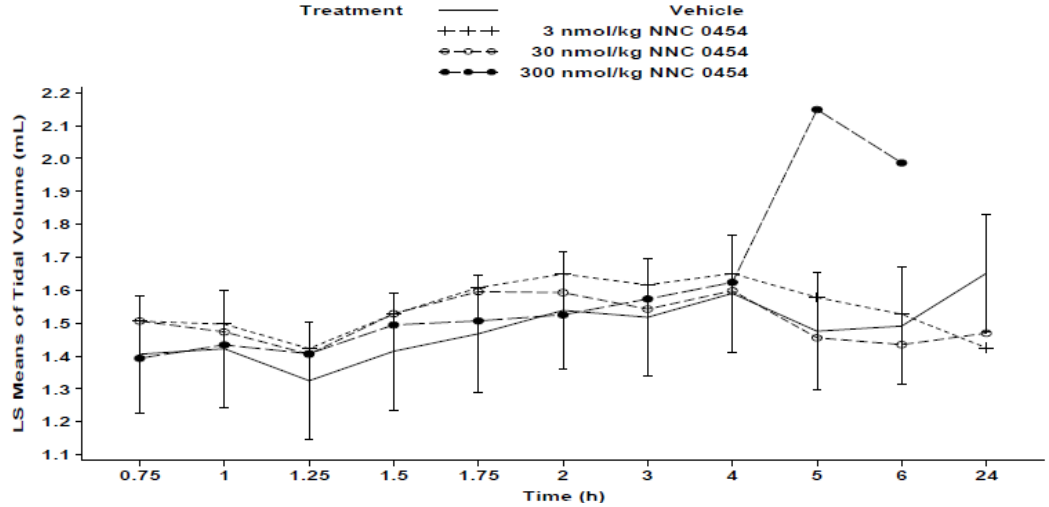
Findings: There were no statistically significant effects of insulin 454 at dose levels of 3 and 30 nmol/kg on respiratory parameters in male rats. However, the 300 nmol/kg insulin 454 resulted in severe clinical signs, which resulted in 1/8, 3/8, and 8/8 rats terminated prior to data collection at 5, 6, and 24 hr, respectively. There were significant effects on respiratory rate and tidal volume at 5 and 6 hr and on minute volume at 6 hr. The effects at 24 hr were not estimated since rats were terminated due to hypoglycaemia-related clinical signs. Reduced blood glucose levels were seen in some HD animals (ranging 1.3-5.4 mmol/L) and these values were lower than the normal range of glucose levels in rats (6.8-14.5 mmol/L), suggesting HD animals were hypoglycemic.

Note: **The 300 nmol/kg produced toxicity in this study but not in the study 205157 above. It is unclear why that was since the same batch of test article and CRO were used in both studies.s**

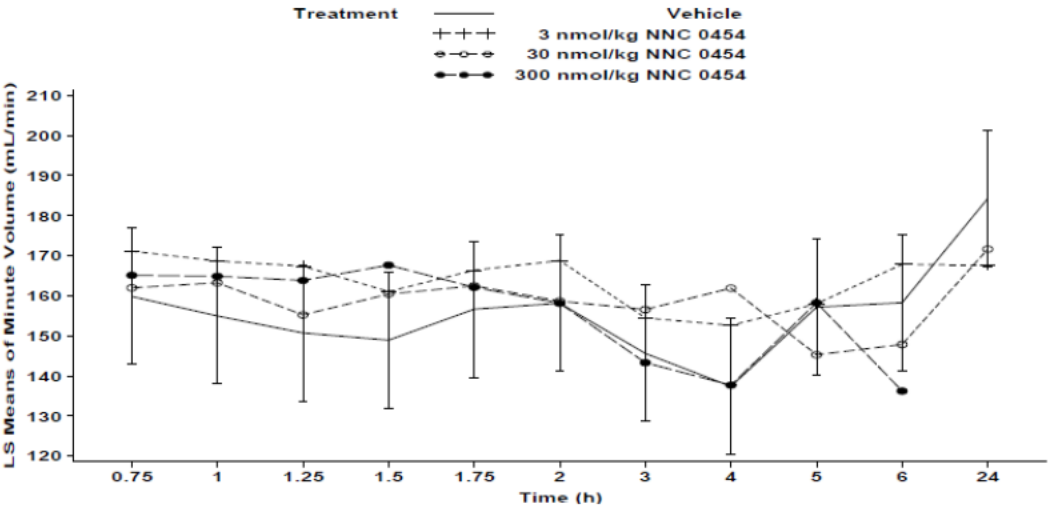
Least Squares Means (± 2 SE for Vehicle) of Respiration Rate (breaths/min) Following Subcutaneous Administration of Vehicle (5 mL/kg), NNC 0454 (3 nmol/kg), NNC 0454 (30 nmol/kg) or NNC 0454 (300 nmol/kg), Study DCZD1017



Least Squares Means (± 2 SE for Vehicle) of Tidal Volume (mL) Following Subcutaneous Administration of Vehicle (5 mL/kg), NNC 0454 (3 nmol/kg), NNC 0454 (30 nmol/kg) or NNC 0454 (300 nmol/kg), Study DCZD1017



Least Squares Means (± 2 SE for Vehicle) of Minute Volume (mL/min) Following Subcutaneous Administration of Vehicle (5 mL/kg), NNC 0454 (3 nmol/kg), NNC 0454 (30 nmol/kg) or NNC 0454 (300 nmol/kg), Study DCZD1017



Study No. 205159: QT prolongation in Purkinje fibers

Effects on action potential parameters in rabbit isolated Purkinje fibers [by (b) (4)] GLP

Study design: This study was to assess the potential of insulin 454 to prolong QT interval by examining its effects on the cardiac action potential in an appropriate in vitro test system. The fiber preparation was electrically paced at 0.5 and 1 Hz. Cardiac Purkinje fibers were isolated from New Zealand white rabbits. The fibers were exposed to each concentration of test article for approx. 30 min. Action potential parameters were monitored and recorded (i.e. action potential duration (APD) at 40, 60, and 90% re-polarization, max. rate of depolarization (MRD), upstroke amplitude (UA), and resting membrane potential (RMP). Findings: In rabbit isolated cardiac Purkinje fibers, paced at stimulation frequencies of 1 and 0.5 Hz, exposure to 10, 100, and 1000 nmol/L insulin 454 had no effect on APD, MRD, UA, and RMP. This data showed that at plasma concentrations of up to 1000 nmol/L, insulin 454 was not expected to have direct effects on QRS complex duration or QT interval.

Effect of NNC 0454 or Vehicle on Action Potential Parameters (1 Hz)

Action Potential Parameter	NNC 0454 Baseline	NNC 0454 10 nmol/L	NNC 0454 100 nmol/L	NNC 0454 1000 nmol/L
RMP (mV)	-90.3 ± 1.0	-89.8 ± 0.7	-88.3 ± 1.3	-91.5 ± 1.2
UA (mV)	120.2 ± 3.5	121.8 ± 2.9	122.7 ± 2.8	122.8 ± 2.8
MRD (V/s)	351.9 ± 20.1	357.4 ± 14.2	376.3 ± 25.3	377.2 ± 25.7
APD_{60} (ms)	204.5 ± 30.1	205.5 ± 30.6	209.9 ± 29.9	213.4 ± 33.6
APD_{90} (ms)	264.7 ± 40.2	266.9 ± 42.7	272.5 ± 41.5	274.9 ± 46.3
APD_{40-90}	146.2 ± 25.8	145.7 ± 26.1	146.6 ± 22.6	143.1 ± 23.7

Effect of NNC 0454 or Vehicle on Action Potential Parameters (0.5 Hz)

Action Potential Parameter	NNC 0454 Baseline	NNC 0454 10 nmol/L	NNC 0454 100 nmol/L	NNC 0454 1000 nmol/L
RMP (mV)	-90.7 ± 1.1	-86.6 ± 1.6	-87.6 ± 0.9	-89.5 ± 1.5
UA (mV)	119.6 ± 3.8	120.2 ± 3.2	121.5 ± 2.9	121.4 ± 3.4
MRD (V/s)	359.8 ± 12.8	392.9 ± 29.4	361.8 ± 22.5	377.4 ± 25.3
APD_{60} (ms)	223.2 ± 37.6	223.0 ± 40.9	225.1 ± 36.6	233.9 ± 43.8
APD_{90} (ms)	305.1 ± 53.3	309.9 ± 57.7	310.3 ± 54.9	314.7 ± 61.3

Study No. 205160: ECG effect in dogs

Effects on general haemodynamics in anaesthetized, mechanically ventilated beagle dogs [by (b) (4)] GLP

Study design: There were two groups of male Beagle dogs. Group 1 had 2 animals which were dosed with 3 doses of the vehicle (0.5 mg/kg) separated by 60 min, whereas Group 2 had 4 animals which were dosed with 3 ascending doses of insulin 454 (4, 8, and 12 nmol/kg in a dose volume of 0.5 mg/kg) separated by 60 min via iv bolus injection. Several parameters were measured continuously (i.e. arterial blood pressure, heart rate, lead II ECG parameters, left ventricular variables, cardiac output, stroke volume, mean femoral arterial blood flow, and conductance were measured continuously). Blood glucose levels and sodium, potassium, and chloride levels were also measured.

Group 1	Vehicle	0.5, 0.5 and 0.5 ml/kg
Group 2	NNC 0100-0000-0454	4, 8 and 12 nmol/kg (total cumulative dose of 24 nmol/kg)

Findings: There were no effects observed following administration of 4, 8, or 12 nmol/kg insulin 454 in any parameters measured, except for heart rate which was noted to increase in 2 animals 20 min after the start of the 8 nmol/kg dose, and in another animal 20 min after the start of the 12 nmol/kg dose.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

STUDY NO. 204342: SINGLE-DOSE IV/SC PIG STUDY

Pharmacokinetics after single intravenous and subcutaneous administration to pigs [by Novo Nordisk, Denmark] non-GLP

Study design: This non-GLP study was to evaluate the PK after IV and SC dosing with insulin 454 (batch AF-IT-K2_001) in LYD pigs. Six female pigs were given a single sc injection at 1, 2, and 4 nmol/kg on the lateral side of the neck in a cross over design. The IV dose of 2 nmol/kg was administered through an ear vein. Blood samples were collected at various time points.

Overview of cross over design (sc injection)

Week No and route	Pig No. 333-334	Pigs No. 335-336	Pigs No. 337-338
Week 48 SC	1 nmol/kg BW	2 nmol/kg BW	4 nmol/kg BW
Week 49 SC	4 nmol/kg BW	1 nmol/kg BW	2 nmol/kg BW
Week 50 SC	2 nmol/kg BW	4 nmol/kg BW	1 nmol/kg BW
Week 51 IV	2 nmol/kg BW	2 nmol/kg BW	2 nmol/kg BW

Findings: Insulin 454 was eliminated fast from plasma following iv administration. Absorption following sc administration took place immediately with maximal plasma concentration 2-3 hrs after dosing in pigs. Bioavailability was in the range of 55-73% following sc administration. The mean plasma Cmax and mean AUC vs. the dose indicated a dose proportional exposure due to linear PK in the range of 1-4 nmol/kg.

Individual and mean (SD) Pharmacokinetic parameters for insulin 454 in pigs after IV administration of 2 nmol/kg

Pig No.	Tmax (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUC (hr*pmol/L)	AUCExtra (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
333	0.1	47397	0.7459	0.9	39796	14	0.0674	0.0503	1.3
334	0.1	41236	0.6501	1.1	38896	14	0.0791	0.0514	1.2
335	0.1	50181	0.7594	0.9	35715	13	0.0737	0.0560	1.2
336	0.1	48995	0.8485	0.8	42520	11	0.0554	0.0470	1.0
337	0.1	47017	0.5360	1.3	37064	23	0.1007	0.0540	1.6
338	0.1	42035	0.5428	1.3	37541	22	0.0982	0.0533	1.6
NObs	6	6	6	6	6	6	6	6	6
Mean	0.1	46143	0.6805	1.0	38589	16	0.0791	0.0520	1.3
SD	0.0	3681	0.1261	0.2	2395	5	0.0176	0.0031	0.2
Min	0.1	41236	0.5360	0.8	35715	11	0.0554	0.0470	1.0
Median	0.1	47207	0.6980	1.0	38218	14	0.0764	0.0523	1.2
Max	0.1	50181	0.8485	1.3	42520	23	0.1007	0.0560	1.6
Harmonic Mean	0.1	45891	0.6606	1.0	38468	15	0.0757	0.0518	1.3

Individual and mean (SD) pharmacokinetic parameters for insulin 454 in pigs after SC administration of 1 nmol/kg

Dose (nmol/kg)	Pig No.	Tmax (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUC (hr*pmol/L)	AUCExtra (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
1	335	1.0	1694	0.1403	4.9	10733	1	0.664	0.093	6.6
	336	1.0	1743	0.1712	4.0	11175	1	0.523	0.089	5.7
	337	0.5	1403	0.0727	9.5	18354	11	0.749	0.054	14.2
	338	3.0	1634	0.1273	5.4	13667	2	0.575	0.073	6.9
	NObs	4	4	4	4	4	4	4	4	4
	Mean	1.4	1619	0.1279	6.0	13482	4	0.628	0.078	8.4
	SD	1.1	150	0.0411	2.4	3496	5	0.100	0.018	3.9
	Min	0.5	1403	0.0727	4.0	10733	1	0.523	0.054	5.7
	Median	1.0	1664	0.1338	5.2	12421	2	0.619	0.081	6.8
	Max	3.0	1743	0.1712	9.5	18354	11	0.749	0.093	14.2
	Harmonic Mean	0.9	1607	0.1157	5.4	12890	1	0.616	0.074	7.4

Individual and mean (SD) pharmacokinetic parameters for insulin 454 in pigs after SC administration of 2 nmol/kg

Dose (nmol/kg)	Pig No.	Tmax (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUC (hr*pmol/L)	AUCExtra (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
2	333	2.0	3388	0.1344	5.2	25788	2	0.577	0.078	7.0
	334	1.0	3574	0.1475	4.7	28350	2	0.478	0.071	7.9
	335	1.0	3448	0.1297	5.3	16356	2	0.943	0.122	6.4
	336	2.0	5033	0.1588	4.4	23065	1	0.546	0.087	5.2
	337	3.0	1151	0.0975	7.1	17113	6	1.198	0.117	11.8
	338	2.0	2263	0.1107	6.3	27685	5	0.653	0.072	10.4
	NObs	6	6	6	6	6	6	6	6	6
	Mean	1.8	3143	0.1297	5.5	23059	3	0.733	0.091	8.1
	SD	0.8	1316	0.0227	1.0	5237	2	0.280	0.023	2.5
	Min	1.0	1151	0.0975	4.4	16356	1	0.478	0.071	5.2
	Median	2.0	3418	0.1320	5.3	24426	2	0.615	0.082	7.4
	Max	3.0	5033	0.1588	7.1	28350	6	1.198	0.122	11.8
	Harmonic Mean	1.6	2527	0.1262	5.3	21970	2	0.661	0.087	7.5

Individual and mean (SD) pharmacokinetic parameters for insulin 454 in pigs after SC administration of 4 nmol/kg

Dose (nmol/kg)	Pig No.	Tmax (hr)	Cmax (pmol/L)	λz (1/hr)	$t_{1/2}$ (hr)	AUC (hr*pmol/L)	AUCExtra (%)	Vz/F (L/kg)	Cl/F (L/hr/kg)	MRT (hr)
4	333	2.0	5784	0.1510	4.6	48803	1	0.543	0.082	7.3
	334	1.0	5085	0.1275	5.4	43563	2	0.720	0.092	8.4
	335	2.0	7374	0.1292	5.4	54239	2	0.571	0.074	7.4
	336	1.0	7163	0.1439	4.8	45187	1	0.615	0.089	6.8
	337	1.0	12334	0.1357	5.1	45065	1	0.654	0.089	5.7
	338	2.0	5728	0.1279	5.4	47592	2	0.657	0.084	7.6
	NObs	6	6	6	6	6	6	6	6	6
	Mean	1.5	7245	0.1359	5.1	47408	2	0.627	0.085	7.2
	SD	0.5	2647	0.0097	0.4	3843	1	0.064	0.006	0.9
	Min	1.0	5085	0.1275	4.6	43563	1	0.543	0.074	5.7
	Median	1.5	6474	0.1325	5.2	46389	2	0.635	0.086	7.4
	Max	2.0	12334	0.1510	5.4	54239	2	0.720	0.092	8.4
	Harmonic Mean	1.3	6664	0.1353	5.1	47164	2	0.621	0.084	7.1

STUDY NO. 206015: SINGLE-DOSE IV/SC DOG STUDY

A 7-day pharmacokinetic study of insulin 454 from 3 Zn and 6 Zn formulation in dogs [by (b) (4)] GLP

Study design: This GLP study was to investigate the PK of insulin 454 in dogs following a single iv administration (on Day 1) and following steady state (6 days; Days 3 to 8) sc administration from 2 formulation of insulin 454 with 3 Zn per 6 insulins (batch 412_NO5169) and insulin 454 with 6 Zn per 6 insulins (batch 412_NO6048).

Dosed with formulation X (3 Zn per 6 insulins).

Group	Dose level	Dose concentration	Animal Nos		Colour code
	(nmol/kg)	(nmol/ml)	Male	Female	
1	2	200	1 – 3	4 – 6	White
2	4	400	7 – 9	10 – 12	Blue
3	8	800	13 – 15	16 – 18	Green
4	12	1200	19 – 21	22 – 24	Red

Dosed with formulation Y (6 Zn per 6 insulins).

Group	Dose level	Dose concentration	Animal Nos		Colour code
	(nmol/kg)	(nmol/ml)	Male	Female	
1	2	200	1 – 3	4 – 6	White
2	4	400	7 – 9	10 – 12	Blue
3	8	800	13 – 15	16 – 18	Green
4	12	1200	19 – 21	22 – 24	Red

Findings: The PK of insulin 454 in dogs following a single iv administration and following steady steate sc administration from 2 formulations of insulin 454 showed no insulin 454-related effect on clinical signs, body weight gain, and food consumption. Based on AUC_{0-24}, dose proportionality was seen for both formulations following iv or sc administration. No consistent differences in Cmax and AUC_{0-24} were observed between two formulations. However, there was a tendency towards a slightly longer apparent half life for 6 Zn per 6 insulin formulation as compared to 3 Zn per 6 insulin formulation after both iv and sc administration. Clearance and Vd were comparable and did not

change with doses. Accumulation was observed for both formulation (more on 6 Zn per 6 insulin formulation). No gender difference was seen based on Cmax and ACU_{0-24}.

Summary of average pharmacokinetic parameters of Insulin 454 with Treatment A (3 Zn per 6 insulins) or Treatment B (6 Zn per 6 insulins)

Treat-ment	Group	Day	Gender	Dose (nmol/kg)	Rsq	C_{max} (pmol/L)	C(0) (pmol/L)	T_{max} (h)	T½* (h)	AUC_{INF} (h*pmol/L)	AUC_{INF} ^ (h*pmol/L)	$AUC_{\%Extrap}$ (%)	AUC_{0-24} (h*pmol/L)	AUC_{0-24} ^ (h*pmol/L)	F	Cl (L/h/kg)	Vz (L/kg)
A	1	1	Female	2	0.99	-	11,979	-	1.85	25,336	152,016	2.83	25,338	152,026	-	0.079	0.211
			Male	2	0.99	-	8,812	-	2.32	27,425	164,550	2.22	27,355	164,130	-	0.074	0.240
A	2	1	Female	4	1.00	-	15,426	-	2.58	37,802	113,407	3.40	37,716	113,147	-	0.135	0.475
			Male	4	0.99	-	20,043	-	2.36	46,084	138,252	5.83	46,022	138,068	-	0.088	0.301
A	3	1	Female	8	1.00	-	26,775	-	2.67	73,313	109,970	1.55	73,150	109,726	-	0.128	0.503
			Male	8	1.00	-	55,064	-	3.31	128,511	192,767	0.14	127,844	191,766	-	0.063	0.298
A	4	1	Female	12	1.00	-	65,414	-	3.18	149,293	149,293	0.14	148,834	148,834	-	0.082	0.379
			Male	12	0.99	-	50,055	-	3.24	179,894	179,894	1.75	178,459	178,459	-	0.068	0.311
B	1	1	Female	2	0.99	-	7,870	-	2.56	21,196	127,174	2.35	21,135	126,810	-	0.102	0.342
			Male	2	0.83	-	8,893	-	3.77	23,505	141,032	10.8	23,108	138,646	-	0.093	0.558
B	2	1	Female	4	0.99	-	15,876	-	3.35	48,192	144,577	2.36	47,712	143,135	-	0.084	0.403
			Male	4	1.00	-	19,902	-	3.06	58,376	175,129	2.02	58,142	174,425	-	0.074	0.316
B	3	1	Female	8	1.00	-	48,581	-	3.21	130,861	196,292	0.17	130,251	195,376	-	0.068	0.302
			Male	8	1.00	-	46,162	-	3.90	142,092	213,137	0.20	140,452	210,679	-	0.069	0.393
B	4	1	Female	12	1.00	-	70,459	-	3.77	142,026	142,026	0.17	140,857	140,857	-	0.088	0.488
			Male	12	0.96	-	56,676	-	4.36	173,382	173,382	2.93	172,357	172,357	-	0.094	0.77

*Exact T½ calculation intervals are described in Table 4.
^ Dose corrected to 12 nmol/kg.

Treat-ment	Group	Day	Gender	Dose (nmol/kg)	Rsq	C_{max} (pmol/L)	C(0) (pmol/L)	T_{max} (h)	T½* (h)	AUC_{INF} (h*pmol/L)	AUC_{INF} ^ (h*pmol/L)	$AUC_{\%Extrap}$ (%)	AUC_{0-24} (h*pmol/L)	AUC_{0-24} ^ (h*pmol/L)	F	Cl (L/h/kg)	Vz (L/kg)
A	1	8	Female	2	0.99	5,336	-	3	3.53	41,263	247,578	0.33	40,262	241,574	1.63	-	-
			Male	2	1.00	5,359	-	3	3.67	39,324	235,942	0.37	38,323	229,938	1.47	-	-
A	2	8	Female	4	0.99	8,051	-	4.3	3.72	80,025	240,075	0.33	77,318	231,953	2.68	-	-
			Male	4	1.00	7,894	-	5	3.54	71,882	215,646	0.24	69,973	209,920	1.59	-	-
A	3	8	Female	8	0.93	11,401	-	5	4.94	136,430	204,646	0.64	129,011	193,517	2.23	-	-
			Male	8	0.98	14,650	-	4	4.41	176,922	265,383	0.53	167,778	251,668	1.39	-	-
A	4	8	Female	12	0.98	32,553	-	3.3	5.50	290,229	290,229	0.85	261,907	261,907	1.99	-	-
			Male	12	0.94	18,557	-	2	3.05	172,634	172,634	0.41	166,238	166,239	0.90	-	-
B	1	8	Female	2	1.00	4,649	-	5	3.49	50,268	301,610	0.38	48,726	292,354	2.55	-	-
			Male	2	1.00	4,429	-	4	3.75	48,805	292,828	0.35	47,129	282,776	2.27	-	-
B	2	8	Female	4	0.96	9,088	-	4.3	4.80	95,138	285,413	0.59	90,629	271,887	1.99	-	-
			Male	4	1.00	6,930	-	6	4.15	76,285	228,857	0.49	72,570	217,709	1.35	-	-
B	3	8	Female	8	0.98	15,221	-	4.3	4.80	141,965	212,948	0.71	133,319	199,979	1.15	-	-
			Male	8	0.96	9,560	-	6	6.41	145,490	218,235	1.22	128,309	192,464	1.24	-	-
B	4	8	Female	12	1.00	16,933	-	6	6.49	225,404	225,403	1.44	197,710	197,710	1.69	-	-
			Male	12	0.93	12,873	-	3.7	6.50	173,856	173,856	1.48	153,450	153,450	1.40	-	-

*Exact T½ calculation intervals are described in Table 4.
^ Dose corrected to 12 nmol/kg.

STUDY NO. 206016: SINGLE-DOSE IV/SC RAT STUDY

A 7-day pharmacokinetic study of insulin 454 from 3 Zn and 6 Zn formulation in rats [by (b) (4)] GLP

Study design: This GLP study was to investigate the PK of insulin 454 in dogs following a single iv administration (on Day 1) and following steady state (6 days; Days 3 to 8) sc administration from 2 formulation of insulin 454 with 3 Zn per 6 insulins (batch 412_NO5169) and insulin 454 with 6 Zn per 6 insulins (batch 412_NO6048).

Animals dosed with formulation X (3 Zn per 6 insulins) were as follows:

Group	Route of administration	Dose nmol/kg	Dose concentration	Animal Nos		Colour code
			(nmol/ml)	Males	Females	
1	IV	6	6	1-9	10-18	White
2	SC	25	25	19-27	28-36	Blue
3	SC	150	150	37-45	46-54	Green
4	SC	250	250	55-63	64-72	Red

Animals dosed with formulation Y (**6 Zn per 6 insulins**) were as follows:

Group	Route of administration	Dose nmol/kg	Dose concentration	Animal Nos		Colour code
			(nmol/ml)	Males	Females	
5	IV	6	6	73-81	82-90	Yellow
6	SC	25	25	91-99	100-108	Orange
7	SC	150	150	109-117	118-126	White with black rim
8	SC	250	250	127-135	136-144	Blue with black rim

Findings: There was no treatment-related effect on clinical signs, body weight gain, and food consumption. No difference in clearance was observed following iv administration in rats. Insulin 454 showed linear PK (as dose proportional exposure in terms of Cmax and AUC) following sc dosing in the range 25-150 nmol/kg. There was no accumulation. In addition, a change of insulin 454 formulation from 3 Zn to 6 Zn per 6 insulins resulted in unchanged values of insulin 454 AUC and slightly lower values of plasma insulin 454 Cmax, suggesting an exposure of a longer duration of exposure to insulin 454 in the blood.

Pharmacokinetic parameters of insulin 454 mean values in male and female rats after intravenous administration of 6 nmol/kg from 3 and 6 Zn per 6 insulin formulation.

Treatment	Gender	AUC ((h)*(nM))	AUCtau ((h)*(nM))	% AUCextra	Thalf (h)	Lz (1/h)	CL/f (L/h/kg)	Vz/f (l/kg)
IV 3 Zn 6 nmol/kg	Female	31.1	22.1	1.08	1.23	0.5656	0.193	0.341
	Male	28.7	21.2	5.17	1.92	0.3601	0.209	0.581
	Mean	29.9	21.6	3.12	1.58	0.4628	0.201	0.461
	Harmean	29.9	21.6	1.79	1.50	0.4400	0.201	0.430
IV 6 Zn 6 nmol/kg	Female	27.5	20.2	0.631	1.11	0.6234	0.218	0.350
	Male	26.0	18.4	2.25	1.49	0.4644	0.230	0.496
	Mean	26.8	19.3	1.44	1.30	0.5439	0.224	0.423
	Harmean	26.7	19.2	0.985	1.27	0.5322	0.224	0.411

Pharmacokinetic parameters of insulin 454 mean values in male and female rats after subcutaneous administration on Day 1 of 25, 150 and 250 nmol/kg from 3 and 6 Zn per 6 insulin formulation.

Treatment	Gender	Cmax (nM)	Tmax (h)	AUC ((h)*(nM)	AUCtau ((h)*(nM)	%AUCextra	Thalf (h)	Lz (1/h)	CL/f (L/h/kg)	Vz/f (L/kg)
SC 3 Zn 025 nmol/kg	Female	51.1	2.00	183	170	7.23	1.83	0.3778	0.137	0.362
	Male	51.8	2.00	222	201	9.29	2.06	0.3370	0.113	0.335
	Mean	51.4	2.00	202	185	8.26	1.95	0.3574	0.125	0.348
	Harmean	51.4	2.00	201	184	8.13	1.94	0.3562	0.124	0.348
SC 3 Zn 150 nmol/kg	Female	369	2.00	1560	1380	11.7	2.24	0.3097	0.0959	0.310
	Male	327	2.00	1360	1230	10.0	2.09	0.3309	0.110	0.333
	Mean	348	2.00	1460	1300	10.9	2.17	0.3203	0.103	0.321
	Harmean	347	2.00	1460	1300	10.8	2.16	0.3200	0.102	0.321
SC 3 Zn 250 nmol/kg	Female	475	2.00	2700	2270	15.9	2.73	0.2535	0.0927	0.366
	Male	423	2.00	2850	2100	26.5	3.73	0.1860	0.0876	0.471
	Mean	449	2.00	2780	2180	21.2	3.23	0.2197	0.0901	0.418
	Harmean	448	2.00	2770	2180	19.9	3.15	0.2145	0.0901	0.412
SC 6 Zn 025 nmol/kg	Female	48.8	2.00	208	186	10.7	2.17	0.3189	0.120	0.376
	Male	41.9	1.00	204	178	12.6	2.43	0.2857	0.123	0.430
	Mean	45.4	1.50	206	182	11.6	2.30	0.3023	0.121	0.403
	Harmean	45.1	1.33	206	182	11.6	2.29	0.3014	0.121	0.401
SC 6 Zn 150 nmol/kg	Female	298	2.00	1560	1290	17.3	2.77	0.2506	0.0960	0.383
	Male	230	4.00	1470	1230	16.2	2.34	0.2960	0.102	0.345
	Mean	264	3.00	1520	1260	16.8	2.55	0.2733	0.0991	0.364
	Harmean	259	2.67	1510	1260	16.7	2.54	0.2714	0.0990	0.363
SC 6 Zn 250 nmol/kg	Female	403	2.00	2790	2140	23.5	3.49	0.1987	0.0895	0.451
	Male	307	2.00	2700	1730	36.0	4.92	0.1410	0.0926	0.657
	Mean	355	2.00	2750	1930	29.7	4.20	0.1698	0.0910	0.554
	Harmean	349	2.00	2750	1910	28.4	4.08	0.1649	0.0910	0.534

Pharmacokinetic parameters of insulin 454 mean values in male and female rats after subcutaneous administration on Day 7 of 25, 150 and 250 nmol/kg from 3 and 6 Zn per 6 insulin formulation.

Treatment	Gender	Cmax(nM)	Tmax(h)	AUC((h)*(nM))	AUCtau(h)*(nM))	%AUCextra	Thalf(h)	Lz(1/h)	CL/f(L/kg/h)	Vz/f/L/kg)	RacPred bs	
SC 3 Zn 025 nmol/kg	Female	76.5	1.00	239	236	0.979	3.87	0.1790	0.106	0.591	1.01	1.39
	Male	59.4	2.00	248	245	1.23	3.81	0.1818	0.102	0.562	1.01	1.22
	Mean	68.0	1.50	243	240	1.10	3.84	0.1804	0.104	0.577	1.01	1.30
	Harmean	66.9	1.33	243	240	1.09	3.84	0.1803	0.104	0.577	1.01	1.30
SC 3 Zn 150 nmol/kg	Female	361	2.00	1580	1540	0.341	2.91	0.2386	0.0975	0.409	1.00	1.11
	Male	338	2.00	1490	1460	0.408	2.99	0.2321	0.103	0.442	1.00	1.19
	Mean	350	2.00	1540	1500	0.375	2.95	0.2353	0.100	0.425	1.00	1.15
	Harmean	349	2.00	1540	1500	0.371	2.95	0.2353	0.100	0.425	1.00	1.15
SC 3 Zn 250 nmol/kg	Female	520	2.00	2510	2500	0.309	2.80	0.2477	0.0998	0.403	1.00	1.10
	Male	531	2.00	2920	2910	0.465	2.97	0.2332	0.0861	0.369	1.00	1.39
	Mean	526	2.00	2720	2700	0.387	2.89	0.2405	0.0929	0.386	1.00	1.24
	Harmean	525	2.00	2700	2690	0.372	2.88	0.2402	0.0924	0.385	1.00	1.23
SC 6 Zn 025 nmol/kg	Female	49.6	2.00	230	228	0.979	3.63	0.1908	0.110	0.575	1.01	1.23
	Male	42.9	2.00	223	220	1.17	3.52	0.1971	0.114	0.576	1.01	1.24
	Mean	46.2	2.00	226	224	1.08	3.57	0.1939	0.112	0.576	1.01	1.23
	Harmean	46.0	2.00	226	224	1.07	3.57	0.1939	0.112	0.576	1.01	1.23
SC 6 Zn 150 nmol/kg	Female	277	2.00	1560	1550	0.405	2.90	0.2393	0.0966	0.404	1.00	1.20
	Male	215	2.00	1460	1450	0.773	3.21	0.2162	0.104	0.479	1.01	1.18
	Mean	246	2.00	1510	1500	0.589	3.05	0.2278	0.100	0.441	1.00	1.19
	Harmean	242	2.00	1510	1500	0.531	3.04	0.2272	0.0999	0.438	1.00	1.19
SC 6 Zn 250 nmol/kg	Female	411	2.00	2530	2520	0.606	3.05	0.2271	0.0994	0.438	1.00	1.18
	Male	308	2.00	2380	2350	1.07	3.45	0.2012	0.106	0.529	1.01	1.36
	Mean	360	2.00	2450	2430	0.840	3.25	0.2141	0.103	0.483	1.01	1.27
	Harmean	352	2.00	2450	2430	0.775	3.24	0.2134	0.103	0.479	1.01	1.26

STUDY NO. 206529: SINGLE-DOSE IV/SC RAT STUDY

[3H]-NNC-01000-0000-0454: a study of pharmacokinetics following a single subcutaneous and intravenous administration to the rat [by (b) (4)] GLP

Study design: This GLP study was to measure PK of radioactivity following either sc or iv administration of [3H]-NNC-0100-0000-0454 to the rat. Six male rats were split into 2 groups – single sc (25 nmol/kg) and iv (6 nmol/kg). Blood samples were collected at 1, 0.5, 1, 2, 3, 5, 8, 12, 24, and 36 hrs after dosing via tail vein. Additional 12 rats were split into 2 groups – single sc (25 nmol/kg) and iv (6 nmol/kg). Blood samples were

collected at 3, 12, and 24 hrs (sc dose) or 30 min, 3, and 12 (iv dose) via cardiac puncture.

Findings: The half-life of radioactivity was about 25 hrs, regardless the dose routes. AUC values were higher for plasma than for whole blood for both routes. When normalized for the dose levels, the AUC values were similar for both routes.

Plasma concentrations and pharmacokinetics of radioactivity following a single subcutaneous dose of [^{3}H]-NNC 0100-0000-0454 to male rats at a nominal dose level of 25 nmol/kg (group A)

	Concentration (pmol equivalents/g)				
Animal number and sex Sampling time	319M	320M	321M	Mean	SD
Pre-dose	ND	ND	ND	ND	NA
0.5 hours	18.53	23.93	37.23	26.56	9.62
1 hour	34.27	27.69	126.5	62.82	55.25
2 hours	43.56	51.97	71.15	55.56	14.14
3 hours	51.03	67.65	60.36	59.68	8.33
5 hours	50.12	60.92	49.89	53.64	6.30
8 hours	47.11	52.32	**38.32**	45.92	7.08
12 hours	**36.13**	**32.03**	NS	34.08	NA
24 hours	**21.39**	**22.88**	**21.34**	21.87	0.88
36 hours	**17.29**	**14.03**	**18.07**	16.46	2.15
C_{max} (pmol equiv/g)	51.03	67.65	126.5	81.73	39.66
T_{max} (hours)	3	3	1	2.333	1.155
$t_{½\,elim}$ * (hours)	22.57	20.15	25.32	22.68	2.586
AUC $_{0-t}$ (pmol equiv.h/mL)	1085	1125	1154	1121	34.38
$AUC_{0-\infty}$ (pmol equiv.h/mL)	1648	1533	1814	1665	140.9

* time points used to determine $t_{½\,elim}$ are shown in **bold type**
ND – Not detected
NS – No sample
NA – Not Applicable

Plasma concentrations and pharmacokinetics of radioactivity following a single intravenous dose of [^{3}H]-NNC 0100-0000-0454 to male rats at a nominal dose level of 6 nmol/kg (group B)

	Concentration (pmol equivalents/g)				
Animal number and sex Sampling time	322M	323M	324M	Mean	SD
Pre-dose	ND	ND	ND	ND	NA
0.5 hours	46.32	31.59	30.94	36.28	8.70
1 hour	29.74	24.64	26.98	27.12	2.55
2 hours	23.98	17.48	20.16	20.54	3.26
3 hours	20.91	8.44	18.28	15.87	6.57
5 hours	14.35	9.78	12.93	12.36	2.34
8 hours	10.42	**8.49**	**8.92**	9.28	1.02
12 hours	**6.40**	NS	**5.97**	6.19	NA
24 hours	**4.50**	**3.74**	NS	4.12	NA
36 hours	**3.84**	**2.65**	**3.15**	3.21	0.60
C_0 (pmol equiv/g)	72.14	40.50	35.48	49.37	19.88
$t_{½\,elim}$ * (hours)	32.57	16.46	20.69	23.24	8.349
AUC $_{0\text{-}t}$ (pmol equiv.h/mL)	316.8	241.5	272.2	276.9	37.88
$AUC_{0\text{-}\infty}$ (pmol equiv.h/mL)	497.3	304.4	366.3	389.3	98.46

* time points used to determine $t_{½\,elim}$ are shown in bold type
ND – Not detected
NS – No Sample
NA – Not Applicable

Blood concentrations and pharmacokinetics of radioactivity following a single subcutaneous dose of [^{3}H]-NNC 0100-0000-0454 to male rats at a nominal dose level of 25 nmol/kg (group A)

	Concentration (pmol equivalents/g)				
Animal number and sex Sampling time	319M	320M	321M	Mean	SD
Pre-dose	0.06	0.99	ND	0.35	0.56
0.5 hours	13.89	14.78	24.39	17.69	5.82
1 hour	23.88	26.71	36.35	28.98	6.54
2 hours	30.27	39.45	44.06	37.93	7.02
3 hours	33.66	42.70	42.67	39.67	5.21
5 hours	39.64	40.48	35.86	38.66	2.46
8 hours	31.38	36.11	29.18	32.22	3.55
12 hours	**25.83**	**29.91**	**24.95**	26.90	2.65
24 hours	**17.76**	**23.25**	**17.84**	19.62	3.15
36 hours	**13.76**	**14.55**	**14.85**	14.38	0.56
C_{max} (pmol equiv/g)	39.64	42.70	44.06	42.13	2.264
T_{max} (hours)	5	3	2	3.333	1.528
$t_{½\,elim}$ * (hours)	26.42	23.09	32.06	27.19	4.537
AUC $_{0\text{-}t}$ (pmol equiv.h/mL)	812.0	958.1	838.4	869.5	77.84
$AUC_{0\text{-}\infty}$ (pmol equiv.h/mL)	1336	1443	1525	1435	94.69

* time points used to determine $t_{½\,elim}$ are shown in bold type
ND – Not detected

Blood concentrations and pharmacokinetics of radioactivity following a single intravenous dose of [^{3}H]-NNC 0100-0000-0454 to male rats at a nominal dose level of 6 nmol/kg (group B)

Animal number and sex / Sampling time	Concentration (pmol equivalents/g) 322M	323M	324M	Mean	SD
Pre-dose	ND	ND	0.03	0.01	0.02
0.5 hours	27.28	21.65	23.59	24.17	2.86
1 hour	19.65	15.38	17.72	17.58	2.14
2 hours	16.25	13.31	14.20	14.59	1.51
3 hours	14.01	10.02	12.02	12.02	2.00
5 hours	10.48	8.13	8.81	9.14	1.21
8 hours	7.76	6.10	6.51	6.79	0.87
12 hours	**5.97**	**4.93**	**4.93**	5.28	0.60
24 hours	**3.97**	**3.09**	**3.30**	3.45	0.46
36 hours	**3.02**	**2.84**	**2.78**	2.88	0.12
C_0 (pmol equiv/g)	37.87	30.48	31.40	33.25	4.029
$t_{½\,elim}$ * (hours)	24.41	30.16	29.04	27.87	3.048
AUC $_{0-t}$ (pmol equiv.h/mL)	240.2	192.2	204.3	212.2	24.97
$AUC_{0-\infty}$ (pmol equiv.h/mL)	346.6	315.8	320.8	327.7	16.52

* time points used to determine $t_{½\,elim}$ are shown in **bold type**
ND – Not detected

Comparative pharmacokinetic data in plasma and blood following a single subcutaneous or a single intravenous administration of [^{3}H]-NNC 0100-0000-0454 to male rats at nominal dose levels of 25 or 6 nmol/kg, respectively (groups A and B)

Sample type	Plasma		Blood	
Dose route	Subcutaneous	Intravenous	Subcutaneous	Intravenous
Dose level	25 nmol/kg	6 nmol/kg	25 nmol/kg	6 nmol/kg
Mean AUC_{0-last} (pmol equiv.h/mL)	1121	276.9	869.5	212.2
Mean $AUC_{0-\infty}$ (pmol equiv.h/mL)	1665	389.3	1435	327.7
Mean AUC_{0-last} normalised for dose level (pmol equiv.h/mL per nmol/kg)	44.85	46.14	34.78	35.37
Mean $AUC_{0-\infty}$ normalised for dose level (pmol equiv.h/mL per nmol/kg)	66.60	64.89	57.39	54.62

STUDY NO. 211057: SINGLE-DOSE IV/SC DOG STUDY

Pharmacokinetics in the Beagle dog after intravenous and subcutaneous administration [by Novo Nordisk] non-GLP

Study design: This non-GLP study was to assess the PK of IDeg administered as a single dose by iv or sc injection to Beagle dogs and to assess the absolute and relative systemic bioavailability. Eight male dogs were dosed in a latin-square cross-over regimen using 2 formulations, 2 different dose levels, and iv or sc dosing. Treatment A was 0.24 nmol/kg formulated in NaCl (iv) and treatment B was 0.24 nmol/kg formulated in standard vehicle (iv). Treatments C (iv) and D (sc) were 2.4 nmol/kg, formulated in standard vehicle.

Treatment schedule

Animal Group	Day	Route of administration	Formulation	Treatment*	Dose	Dose formulation concentration*	Dose volume	Animal Nos
					(nmol /kg)	(nmol/mL)	(mL/kg)	
1	1	IV	I	A	0.24	30	0.008	1-4
2	1	IV	II	B	0.24	30	0.008	5-8
2	8	IV	I	A	0.24	30	0.008	5-8
1	8	IV	II	B	0.24	30	0.008	1-4
1	15	IV	II	C	2.4	30	0.08	1-4
2	15	SC	II	D	2.4	30	0.08	5-8
2	22	IV	II	C	2.4	30	0.08	5-8
1	22	SC	II	D	2.4	30	0.08	1-4
1+2	50	IV	I	E	0.24	30	0.008	1-8

*Diluted from stock solution (600 nmol/mL), a – refer to Section 4.3 for definitions

Findings: IV route/std vehicle: The PK of IDeg was similar regardless dose. Systemic exposure increased proportionally to dose after single iv bolus administration of 0.24 and 2.4 nmol/kg in standard vehicle to dogs. IDeg levels declined with IDeg being essentially cleared 12 hrs post dosing. Clearance was relatively low and Vd was small. The terminal t ½ was 3-4 hrs. SC route/std vehicle: Maximum IDeg levels were observed 6-8 hrs post dose after sc administration of 2/4 nmol/kg in standard vehicle, with IDeg being eliminated after 36 hrs, The t ½ was 5-6 hrs and the mean absorption time was 6-10 hrs, suggesting absorption-rate limited PK for IDeg. The bioavailability was 60-80%. IV route/NaCl: Systemic exposure to IDeg after iv dosing of 0.24 nmol/kg IDeg in NaCl was lower compared to that in standard vehicle. A NaCl stored for 20 hrs at RT showed approx. 20-30% lower exposure, suggesting a consequence of higher molecular weight compounds of IDeg, such as tetra-, hexa-, and multi-hexamers, forming by self-assembly of IDeg hexamers in the NaCl solution. This was not observed when IDeg was formulated in vehicle.

Distribution

STUDY NO. 204209: SINGLE-DOSE IV RAT STUDY

[^{125}I]-O454: a study of distribution in the rat after intravenous dosing by quantitative whole-body autoradiography [by (b) (4)] GLP

Study design: This GLP study was to determine the tissue distribution of radioactivity in the albino rat following single administration of [^{125}I]-O454, using quantitative whole-body autoradiography. Six female rats received a single iv administration of IDeg, at a nominal dose level of 6 nmol/kg. Duplicate animals were sacrificed at 5, 10, and 20 min after dosing for whole-body autoradiography.

Findings: Radioactivity was widely distributed at all sample times (up to 20 min postdose) after administration with levels in tissues generally increasing over the study period (except plasma, blood, aorta, kidney medulla, liver, adrenal, intra-orbital lachrymal gland, myocardium, lung, pancreas, and spleen, and oesophaqus). At all sampling times, the highest concentration of radioactivity was present in the plasma. Concentrations of radioactivity in the thyroid were comparable to other tissues at 5 and 10 min and only slightly higher at 20 min, indicating relatively low levels of free ^{125}I.

Mean concentrations of radioactivity in the tissues of female albino rats after a single intravenous administration of [^{125}I]-O454 at a nominal dose level of 6 nmol/kg body weight

			mean pmol equivalents of O454/g of tissue		
Tissue type	Tissue	Sampling time	5 min	10 min	20 min
Vascular/ lymphatic	Plasma[1]		128	107	89.3
	Blood		58.7	52.9	38.4
	Aorta		37.7	34.3	29.5
	Mandibular lymph nodes		3.17	8.73	7.95
Metabolic/ excretory	Kidney cortex (inner)		21.9	23.5	18.3
	Kidney cortex (outer)		36.5	49.8	48.8
	Kidney medulla		26.4	24.5	18.9
	Liver		20.7	15.5	11.3
CNS	Brain		1.29	1.51	1.40
	Choroid plexus		4.63	5.35	4.36
	Meninges		8.69	10.1	5.97
	Pineal body		11.5	13.2	12.4
	Spinal cord		1.05	2.03	2.27
Endocrine	Adrenal		31.2	17.0	11.7
	Adrenal cortex		31.1	16.8	11.3
	Adrenal medulla		36.2	29.0	17.1
	Pituitary		6.82	9.63	11.1
	Thymus		4.15	4.72	4.47
	Thyroid		15.4	18.2	43.2
Secretory	Exorbital lachrymal gland		11.7	NS	11.0
	Harderian gland		6.22	10.4	10.4
	Intra-orbital lachrymal gland		12.0	11.9	10.5
	Salivary glands		18.4	17.3	17.8
Fatty	Brown fat		6.40	10.9	11.1
	White fat		3.38	3.54	4.70
Gonads	Clitoris		10.0	11.1	8.38
	Mammary tissue		8.14	7.28	9.18
	Ovary		29.3	19.8	20.3
	Uterus		5.88	10.9	7.94
Muscular	Muscle		1.74	2.53	2.67
	Myocardium		20.0	17.3	15.4
	Tongue		3.85	5.69	9.72
Ocular	Lens		0.215	0.261	0.340
	Uveal tract		5.48	6.25	7.86
Unclassified	Bone marrow		7.54	10.4	11.1
	Epimysium		3.70	6.99	7.29
	Lung		36.8	33.4	24.9
	Nasal mucosa		4.18	6.78	13.9
	Pancreas		15.3	14.2	11.5
	Periosteum		3.29	4.93	4.99
	Skin		2.63	3.68	5.85
	Spleen		11.4	10.8	8.77
	Tooth pulp		15.5	14.7	19.1
	Trachea		5.30	9.01	7.88
Gastrointestinal	Oesophagus wall		15.9	14.3	10.5
	Stomach mucosa (fundus)		9.58	10.0	11.8
	Stomach mucosa (non-fundic)		3.91	5.37	4.93
	Small intestine mucosa		6.63	7.95	7.72
	Caecum mucosa		4.31	5.02	6.87
	Large intestine mucosa		6.59	8.20	7.49
	Rectum mucosa		7.48	7.10	8.66
	Upper limit of quantification =		81.0	pmol equiv/g for all measurements	
	Lower limit of quantification =		0.149	pmol equiv/g for all measurements	

[1] - Plasma concentrations of radioactivity determined by gamma counting methods
Limit of detection = 0.015 pmol equiv/g
NS - Tissue not sectioned

STUDY NO. 205521: SINGLE-DOSE IV RAT STUDY

[^{125}I]-NNC 100-054: a study of distribution by quantitative whole-body autoradiography following intravenous administration to the Zucker fa/fa rat [by (b) (4)] GLP

Study design: This GLP study was to determine the tissue distribution of radioactivity in the male Zucker fa/fa strain rat following a single iv administration of radiolabeled NN5401. Nine rats were divided into 3 groups of three. Each group received a single iv administration with all doses being administered by injection into a lateral tail vein at a

nominal dose level of 6 nmol/kg. Animals were sacrificed at 5, 10, and 20 min after dosing and prepared for whole-body autoradiography.

Findings: Radioactivity was widely distributed at all sample times, although peak levels occurred in most tissues at 20 min post-dose. Concentrations of radioactivity in tissues were less than that in plasma at all sampling times. Highest concentrations of radioactivity in other tissues were mostly associated with the blood, lung, and kidney. Lowest concentrations of radioactivity were generally present in the CNS, white fat, brown fat, testis, and the lens of the eyes. Systemically available test substance was eliminated by both the renal and biliary routes.

Mean concentrations of radioactivity in the tissues of male Zucker *fa/fa* rats after a single intravenous administration of [^{125}I]-NNC 100-0454 at a nominal dose level of 6 nmol/kg body weight

		pmol equivalents of NNC 100-0454/g of tissue		
Tissue type	Tissue Sampling time	5 minutes	10 minutes	20 minutes
Vascular/ lymphatic	Plasma[1]	217	179	142
	Blood	99.5+	85.9+	67.0+
	Aorta	58.7+*	38.6*	28.9*
	Mandibular lymph nodes	8.19	11.1	9.19
Metabolic/ excretory	Kidney cortex (inner)	51.8	47.8*	41.2*
	Kidney cortex (outer)	45.9	69.5+	74.0+
	Kidney medulla	59.4+	42.1*	41.8*
	Liver	29.5	21.0	21.1
CNS	Brain	2.35	2.18	1.81
	Choroid plexus	7.45	6.19*	10.2*
	Meninges	4.72	4.67	5.54
	Pineal body	40.7*	36.8*	22.9*
	Spinal cord	2.33	2.45	2.35
Endocrine	Adrenal	37.1	29.0	27.5
	Pituitary	17.2	16.1	12.6
	Thymus	4.73	5.45	3.97
	Thyroid	17.9	20.7	32.1
Secretory	Exorbital lachrymal gland	15.9	16.3	14.5
	Harderian gland	6.36	8.58	8.24
	Intra-orbital lachrymal gland	11.2	12.7	13.1
	Salivary glands	12.2	10.9	13.2
Fatty	Brown fat	2.00	2.90	3.34
	Inguinal fat	0.891	1.34	1.12
	Peri-renal fat	1.52	3.05	3.32
	Subcutaneous fat	0.981	1.48	1.96
Reproductive	Bulbo-urethral gland	20.3	17.7	19.5
	Epididymis	2.50	3.75	5.32
	Preputial gland	7.62	8.10	10.0
	Prostate	5.03	5.80	6.20
	Seminal vesicles	2.11	3.61	3.08
	Testis	1.49	2.59	3.79
Muscular	Muscle	3.51	4.22	4.69
	Myocardium	29.4*	23.8*	22.1*
	Tongue	5.29	7.59	9.12
	Upper limit of quantification =	72.2	pmol equiv/g for all measurements	
	Lower limit of quantification =	0.089	pmol equiv/g for all measurements	

[1] - Plasma concentrations determined by liquid scintillation counting. Limit of detection = 0.033 pmol equiv/g

* - One or more individual measurements affected by diffusion of image due to path length of I^{125} (see Diffusion of Image)

+ - One or more individual measurements above the upper limit of quantification.

(continued) Mean concentrations of radioactivity in the tissues of male Zucker *fa/fa* rats after a single intravenous administration of [^{125}I]-NNC 100-0454 at a nominal dose level of 6 nmol/kg body weight

Tissue type	Tissue	Sampling time	pmol equivalents of NNC 100-0454/g of tissue 5 minutes	10 minutes	20 minutes
Dermal	Non-pigmented skin		1.84	2.35	4.02
	Pigmented skin		3.18	3.65	5.24
Ocular	Lens		0.682	0.748	0.733
	Uveal tract		5.21	6.74	11.1
Other	Bone marrow		11.9	11.0	9.91
	Lung		68.1+	56.3	51.9
	Nasal mucosa		8.62	7.28	6.86
	Pancreas		23.7*	21.7*	21.0*
	Periosteum		4.38	5.78	5.45
	Spleen		12.1	16.7	12.1
	Tooth pulp		23.5	23.9	23.7
Gastrointestinal	Stomach mucosa (fundus)		13.0	10.8	10.1
	Stomach mucosa (non-fundic)		2.79	3.68	5.75
	Small intestine mucosa		8.20	8.27	7.70
	Caecum mucosa		NS	3.78	NS
	Large intestine mucosa		5.94	6.61	8.15
	Rectum mucosa		5.96	8.65	7.45
	Upper limit of quantification =		72.2	pmol equiv/g for all measurements	
	Lower limit of quantification =		0.089	pmol equiv/g for all measurements	

* - One or more individual measurements affected by diffusion of image due to path length of I^{125} (see Diffusion of Image)

+ - One or more individual measurements above the upper limit of quantification.

NS - tissue not sectioned

STUDY NO. 207366: SINGLE-DOSE SC RAT STUDY

[^{3}H]-insulin 454: a study of distribution by quantitative whole-body autoradiography following subcutaneous administration to the rat [by (b) (4)] GLP

Study design: This GLP study was to determine the tissue distribution of radioactivity in the male Wistar rats following a single sc administration of radiolabeled NN5401. Twelve rats were divided into 4 groups of three. Each groups received a single sc administration with all doses being administered by injection at a nominal dose level of 25 nmol/kg. Animals were sacrificed at 0.5, 2, 5, and 8 hrs after dosing after dosing and prepared for whole-body autoradiography.

Findings: The test article appeared to be slowly absorbed from the sc dosing site. However, peak levels, in most tissues, were achieved at 2 or 5 hrs after dosing. After each sampling time, high levels of radioactivity were associated with the plasma and whole blood. The highest level of radioactivity was detected in the kidney cortex followed by liver, tooth pulp, lung, kidney medulla and bulbo-urethral gland. Elimination was via the faecal and renal routes, substantiated by the presence of radioactivity in the bile ducts and GI contents and the urinary system.

Mean concentrations of radioactivity in the tissues of male Wistar rats after a single subcutaneous administration of [^{3}H]-insulin 454 at a nominal dose level of 25 nmol/kg body weight

		pmol equivalents of insulin 454/g of tissue			
Tissue type	Tissue Sampling time	0.5 hours	2 hours	5 hours	8 hours
Vascular/ lymphatic	Plasma[1]	14.0	35.3	37.0	26.7
	Blood	11.4	24.9	22.9	12.6
	Mandibular lymph nodes	1.40	4.24	4.64	2.35
Metabolic/ excretory	Kidney cortex	8.71	63.2	114	98.3
	Kidney medulla	5.23	13.3	13.4	8.92
	Liver	4.46	20.8	52.8	68.7
CNS	Brain	BLQ	BLQ	BLQ	BLQ
	Choroid plexus	1.89	4.91	BLQ	BLQ
	Meninges	1.65	4.27	3.43	3.08
	Pineal body	1.88	5.23	4.24	3.11
	Spinal cord	BLQ	BLQ	BLQ	BLQ
Endocrine	Adrenal	2.98	5.19	4.36	2.58
	Pituitary	1.80	9.28	5.28	2.64
	Thymus	0.252	1.43	1.39	0.820
	Thyroid	2.10	11.0	4.10	2.41
Secretory	Exorbital lachrymal gland	1.56	5.58	3.65	2.80
	Harderian gland	0.858	2.53	3.70	1.52
	Intra-orbital lachrymal gland	1.86	6.11	4.26	1.75
	Salivary glands	2.44	7.22	4.31	3.60
Fatty	Brown fat	2.37	4.90	3.22	1.80
	White fat	BLQ	BLQ	BLQ	BLQ
Reproductive	Bulbo-urethral gland	3.86	11.3	10.3	7.69
	Epididymis	0.520	4.29	6.24	6.82
	Preputial gland	0.924	5.36	2.23	1.39
	Prostate	1.73	4.19	3.60	2.81
	Seminal vesicles	0.427	1.62	2.42	1.07
	Testis	0.503	3.32	4.51	2.83
Muscular	Muscle	0.292	1.89	1.29	1.00
	Myocardium	2.98	8.59	6.59	4.17
	Tongue	1.50	7.38	5.79	3.90
	Lower limit of quantification =	0.433	0.433	0.433	0.433
	Upper limit of quantification =	388	388	388	388

[1] - Plasma concentrations determined by liquid scintillation counting
BLQ – below level of quantification

(continued) Mean concentrations of radioactivity in the tissues of male Wistar rats after a single subcutaneous administration of [^{3}H]-insulin 454 at a nominal dose level of 25 nmol/kg body weight

			pmol equivalents of insulin 454/g of tissue			
Tissue type	Tissue	Sampling time	0.5 hours	2 hours	5 hours	8 hours
Other	Bone marrow		1.56	1.66	3.10	2.20
	Lung		6.99	14.8	13.4	9.44
	Nasal mucosa		4.07	4.55	2.89	2.18
	Pancreas		3.79	7.33	5.70	3.60
	Periosteum		0.738	10.9	3.20	1.82
	Skin		0.462	3.99	3.94	2.86
	Spleen		1.41	3.88	2.69	1.90
	Tooth pulp		5.72	20.8	22.1	13.6
	Uveal tract		1.58	7.44	7.30	3.84
Gastrointestinal	Oesophagus wall		2.17	10.2	6.10	5.68
	Stomach mucosa (fundus)		2.03	7.47	6.42	3.21
	Stomach mucosa (non-fundic)		0.619	3.46	5.20	2.25
	Small intestine mucosa		1.64	6.05	3.97	5.39
	Caecum mucosa		0.895	4.30	2.00	6.75
	Large intestine mucosa		2.41	5.75	4.96	6.40
	Rectum mucosa		1.75	6.55	5.73	3.58
	Lower limit of quantification =		0.433	0.433	0.433	0.433
	Upper limit of quantification =		388	388	388	388

STUDY NO. 208222: REPEAT-DOSE SC FETAL RAT STUDY

Fetal exposure study in the Han Wistar rat by subcutaneous administration [by (b) (4)] GLP

Study design: This GLP study was to provide an assessment of fetal exposure to insulin 454 following daily administration during the organogenesis phase and up to Day 20 of pregnancy in rats. A single group comprising 8 female rats received insulin 454 by sc administration at a dose level of 125 nmol/kg/day during Days 6-20 after mating. Animals were sacrificed on Day 20 after mating for pregnancy outcome and embryo-fetal survival assessment.

Findings: The data showed that exposure to the test item at either 3 hrs or 9 hrs post-dosing on GD20. All fetal samples showed exposure to the test item at either 3 hrs or 9 hrs after dosing on GD20. Fetal exposure levels were approx. 4% of maternal exposure at 2 hrs after adult dosing, and approx. 0.8% of maternal exposure at 9 hrs after dosing of the adult females.

Exposure assessment - group mean values (pM) for adult females on Day 5 and Day 20 of gestation

Group /Sex		Day 5	Day 20	
			3 hours after dosing	9 hours after dosing
1F	Mean	-	378300	44090
	SD	-	80400	12770
	CV(%)	-	21.3	29.0

Exposure assessment - group mean values (pM) for fetuses

Group /Sex		Day 20 3 hours after dosing	Day 20 9 hours after dosing
1F	Mean	1530.1	356.4
	SD	639	47
	CV(%)	41.7	13.1

Litter data - individual values on Day 20 of gestation

Group /Sex	Animal Number	Corpora Lutea	Implantations	Resorptions Early	Resorptions Late	Resorptions Total	Live fetuses	Implantation Loss (%) Pre-	Implantation Loss (%) Post-
1F	1	15	13	0	0	0	13	13.3	0.0
	2	14	14	2	0	2	12	0.0	14.3
	3	14	12	0	0	0	12	14.3	0.0
	4	14	14	0	0	0	14	0.0	0.0
	5	12	12	0	0	0	12	0.0	0.0
	6#	12	11	1	0	1	10	8.3	9.1
	7	15	14	0	1	1	13	6.7	7.1
	8	14	13	1	0	1	12	7.1	7.7

Female found dead at necropsy on Day 20 of gestation prior to blood sampling

Metabolism

STUDY NO. 204244: METABOLITE PROFILE VIA IN VITRO ASSAYS

In vitro metabolic stability of NNC 0100-0000-0454 and human insulin after incubation with Cathepsin D [by Novo Nordisk, Denmark] non-GLP

Study design: This non-GLP study was to establish the metabolite profile formed in vitro following incubation of insulin 454 (batvh AF-1T-K2-001) or human insulin with Cathepsin D (acidic endopeptidase) and to identify any metabolite. Human insulin (20 µM) or insulin 454 (20 µM) was incubated with 1 unit/ml Cathepsin D in 0.1 M citrate-phosphate buffer at pH 4 for 0, 10, 30, and 60 min. All samples were analyzed on HPLC using UV detection to obtain the metabolite profiles. Selected samples were further analyzed using LC-MS to identify initial metabolites.

Findings: Based on this study result, the metabolism pathway of insulin 454 and human insulin were found to be the same. The first steps of metabolism (b) (4)

STUDY NO. 206618: METABOLITE PROFILE VIA SINGLE-DOSE IV/SC RAT STUDY

[3H]-NNC 0100-0000-0454: investigation of metabolites in the Wistar rat following single dose administration [by Novo Nordisk, Denmark]

Study design: This study investigated the metabolites formed form insulin 454 in rat urine, feces, and bile. One group of animals received a single sc dose of 25 nmol/kg [3H]-insulin 454 and 9.25 MBq/kg, prior to plasma urine, feces and bile collection. Another group of animals received a single iv dose of 6 nmol/kg [3H] insulin 454 and

2.22 MBq/kg, prior to plasma collection. Plasma was isolated 3, 12, and 24 hrs after sc administration and 0.5, 3, and 12 hrs after iv administration. The selected urine samples were collected 0-12, 12-24, 24-48, and 48-72 hrs after sc administration. The selected feces samples were collected 0-24, 24-48, and 48-72 hrs after sc administration. The selected bile samples were collected 0-2, 2-4, 6-12, and 12-24 hrs after sc administration.

Findings: In urine and feces collected 0-72 hrs and bile collected 0-24 hrs after sc administration, a large number of components (total of 10, 11, and 10 chromatographic regions respectively) were detected. The most abundant compound, 6% of the administered dose, was excreted in the urine sample. The most abundant compound, 4 and 2 % of the administered dose, was excreted in feces and bile samples. In urine, feces, and bile samples, one component most likely tritiated water was eliminated more slowly than the other components.

The relative peak area (%) from all peaks/regions detected in urine collected 0-12, 12-24, 24-48 and 48-72 hours after subcutaneous administration of 25 nmol/kg NNC 0100-0454. The values are presented in the table together with mean retention times.

		Relative peak area (%)			
Peak/Region	Mean Retention time (min)	0-12 hours	12-24 hours	24-48 hours	48-72 hours
U1	3.1	6.14	9.04	27.64	100.00
#U2	3.7-10.5	19.67	23.01	29.16	n/a
U3	11.1	9.34	7.67	2.95	n/a
U4	13.5	19.27	22.07	21.98	n/a
U5	14.6	2.62	2.45	2.96	n/a
U6	15.4	7.40	6.83	4.29	n/a
U7	17.5	3.12	1.41	n/a	n/a
U8	22.1	18.04	6.86	n/a	n/a
U9	24.2	4.30	1.48	n/a	n/a
U10	25.3	10.10	19.17	11.02	n/a

#Integrated region of interest

n/a = not applicable

The relative peak area (%) from all peaks detected in faeces collected 0-24, 24-48 and 48-72 hours after subcutaneous administration of 25 nmol/kg NNC 0100-0454. The values are presented in the table together with mean retention times.

Peak/Region	Mean Retention time (min)	Relative peak area (%)		
		0-24 hours	24-48 hours	48-72 hours
F1	3.2	9.42	48.92	100.00
F2	14.8	5.15	n/a	n/a
F3	24.6	4.30	18.25	n/a
F4	25.6	19.96	32.84	n/a
F5	35.7	5.26	n/a	n/a
F6	43.0	4.35	n/a	n/a
F7	45.5	10.94	n/a	n/a
F8	54.4	31.48	n/a	n/a
F9	57.7	1.33	n/a	n/a
F10	63.4	1.88	n/a	n/a
F11	67.5	5.92	n/a	n/a

n/a = not applicable

The relative peak area (%) from all peaks detected in bile collected 0-6, 6-12 and 12-24 hours after subcutaneous administration of 25 nmol/kg NNC 0100-0454. The values are presented in the table together with mean retention times.

Peak/Region	Mean Retention time (min)	Relative peak area (%)		
		0-6 hours	6-12 hours	12-24 hours
B1	3.2	3.00	12.32	41.60
B2a	6.0	1.77	n/a	4.46
B2b	6.8	n/a	5.19	11.45
B3a	13.5	7.28	n/a	n/a
B3b	14.5	n/a	8.39	7.25
B4	24.8	1.53	1.30	n/a
B5	27.9	6.22	6.68	3.14
B6	37.6	27.26	30.48	14.01
B7	46.7	22.68	21.05	11.28
NNC-0100-0454	52.5	5.53	n/a	n/a
B8	54.6	23.07	12.55	6.81
B9	59.9	1.66	2.04	n/a

n/a = not applicable

STUDY NO. 207309: METABOLITE PROFILE VIA SINGLE-DOSE SC RAT STUDY

[3H]-insulin 454: metabolite profiling of plasma from male and female Wistar rats following single subcutaneous administration [by Novo Nordisk, Denmark] GLP

Study design: This GLP study investigated the levels of metabolites and unchanged insulin 454 in plasma from rats administered with [3H]-insulin 454. Animals were administered with nominal single sc dose of 25 nmol/kg [3H]-insulin 454 (males in group A and females in group B) and 250 nmol/kg [3H]-insulin 454 (males in group C), and radiochemical dose in the range 22-23 MBq/kg. Terminal blood was collected 1, 3, 5, 8, and 12 hr after administrated and mixed with 0.5 mM diamide.

Findings: Unchanged insulin 454 was the major component in the analyzed plasma samples. Insulin 454 accounted for 69-76% of total AUC, whereas the AUCs for the two metabolites P2 and P3 corresponded to 12-15 and 12-17% respectively (0-12 h after

administration). A component, most likely drug-related water, was detected in plasma which was regarded to have no impact on safety studies.

Concentration equivalents from total radioactivity in plasma samples isolated 1, 3, 5, 8 and 12 h after single subcutaneous administration of [^{3}H]-insulin 454.

Group / Gender / dose	Time (h)	Concentration equivalents (nmol eq./L)
A	1	14.39
Male rats	3	23.24
25 nmol/kg	5	19.38
	8	16.94
	12	9.37
B	1	34.77
Female rats	3	23.96
25 nmol/kg	5	21.93
	8	15.41
	12	7.37
C	1	331.23
Male rats	3	341.16
250 nmol/kg	5	253.33
	8	187.67
	12	157.86

Pharmacokinetic parameters, AUC_{0-12h} and fractions of total AUC, obtained from male rats (group A) after a single subcutaneous administration of 25 nmol/kg [^{3}H]-insulin 454

Peak/Region	AUC_{0-12h} (nmol x h/L)	Fraction of total AUC (%)	[a]Fraction of total AUC_{-P1} (%)
P1	53	33	NA
Insulin 454	75	46	69
P2	16	10	15
P3	18	11	16
Total	162	100	100

[a] Total AUC_{-P1} includes AUC from insulin 454, P2 and P3 (P1 excluded)
NA = not applicable

Pharmacokinetic parameters, AUC_{0-12h} and fractions of total AUC, obtained from female rats (group B) after a single subcutaneous administration of 25 nmol/kg [^{3}H]-insulin 454

Peak/Region	AUC_{0-12h} (nmol x h/L)	Fraction of total AUC (%)	[a]Fraction of total AUC_{-P1} (%)
P1	41	25	NA
Insulin 454	85	51	69
P2	18	11	14
P3	21	13	17
Total	165	100	100

[a] Total AUC_{-P1} includes AUC from insulin 454, P2 and P3 (P1 excluded)
NA = not applicable

Pharmacokinetic parameters, AUC_{0-12h} and fractions of total AUC, obtained from male rats (group C) after a single subcutaneous administration of 250 nmol/kg [^{3}H]-insulin 454

Peak/Region	AUC_{0-12h} (nmol x h/L)	Fraction of total AUC (%)	[a]Fraction of total AUC_{-P1} (%)
P1	438	18	NA
Insulin 454	1526	63	76
P2	240	10	12
P3	234	10	12
Total	2438	100	100

[a] Total AUC_{-P1} includes AUC from insulin 454, P2 and P3 (P1 excluded)
NA = not applicable

STUDY NO. 207374: METABOLITE PROFILE VIA SINGLE-DOSE IV/SC DOG STUDY

[3H]-insulin 454: a study of serum pharmacokinetics following subcutaneous and intravenous administration to the dog [by (b) (4)] GLP

Study design: This GLP study investigated the levels of metabolites and unchanged insulin 545 in serum in male dogs following a single sc (Group A) or iv (Group B) dose of [3H]-insulin 454 at a nominal dose level of 4 nmol/kg. Radioactivity in serum and whole blood was determined. Serum samples were analyzed for unchanged insulin 454 concentrations. The metabolite profiles of selected serum samples at 3, 6, 12, 24, and 30 hr post-dose (Group A) and 1, 3, 6, and 12 hr (Group B) post-dose were determined by radio-HPLC. PK parameters were determined based on serum concentrations of total radioactivity and unchanged insulin 454.

Findings: Serum concentrations of unchanged insulin 454 and total radioactivity were similar immediately following iv administration. Mean serum concentration of insulin were approx. 2.4X less than for total radioactivity concentrations at Cmax following sc administration, indicating the presence of circulating metabolites. The terminal sc and iv t ½ of insulin 454 and those for total radioactivity were similar. However, the mean t ½ of total radioactivity was approx 4X greater than for unchanged insulin 454, indicating the presence of a component, possibly tritiated water, with a longer half-life. The mean sc bioavailability of insulin 454 was 73.9%.

Serum concentrations of Insulin 454 and pharmacokinetics following a single subcutaneous administration of [^{3}H]-Insulin 454 to male dogs at a nominal dose level of 4 nmol/kg body weight – Group A

Time point (h)	Concentration (pmol/L)				
	101M	102M	103M	Mean	SD
Pre-dose	<27.0	<27.0	<27.0	NC	NC
0.5	186	70.5	68.0	108	67.4
1	697	364	202	421	252
3	5120	3090	3030	3750	1190
6	5570	4620	5790	5320	620
12	3050	2750	7080	4300	2420
24	267	443	443	384	102
30	142	186	186	171	25.4
C_{max} (pmol equiv/L)	5570	4620	7080	5760	1240
T_{max} (h)	6	6	12	8	3.5
$t_{½\ elim}$ * (h)	3.96	4.62	3.36	3.98	0.63
AUC $_{0-t}$ (pmol equiv.h/L)	62100	53700	85700	67200	16600
AUC (pmol equiv.h/L)	62900	54900	86600	68200	16400
% extrapolated	1.29	2.26	1.04	1.53	0.642
% F	57.1	58.7	106	73.9	27.8

NC – Not calculable

Serum concentrations of Insulin 454 following a single intravenous administration of [^{3}H]-Insulin 454 to male dogs at a nominal dose level of 4 nmol/kg body weight – Group B

Time point (h)	Concentration (pmol/L)				
	201M	202M	203M	Mean	SD
Pre dose	<27.0	<27.0	<27.0	NC	NC
0.25	41400	40600	38200	40100	1650
0.5	33300	33200	27200	31200	3520
1	22000	20600	18100	20300	1970
3	10500	7610	7170	8430	1810
6	6090	4090	2940	4370	1590
12	924	993	918	945	41.7
24	99.8	76.4	73.7	83.3	14.4
30	44.3	36.4	36.8	39.2	4.45
C_t (pmol equiv/L)	41400	40600	38200	40100	1650
$t_{½\ elim}$ * (h)	3.35	3.41	3.53	3.43	0.0909
AUC $_{0-t}$ (pmol equiv.h/L)	99600	83300	71900	84900	13900
AUC (pmol equiv.h/L)	110000	93600	81600	95100	14300
% extrapolated	0.194	0.191	0.229	0.205	0.0213
V_z (L/kg)	0.175	0.210	0.249	0.212	0.037
CL (L/hr/kg)	0.0363	0.0427	0.0490	0.0427	0.00635

NC – Not calculable

Up to 5 regions of radioactivity were resolved in serum by HPLC. Unchanged insulin 454 was the principal component identified at time points up to 6 hrs post iv and 12 hrs post sc administration. Region S1 was likely to be tritiated water. The relative system exposure of S1 compared to that from total radioactivity was approx 17 and 7% following sc and iv administration respectively. In addition, the other major regions, S2, S3, and S4, showed systemic exposures relative to that from total radioactivity of between 11-13% following sc and 9 and 10% following iv administration.

Quantification of the radioactive components in serum following subcutaneous administration of [^{3}H]-Insulin 454 to male dogs at a nominal dose level of 4 nmol/kg body weight – Group A

		% Peak area						
Peak no.	Mean retention time (min)	103M 3 h	101M 6 h	102M 6 h	103M* 12 h	101M 24 h	103M 24 h	103M 30 h
S1	1.8-3.4	2.50	2.08	3.16	11.4	31.1	22.3	43.1
Insulin 454	28.7-29.4	64.5	52.8	50.2	48.3	20.3	18.4	9.71
S2	29.2-29.8	7.53	8.99	16.9	†	12.6	10.5	10.4
S3	31.0-31.6	7.14	7.96	10.1	13.4	ND	4.98	ND
S4	34.2-34.9	2.77	5.48	5.80	11.0	13.6	10.7	8.80

ND – not detected
* - sample with slight haemolysis
†- peak S2 merges with Insulin 454

		Concentration pmol equiv./L						
Peak no.	Mean retention time (min)	103M 3 h	101M 6 h	102M 6 h	103M* 12 h	101M 24 h	103M 24 h	103M 30 h
S1	1.8-3.4	146	324	380	1650	1740	1740	2330
Insulin 454	28.7-29.4	3760	8220	6030	6980	1140	1440	525
S2	29.2-29.8	439	1400	2030	†	705	820	562
S3	31.0-31.6	417	1240	1210	1940	ND	389	ND
S4	34.2-34.9	162	853	697	1590	762	838	476

ND – not detected
* - sample with slight haemolysis
†- peak S2 merges with Insulin 454

Area under the curve, $AUC_{0\text{-}30h}$ and fractions of total AUC in serum following subcutaneous administration of [^{3}H]-Insulin 454 to male dogs at a nominal dose level of 4 nmol/kg body weight – Group A

Peak/Region	$AUC_{0\text{-}30h}$ (pmol.h/L)	Fraction of total AUC (%)	[a]Fraction of total AUC_{S1} (%)
S1	39500	17.2	NA
Insulin 454	110000	48.0	57.9
S2	29000	12.7	15.3
S3	24200	10.6	12.7
S4	26300	11.5	13.8
Total	229000	100	100

[a] Total AUC_{S1} includes AUC from insulin 454, S2, S3 and S4 (S1 excluded)
NA - not applicable

Quantification of the radioactive components in serum following intravenous administration of [^{3}H]-Insulin 454 to male dogs at a nominal dose level of 4 nmol/kg body weight – Group B

		% Peak area				
Peak no.	Mean retention time (min)	202M * 1 h	202M* 3 h	202M** 6 h	201M* 12 h	203M 12 h
S1	1.8-3.4	ND	2.37	8.44	19.1	25.0
Insulin 454	28.7-29.4	72.2	59.5	28.7	17.4	19.4
S2	29.2-29.8	ǂ	ǂ	14.5	12.6	14.0
S3	31.0-31.6	8.68	13.5	9.29	7.02	ND
S4	34.2-34.9	3.39	8.32	10.9	15.2	10.7

ND – not detected
* - samples with slight haemolysis
ǂ- peak S2 merges with Insulin 454
** - haemolysed samples

		Concentration pmol equiv./L				
Peak no.	Mean retention time (min)	202M* 1 h	202M* 3 h	202M** 6 h	201M* 12 h	203M 12 h
S1	1.8-3.4	ND	377	832	1010	1130
Insulin 454	28.7-29.4	18500	9470	2830	916	877
S2	29.2-29.8	ǂ	ǂ	1430	665	631
S3	31.0-31.6	2230	2150	916	370	ND
S4	34.2-34.9	869	1320	1080	799	484

ND – not detected
* - samples with slight haemolysis
ǂ- peak S2 merges with Insulin 454
** - haemolysed samples

Area under the curve, $AUC_{0\text{-}12h}$ and fractions of total AUC in serum following intravenous administration of [^{3}H]-Insulin 454 to male dogs at a nominal dose level of 4 nmol/kg body weight – Group B

Peak/Region	$AUC_{0\text{-}12h}$ (pmol.h/L)	Fraction of total AUC (%)	[a]Fraction of total $AUC_{\text{-}S1}$ (%)
S1	8090	6.8	NA
Insulin 454	75700	63.6	68.2
S2	10200	8.6	9.2
S3	13400	11.3	12.1
S4	11300	9.5	10.2
Total	119000	100	100

[a] Total $AUC_{\text{-}S1}$ includes AUC from insulin 454, S2, S3 and S4 (S1 excluded)
NA - not applicable

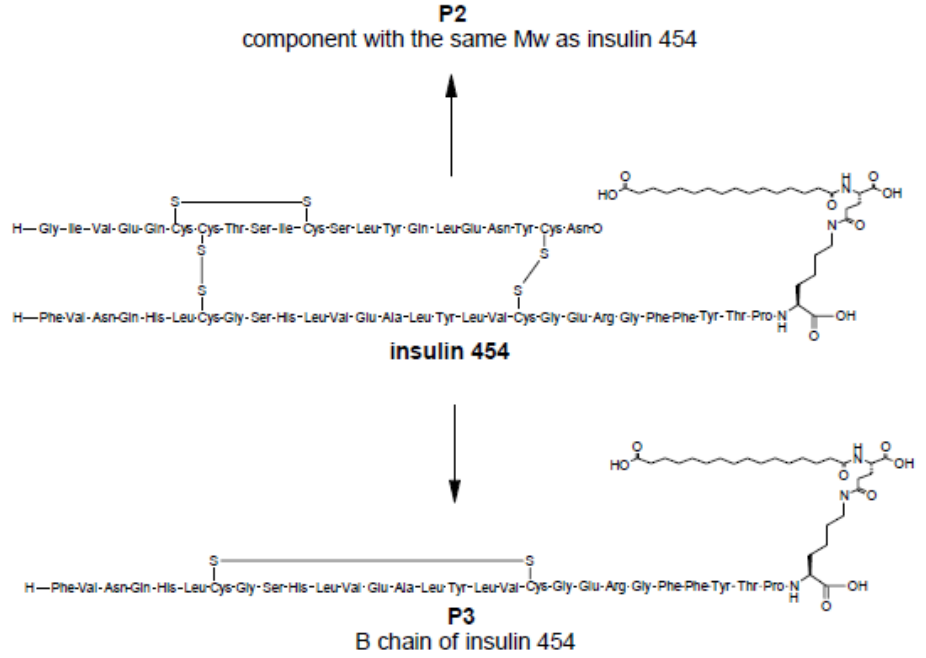
STUDY NO. 209425: METABOLITE PROFILE (STRUCTURAL CHARACTERIZATION) VIA SINGLE-DOSE SC RAT STUDY

[3H]-insulin 454: structural characterization of metabolites in plasma from the male Wistar rat following single subcutaneous administration [by Novo Nordisk, Denmark] non-GLP

Study design: This study was to obtain information on the structure of metabolites form insulin 454 in plasma after administration with [3H]-insulin 454 in rats. Ten male rats were given a nominal single sc dose of 250 nmol/kg [3H]-insulin 454 and a nominal radiochemical dose of 50 MBq/kg. Terminal blood was collected 3 hr after dosing. The plasma components of insulin were analyzed by HPLC and UPLC-MS/RAM and UPLC-MS/MS were used for further structure characterization.

Findings: The structures of 3 components in plasma from rats administered with [3H]-insulin 454 were characterized. The most abundant plasma component was insulin 454, one of the minor components P2 (a molecule with the same molecular weight, primary aa structure and fatty acid moiety as insulin 454), and second minor component P3 (the B chain of insulin 454 with a crosslink between the B chain cysteines and intact fatty acid moiety).

Components in rat plasma after administration of [^{3}H]-insulin 454



STUDY NO. 210241: CYP450 ENZYME PROFILE VIA REPEAT-DOSE SC RAT STUDY

Study to investigate the effect on liver cytochrome P450 enzymes after subcutaneous administration to Han Wistar rats for 2 weeks, with NPH insulin as comparator [by (b) (4)] GLP

Study design: This study was to determine the effect on CYP450 enzymes from once daily sc treatment with IDeg for 2 week (vs. NPH insulin). Three groups (5 males and 5 females) received IDeg at 25, 100, 0r 150 nmol/kg/day and one group (5 males and 5 females) received NPH insulin at a dose of 100 nmol/kg/day. Endpoints included clinical signs, body weight, food consumption, organ weight, cytochrome P450 assays, and gross pathology.

Findings: The data showed that following sc administration of IDeg at 25, 100, and 150 nmol/kg/day to rats for 2 weeks, only a minor response to treatment was observed. The CYP450 activities (expressed per g liver) were unchanged at the lowest dose. None of the activities in the mid and high dose groups were changed higher than 1.9X or lower

than 0.8X. All effects of IDeg were quantitatively similar to those elicited by NPH insulin. All in all, IDeg and NPH insulin administrations to the rat did not have a major impact on the regulation of CYP enzymes at the dose levels tested.

Summary of liver enzyme changes in males expressed as group mean results from fractions of the corresponding control group mean.

The data are group mean results expressed as fractions of the corresponding control group mean[1].

Biomarker for	Parameter	Insulin Degludec (nmol/kg/day)			NPH Insulin (nmol/kg/day)
		25	100	150	100
NA	Microsomal protein - (mg/g liver)	1.0	1.1	1.2*	1.3**
NA	Cytochrome P450 - (nmoles/mg protein) - (nmoles/g liver)	1.0 1.0	1.0 1.1	1.1 1.3*	1.1* 1.4*
CYP1A	7-Ethoxyresorufin O-deethylase - (pmoles/min/mg protein) - (nmoles/min/g liver)	1.1 1.1	1.1 1.2	1.2 1.4	1.3 1.6*
CYP2B	7-Pentoxyresorufin O-depentylase - (pmoles/min/mg protein) - (pmoles/min/g liver)	1.0 1.0	1.1 1.2	1.2 1.4*	1.3** 1.6***
CYP2C	Tolbutamide methylhydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	1.0 1.0	0.9 1.0	0.9 1.1	0.9 1.1
CYP2C	Testosterone 16(-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	0.8 0.8	1.0 1.0	1.0 1.1	1.0 1.2
CYP2C	Testosterone 2α- (+ 2β-)hydroxylases - (nmoles/min/mg protein) - (nmoles/min/g liver)	0.8 0.8	1.0 1.1	1.0 1.2	1.0 1.3*
CYP2B, 2C	Testosterone 17β-dehydrogenase - (nmoles/min/mg protein) - (nmoles/min/g liver)	0.9** 0.9	0.8** 0.9	0.9** 1.0	0.8*** 1.0
CYP2E	Lauric acid 11-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	1.0 1.0	0.9 0.9	1.0 1.1	0.9 1.1
CYP3A	Testosterone 6β-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	0.9 0.9	0.9 1.0	1.0 1.2	0.9 1.1

* Significance level of comparison with control, using a Williams' test for Groups 2 to 4 and *t* test for Group 5: $p<0.05$

** Significance level of comparison with control, using a Williams' test for Groups 2 to 4 and *t* test for Group 5: $p<0.01$

*** Significance level of comparison with control, using a Williams' test for Groups 2 to 4 and *t* test for Group 5: $p<0.001$

NA Not applicable

[1] In male groups (7α-hydroxylase activities, a marker for CYP2A), all mean values were below the limit of quantification; 0.175 nmoles/min/mg protein

Summary of liver enzyme changes in females expressed as group mean results from fractions of the corresponding control group mean.

The data are group mean results expressed as fractions of the corresponding control group mean[1].

Biomarker for	Parameter	Insulin Degludec (nmol/kg/day)			NPH Insulin (nmol/kg/day)
		25	100	150	100
NA	Microsomal protein - (mg/g liver)	1.1	1.2**	1.2**	1.1*
NA	Cytochrome P450 - (nmoles/mg protein) - (nmoles/g liver)	1.2 1.3	1.1 1.3	1.1 1.4**	1.2 1.3*
CYP1A	7-Ethoxyresorufin O-deethylase - (pmoles/min/mg protein) - (nmoles/min/g liver)	1.0 1.1	1.2 1.4	1.3 1.6*	1.0 1.2
CYP2B	7-Pentoxyresorufin O-depentylase - (pmoles/min/mg protein) - (pmoles/min/g liver)	1.1 1.2	1.4 1.7*	1.5* 1.9**	1.4 1.6
CYP2C	Tolbutamide methylhydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	1.0 1.1	1.1 1.3	1.1 1.4	1.1 1.2
CYP2A	Testosterone 7(-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	1.1 1.2	0.9 1.1	1.0 1.3	1.0 1.1
CYP2B, 2C	Testosterone 17β-dehydrogenase - (nmoles/min/mg protein) - (nmoles/min/g liver)	0.9 0.9	0.8 0.9	1.1 1.3	0.8 0.9
CYP2E	Lauric acid 11-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	1.0 1.1	1.1 1.3	1.1 1.4	1.1 1.2
CYP3A	Testosterone 6β-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	1.0 1.0	1.0 1.2	1.0 1.2	1.0 1.1

* Significance level of comparison with control: $p<0.05$
** Significance level of comparison with control: $p<0.01$
NA Not applicable
[1] In female groups (testosterone 2α- (+ 2β-) hydroxylase activities (a marker for CYP2C) mean values were below the limit of quantification 0.0875 nmoles/min/mg protein
16α -hydroxylase activities, also a marker for CYP2C) mean values were predomonantly below the limit of quantification: 0.0875 nmoles/min/mg protein and therfore not summarised.

STUDY NO. 210344: METABOLITE PROFILE VIA SINGLE-DOSE SC LACTATING RAT STUDY

[3H]-insulin degludec: metabolite profile analysis of milk and plasma from lactating rats following single subcutaneous administration [by Novo Nordisk, Denmark]

Study design: This study was to obtain information on the relative proportions of metabolites and unchanged IDeg in milk and plasma from lactating rats following sc administration of 25 nmol/kg [3H]-insulin 454, a radiochemical dose of 70 MBq/kg and a target dose volume of 5 mg/kg. The milk and blood were collected at various time points.

Findings: In milk, 4 components were detected including unchanged IDeg, 2 metabolites, and water formed from the IDeg metabolism. IDeg and on of the metabolites were the principal components after water. IDeg and 2 metabolites accounted for 26, 26, and 2% of the total AUC, respectively. In plasma, 7 components were detected including unchanged IDeg as the principal component, 5 metabolites,

and water. IDeg and 2 major metabolites accounted for 49, 15, and 8% of the total AUC0-8 h, respectively. In comparison, the AUC0-8hr of IDeg was 3X higher in plasma than in milk.

Pharmacokinetic parameters in milk, AUC_{0-8h} and fractions of total AUC, obtained from rat after a single subcutaneous administration of 25 nmol/kg [^{3}H]-insulin degludec

Peaks	AUC_{0-8h} (nmol*h/l)	Fraction of total AUC (%)
Mi1	34.4	46
Mi2	19.8	26
[a]insulin degludec	19.9	26
Mi3	1.44	2

[a] The Degludec peak component was characterised as insulin degludec

Pharmacokinetic parameters in plasma, AUC_{0-8h} and fractions of total AUC, obtained from rat after a single subcutaneous administration of 25 nmol/kg [^{3}H]-insulin degludec

Peaks	AUC_{0-8h} (nmol*h/l)	Fraction of total AUC (%)
P1	28.7	21
PL1	1.47	1
PL2	1.35	1
PL3	5.61	4
[a]insulin degludec	65.2	49
P2	11.2	8
P3	20.2	15

[a] The Degludec peak component was characterised as insulin degludec

Excretion

STUDY NO. 206530: SINGLE-DOSE SC RAT STUDY

[3H]-NNC 0100-0000-0454: a study of absorption and excretion following a single subcutaneous administration to the rat [by (b) (4)] GLP

Study design: This study was to characterize the routes of rates of excretion of total radioactivity from administered [3H]-insulin 454 in urine and feces and to determine the extent of biliary excretion of total radioactivity from administered [3H]-insulin 454 and its radiolabeled metabolites. Six male rats were divided into 2 groups: one group was dosed intake and the other group underwent surgery to cannulated the bile duct. All animals received a single sc administration of [3H]-insulin 454 at a nominal dose level of 25 nmol/kg. With intact animals, excreta were collected at 0-12 hr (urine only), 12-24 (feces only), 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hrs after dosing. With biliary cannulated animals, bile was collected at 0-2, 2-4, 4-6, 6-12, 12-24, 24-48, 48-72, 72-96 hrs postdose, with urine and feces being collected at daily intervals over the study period.

Findings: Total recovery of radioactivity was 87.7% in intact animals and 88.3% in biliary cannulated animals. In intact animals, radioactivity was principally present in urine (37.4%), feces (22.3%), and the carcass (22.6%). In biliary cannulated animals,

highest levels of radioactivity were contained in the carcass (25.2%), urine (24.6%), and bile (18.1%), with only 8.9% present in feces. In addition, elimination of radioactivity occurred mostly in the 1st 48 hrs in all animals.

Excretion of radioactivity from male rats following subcutaneous administration of [^{3}H]-NNC 0100-0000-0454 at a nominal dose level of 25 nmol/kg body weight (group A)

		Percent of administered radioactivity				
	Animal number and sex	307M	308M	309M	Mean	SD
Sample	Collection interval					
Urine	0-12 h	9.383	3.816	9.268	7.489	3.182
	12-24 h	10.63	10.49	10.55	10.56	0.070
	24-48 h	7.674	12.23	7.471	9.125	2.691
	48-72 h	3.213	4.808	2.922	3.648	1.015
	72-96 h	2.112	2.464	1.674	2.083	0.396
	96-120 h	2.005	1.862	1.408	1.758	0.312
	120-144 h	1.708	1.601	1.266	1.525	0.231
	144-168 h	1.393	1.251	1.106	1.250	0.143
	Sub-total	38.12	38.53	35.67	37.44	1.547
Faeces	0-24 h	8.259	2.506	19.56	10.11	8.674
	24-48 h	2.580	5.953	5.513	4.682	1.834
	48-72 h	2.392	2.074	2.062	2.176	0.187
	72-96 h	1.658	1.685	1.665	1.669	0.014
	96-120 h	1.198	1.454	1.057	1.236	0.201
	120-144 h	1.460	1.371	1.217	1.349	0.123
	144-168 h	1.260	1.164	0.832	1.085	0.225
	Sub-total	18.81	16.21	31.90	22.30	8.413
Cage Wash	0-24 h	3.373	4.982	1.431	3.262	1.778
	24-48 h	0.537	1.567	0.412	0.839	0.634
	48-72 h	0.460	0.373	0.245	0.359	0.108
	72-96 h	0.132	0.165	0.242	0.18	0.056
	96-120 h	0.233	0.294	0.207	0.245	0.045
	120-144 h	0.130	0.304	0.219	0.217	0.087
	144-168 h	0.124	0.098	0.044	0.089	0.041
	Sub-total	4.988	7.783	2.800	5.190	2.498
Cage Debris	0-168 h	0.290	0.225	0.134	0.217	0.079
Final Cage Wash	168 h	0.070	0.046	0.021	0.046	0.024
Carcass	168 h	23.47	26.12	18.20	22.59	4.034
Total		**85.74**	**88.91**	**88.72**	**87.79**	**1.777**

Excretion of radioactivity from male rats following subcutaneous administration of [^{3}H]-NNC 0100-0000-0454 at a nominal dose level of 25 nmol/kg body weight (group A – freeze-dried samples)

		Percent of administered radioactivity				
	Animal number and sex	307M	308M	309M	Mean	SD
Sample	Collection interval					
Urine	0-12 h	8.977	3.651	8.738	7.122	3.008
	12-24 h	9.497	9.805	10.40	9.900	0.458
	24-48 h	5.603	10.92	4.890	7.138	3.295
	48-72 h	1.189	2.228	1.194	1.537	0.599
	72-96 h	0.459	0.888	0.361	0.569	0.280
	96-120 h	0.287	0.396	0.164	0.283	0.116
	120-144 h	0.177	0.197	0.110	0.161	0.045
	144-168 h	0.091	0.092	0.053	0.079	0.022
	Sub-total	26.28	28.18	25.91	26.79	1.217
Faeces	0-24 h	8.134	2.280	19.43	9.946	8.715
	24-48 h	2.250	3.757	4.421	3.476	1.113
	48-72 h	0.994	0.85	0.803	0.882	0.100
	72-96 h	0.344	0.454	0.362	0.387	0.059
	96-120 h	0.182	0.268	0.162	0.204	0.056
	120-144 h	0.232	0.201	0.168	0.200	0.032
	144-168 h	0.138	0.133	0.097	0.123	0.023
	Sub-total	12.27	7.943	25.44	15.22	9.112
Cage Wash	0-24 h	3.022	3.165	1.115	2.434	1.145
	24-48 h	0.391	1.387	0.373	0.717	0.581
	48-72 h	0.209	0.306	0.191	0.236	0.062
	72-96 h	0.080	0.148	0.069	0.099	0.043
	96-120 h	0.128	0.154	0.049	0.110	0.055
	120-144 h	0.047	0.127	0.118	0.097	0.044
	144-168 h	0.065	0.057	0.016	0.046	0.026
	Sub-total	3.942	5.345	1.931	3.740	1.716
Cage Debris	0-168 h	0.047	0.038	0.029	0.038	0.009
Final Cage Wash	168 h	0.070	0.039	0.031	0.046	0.021
Carcass	168 h	5.384	6.433	5.407	5.741	0.599
Total		**48.00**	**47.98**	**58.74**	**51.57**	**6.211**

Excretion of radioactivity from male biliary cannulated rats following subcutaneous administration of [^{3}H]-NNC 0100-0000-0454 at a nominal dose level of 25 nmol/kg body weight (group B)

		Percent of administered radioactivity				
Sample	Animal number and sex Collection interval	469M	470M	471M	Mean	SD
Bile	Pre-dose	ND	ND	ND	ND	NA
	0-2 h	0.086	0.090	0.268	0.148	0.104
	2-4 h	1.511	0.933	2.597	1.680	0.845
	4-6 h	0.937	1.185	2.661	1.595	0.932
	6-12 h	3.223	3.936	5.884	4.348	1.378
	12-24 h	4.306	3.157	3.846	3.770	0.578
	24-48 h	1.624	3.611	3.912	3.049	1.243
	48-72 h	0.855	2.257	2.135	1.749	0.777
	72-96 h	2.288	1.505	1.538	1.777	0.443
	Sub-total	14.83	16.67	22.84	18.12	4.196
Urine	0-24 h	16.62	14.02	11.83	14.15	2.399
	24-48 h	7.235	7.173	4.757	6.389	1.413
	48-72 h	2.921	2.517	2.963	2.800	0.246
	72-96 h	1.517	0.983	1.202	1.234	0.269
	Sub-total	28.29	24.69	20.75	24.58	3.773
Faeces	0-24 h	3.710	2.275	3.627	3.204	0.806
	24-48 h	2.590	2.520	2.869	2.660	0.185
	48-72 h	2.367	1.923	1.532	1.941	0.418
	72-96 h	1.202	1.215	0.928	1.115	0.162
	Sub-total	9.869	7.934	8.956	8.920	0.968
Cage Wash	0-24 h	9.354	5.357	5.370	6.694	2.304
	24-48 h	3.404	4.323	4.215	3.981	0.502
	48-72 h	0.389	0.402	0.307	0.366	0.051
	72-96 h	0.269	0.164	0.236	0.223	0.054
	Sub-total	13.42	10.25	10.13	11.26	1.865
Cage Debris	0-96 h	0.167	0.158	0.183	0.170	0.013
Final Cage Wash	96 h	0.048	0.113	0.125	0.095	0.041
Carcass	96 h	25.43	24.54	25.51	25.16	0.540
Total		**92.06**	**84.36**	**88.49**	**88.30**	**3.854**

NA – Not Applicable
ND – Not Detected

Excretion of radioactivity from male biliary cannulated rats following subcutaneous administration of [^{3}H]-NNC 0100-0000-0454 at a nominal dose level of 25 nmol/kg body weight (group B – freeze-dried samples)

Sample	Animal number and sex / Collection interval	Percent of administered radioactivity 469M	470M	471M	Mean	SD
Bile	Pre dose	ND	ND	ND	ND	NA
	0-2 h	0.075	0.081	0.239	0.132	0.093
	2-4 h	1.097	0.773	2.534	1.468	0.937
	4-6 h	0.680	0.983	2.556	1.406	1.007
	6-12 h	2.439	3.478	4.476	3.465	1.019
	12-24 h	1.751	1.794	2.113	1.886	0.198
	24-48 h	0.511	0.806	1.663	0.993	0.598
	48-72 h	0.035	0.275	0.282	0.198	0.141
	72-96 h	0.058	0.078	0.082	0.073	0.013
	Sub-total	6.647	8.268	13.94	9.620	3.831
Urine	0-24 h	9.732	9.334	12.07	10.38	1.476
	24-48 h	4.149	4.768	3.521	4.146	0.623
	48-72 h	1.154	0.923	1.088	1.055	0.119
	72-96 h	0.433	0.281	0.340	0.351	0.076
	Sub-total	15.47	15.31	17.01	15.93	0.943
Faeces	0-24 h	1.761	0.930	2.051	1.581	0.582
	24-48 h	0.903	0.876	1.074	0.951	0.107
	48-72 h	0.650	0.499	0.569	0.573	0.076
	72-96 h	0.374	0.193	0.336	0.301	0.096
	Sub-total	3.688	2.498	4.030	3.405	0.804
Cage Wash	0-24 h	7.617	4.341	3.250	5.069	2.273
	24-48 h	2.625	3.588	2.908	3.040	0.495
	48-72 h	0.256	0.283	0.200	0.246	0.042
	72-96 h	0.151	0.115	0.160	0.142	0.024
	Sub-total	10.65	8.326	6.519	8.498	2.070
Cage Debris	0-96 h	0.114	0.127	0.115	0.119	0.007
Final Cage Wash	96 h	0.062	0.096	0.097	0.085	0.020
Carcass	96 h	6.585	4.828	4.565	5.326	1.098
Total		43.21	39.45	46.28	42.98	3.424

NA – Not Applicable
ND – Not Detected

STUDY NO. 210229: SINGLE-DOSE SC LACTATING RAT STUDY

[3H]-insulin degludec: a study of lacteal secretion in the rat following subcutaneous administration [by (b) (4)] GLP

Study design: This study was to determine the levels of radioactivity in plasma and milk in the albino lactating rat following a single sc administration of [3H]-insulin degludec. Rats received a single sc dose of [3H]-insulin 454 at a nominal dose level of 25 nmol/kg. Animals were dosed on approx. Day 9. Milk samples were collected from groups of 3 or 4 rats at 1, 3, 5, and 8 hrs after dosing. Right after milk collection, the dams were sacrificed and blood was collected by cardiac puncture.

Findings: The highest concentrations of radioactivity in plasma were reached at 3 hrs and declined thereafter. In milk, levels increased over time and the highest radioactivity

concentrations were achieved at 8 hrs. The highest milk: plasma ratios of radioactivity concentration occurred at 8 hrs postdose.

Concentrations of radioactivity in milk and plasma from albino rats (on about day 9 *post-partum*) following a single subcutaneous administration of [^{3}H]-insulin degludec at a dose level of 25 nmol/kg body weight - Groups A and B

Animal number and sex	Sampling time	Radioactivity concentration (nmol equiv/L)					
		Plasma		Milk		Milk : plasma ratio	
		Individual	Mean (SD)	Individual	Mean (SD)	Individual	Mean (SD)
101F	1 hour	26.8	18.5 (7.18)	3.92	1.62 (2.00)	0.146	0.070 (0.066)
102F		14.6		0.361		0.025	
103F		14.1		0.566		0.040	
104F	3 hours	33.9	37.5 (8.36)	7.73	9.12 (2.96)	0.228	0.239 (0.023)
105F		47.1		12.5		0.266	
106F		31.6		7.10		0.225	
107F	5 hours	28.8	31.7 (4.00)	22.4	15.5 (6.05)	0.780	0.523 (0.229)
108F		36.5		NS		NC	
109F		28.1		12.5		0.444	
113F		33.6		11.5		0.343	
110F	8 hours	31.3	24.6 (5.05)	15.6	17.1 (3.22)	0.498	0.669 (0.211)
111F		24.7		14.9		0.603	
112F		23.0		20.8		0.905	
114F		19.3		NS		NC	
201F	Control	ND	ND (NA)	ND	ND (NA)	NC	NC (NA)
202F		ND		ND		NC	
203F		ND		ND		NC	

NA – Not Applicable NC – Not Calculable
ND – Not Detected NS – No Sample

5.2 Toxicokinetics

TK analysis was included in several repeat-dose toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Study No. 205246: single-dose SC toxicity study in rats

Single dose toxicity study in the rat with subcutaneous administration of NNC 0100-0000-0454 [by (b) (4)]

Study design: This GLP study was to assess the acute toxicity of NNC 0100-0000-0454 (batch 412_N05230) in rats when administered as a single sc dose (*sighting study*: 3000, 9000, 18000, or 24000 nmol/kg and *main study*: 24000 nmol/kg in the volume of 10 mg/kg) followed by a 14-day observation period. Slighting study was done in 1 M and 1 F. Treatment was sequential, allowing 7 days before treatment of the next

animals. Main study was done in 5 M and 5 F. Clinical signs (at 15 min, 1, 3, 5, and 8 hrs on Day 1 and once daily for 14 days) and body weight (Days 1, 2, 3, 8, and 15) were recorded. Macroscopic examination was done in these animals.

Findings: A single sc injection up to 24000 nmol/kg of NNC 0100-0000-0454 in rats followed by a 14-day observation period showed that there was no test article-related toxicity.

6.2 Repeat-Dose Toxicity

Study No. 204316: 2 wk SC DFR study in rats

2-week dose range finding study by subcutaneous administration and single dose pharmacokinetic study by intravenous administration in Wistar rats [by Novo Nordisk A/S, Denmark]

Study design: This non-GLP study was to assess the PK parameters of PASIA 0100-0000-0454 (batch AF-IT-K2-001) in rats based on a 2-week DRF sc study (at 0, 37.5, 75, 150, and 300 nmol/kg) and a single dose iv study (at 7.5 nmol/kg). Endpoints included clinical signs, body weight, blood glucose, organ weight, macroscopic/microscopic examination, and TK.

Group	Compound	Route of administration	Dosing period	Dose level (nmol/kg/day)	Conc. (nmol/ml)	Animal numbers		Colour code
						Males	Females	
1	Vehicle	SC	Day 0 - Day 13/14	0	0	1-8	101-108	White
2	NNC 0100-0000-0454			37.5	37.5	9-16	109-116	Blue
3				75	75	17-24	117-124	Green
4				150	150	25-32	125-132	Red
5				300	300	33-40	133-140	Yellow
6	NNC 0100-0000-0454	IV	Day 9	7.5	7.5	41-55	141-155	Purple

SC: subcutaneous, IV: intravenous

Findings:

- NNC-0100-0000-0454 given by sc injection to rats for 14 days up to 300 mg/kg/day was well tolerated. No mortalities and clinical signs were observed. Mild local reactions were seen at the injection sites but there was no difference between control and treated animals.
- Blood glucose lower effect was observed approx. 1 hr after dosing and up to 8 hrs after dosing in animals treated at > 37.5 nmol/kg/day (Day 0) and in animals treated at > 75 nmol/kg/day (Day 13).
- The relative liver weight of animals treated at 150 and 300 nmol/kg/day were lower compared to the control. Decreased rarefaction (glycogen vacuolization) was seen at the 300 mg/kg/day group.
- The hemorrhage and cell minimal infiltration in the skin were caused by the injection and there was no difference between the control and treated groups.

- TK analysis (sc): systemic exposure of rats to NNC-0100-0000-0454 was confirmed in all treated groups. Exposure increased proportionally with dose.

Study No. 204317: 2 wk SC MTD study in dogs

Maximum tolerated dose study by subcutaneous administration and pharmacokinetic study by intravenous administration to Beagle dogs [by Novo Nordisk A/S, Denmark]

Study design: This non-GLP study was to investigate the tolerance and toxicity of NNC 0100-0000-0454 (batch AF-IT-K2-001) in dogs from a MTD sc study (dose-escalation phase - 1.5-30 nmol/kg single dose for 4 consecutive days followed by a 3-day treatment-free period and fixed-dose phase – 24/18/12 nmol/kg daily repeat dose for 14 days) and a single dose iv study (at 1.5 nmol/kg). Endpoints included clinical signs, body weight, food consumption, haematology and clinical chemistry, urinalysis, antibody analysis, organ weight, macroscopic/microscopic examination, and TK analysis.

MTD Phase I study (subcutaneous administration)

Animal no.	Week	Day	Dose, actual (nmol/kg/day)	Concentration, actual (nmol/ml)	Dose Volume ml/kg
1 and 2	Week 1	0-3	1.5	150	0.01
	Week 2	7-10	3.0	300	0.01
	Week 3	14-17	6.0	600	0.01
	Week 4	21-22	12.0	1200	0.01
		23-24	18.0	1800	0.01
	Week 5	28[a]-29	30.0*	2397*	0.0125
		30-31[b]	28.2*	2707*	0.0104

* Due to formulation failure, the actual concentration was not identical to the labelled concentration. Therefore, the actual dose of 30.0 and 28.2 nmol/kg varied from the planned dose of 24 and 36 nmol/kg.

[a] Animal no. 1 was not dosed on day 28 (bioanalysis showed no insulin 454 in the plasma on this day)

[b] Animal no. 2 was not dosed on day 31 (due to hypoglycaemic attack on day 30)

MTD Phase II study (subcutaneous administration)

Animal no.	Week	Day	Dose (nmol/kg/day)	Concentration (nmol/ml)	Dose Volume ml/kg
1,2, 3 and 4	Week 1	0*-2	24	2400	0.01
	Week 2	6[#]-9	18	2400	0.0075
	Week 3-4	14-27	12	1200	0.01

*: Animal no. 1 was not dosed on day 0 (bioanalysis showed no Insulin 454 in the plasma on this day)

[#] Animal no. 3 was not dosed on day 6 (bioanalysis showed no Insulin 454 in the plasma on this day)

The pharmacokinetic study (intravenous administration)

Animal no.	Dose (nmol/kg)	Concentration (nmol/ml)	Dose Volume ml/kg
1,2,3 & 4	1.5	15 nmol/ml	0.1

Findings:

- Single doses up to 30 nmol/kg were well tolerated. One dog showed severe signs of hypoglycaemia after 3 days doing with 30 nmol/kg.
- Repeated dose > 18 nmol/kg produced marked hypoglycemia and/or clinical signs of hypoglycaemia. Hypoglycemia was observed after 2-3 days dosing of first 24 and than 18 nmol/kg and dosing was therefore discontinued. The dosing was resumed at 12 nmol/kg. The 12 nmol/kg for 2 weeks were well tolerated in all 4 dogs. No test article-related toxicity was observed (except minimal inflammatory reaction at injection site was observed in 2 out of 4 dogs).
- There were no antibodies against NNC 0100-0000-0454.
- TK analysis: the exposure of the dogs to NNC 0100-0000-0454 following sc administration was proportional to the dose. The plasma half life was 4-6 hrs. No accumulation in the dogs was seen during dosing with 12 and 18 nmol/kg/day and bioavailability following SD ranged from 73 to 104%.

Study No. 205238: 4 wk SC toxicity study in dogs

A 4-week toxicity study in dogs with daily subcutaneous administration of NNC 0100-0000-0454 [by (b) (4)]

Study design: This GLP study was to assess the toxicity of NNC 0100-0000-0454 (batch 412-NO5169) in dogs administered daily by sc injection for 4 weeks at 0, 4, 8, or 12 nmol/kg. Endpoints included clinical signs, body weight, food consumption, ophthalmoscopy, ECG, hematology and coagulation, clinical chemistry, urinalysis, organ weight, antibody analysis, macroscopic/microscopic examination, and TK analysis.

Group	Dose* Concentration nmol/ml	Dose level (nmol/kg)	Animal Nos (Main study)		Colour code
			Male	Female	
1	0	0	1-3	4-6	White
2	400	4	7-9	10-12	Blue
3	800	8	13-15	16-18	Green
4	1200	12	19-21	22-24	Red

*Material as supplied in vials

Findings:
- Subcutaneous dosing once daily for 28 days with NNC 0100-0000-0454 in dogs at the following doses of 0, 4, 8, and 12 nmol/kg, did not cause any toxicity.
- Plasma glucose levels were affected at 12 nmol/kg/day, which was the expected pharmacological effect of NNC 0100-0000-0454.
- Mild subcutaneous changes at the injection site showed no difference between the control and treated groups.
- The NOAEL was > 12 nmol/kg.
- TK analysis: the exposure of the dogs to NNC 0100-0000-0454 following sc administration was reasonably dose proportional with no clear gender differences.

Study No. 205239: 4 wk SC toxicity study in rats

A 4-week toxicity study in rats with daily subcutaneous administration of NNC 0100-0000-0454 [by (b) (4)]

Study design: This GLP study was to assess the toxicity of NNC 0100-0000-0454 (batch 412-NO5169) in rats administered daily by sc injection for 4 weeks at 0, 25, 150, or 250 nmol/kg. Endpoints included clinical signs, body weight, food consumption, ophthalmoscopy, hematology and coagulation, clinical chemistry, urinalysis, organ weight, antibody analysis, macroscopic/microscopic examination, and TK/glucose analysis.

Group	Dose nmol/kg	Dose* concentration (nmol/ml)	Animal Nos (Main study)		Animal Nos (Kinetic)		Colour code
			Males	Females	Males	Females	
1	0	0	1-10	11-20	81 – 89	90 – 98	White
2	25	25	21-30	31-40	99 -107	108 – 116	Blue
3	150	150	41-50	51-60	117 – 125	126 – 134	Green
4	250	250	61-70	71-80	135 - 143	144 – 152	Red

Findings:

- Subcutaneous dosing once daily for 28 days with NNC 0100-0000-0454 in rats at the following doses of 0, 25, 150, or 250 nmol/kg/day, resulted in test article related clinical signs of hypoglycaemia and death at 150 nmol/kg/day (1F) and 250 nmo/kg/day (1 M and 3 F).
- A test article related effects were also seen on body weights and food consumption.
- Changes in clinical chemistry, organ weight (decreased relative liver weight), microscopic findings (lower degree of hepatic rarefaction) were observed at > 150 mg/kg/day. These findings were attributed to the expected pharmacological effect of NNC 0100-0000-0454.
- Mild subcutaneous changes at the injection site showed no difference between the control and treated groups.
- Few treated animals (7 out of 54, distributed evenly among treated groups) found positive for anti- NNC 0100-0000-0454 antibodies.
- The plasma glucose levels were affected in a dose-dependent manner in both genders.
- The NOAEL was 25 nmol/kg/day due to 1 female found dead at 150 nmol/kg/day *(note: The sponsor's NOAEL = 250 nmol/kg/day).*
- TK analysis: The exposure of the rats to NNC 0100-0000-0454 following sc administration was reasonably dose proportional although AUC increased slightly more than proportional and there was no real accumulation. Female seemed to have a higher exposure than males.

Study No. 206072: 2 wk SC pilot study in rabbits

Pilot study in the rabbit by subcutaneous administration [by (b) (4)]

Study design: This non-GLP study was to assess the systemic toxicity and TK of NNC 0100-0000-0454 (batch 412-NO6048) in New Zealand White female rabbits. Animals in Groups 1-4 were treated at dosages of 25, 75, 50 or 30 nmol/kg/day, respectively, via sc administration once daily for up to 14 days. Endpoints included clinical signs, body weight, food consumption, macroscopic exanimation, and TK/glucose analysis. The study results were intended to be used to aid selection of dosages for a preliminary Seg II toxicity study in this species.

Findings:

- This study concluded that 25 nmol/kg/day was well tolerated by female rabbits.
- The doses > 25 nmol/kg was not tolerated and resulted in hypoglycemia-related clinical signs.
- TK analysis: exposure of the female rabbits to NNC 0100-0000-0454 was seen in all treated groups. Exposure increased proportionally with dose. Plasma half lives were between 7-17.5 hrs.

Study No. 206314: 6 mo (4 wk recovery) SC toxicity study in dogs [PIVOTAL]

A 6-month study with a 4-week recovery period in dogs with daily subcutaneous administration of insulin 454

Study no.:	206314
Study report location:	Novo Nordisk, Denmark
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 15 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Insulin 454, batch number 412-NO6140 (purity 96.9%); NPH insulin comparator, batch number RW 52236

KEY STUDY FINDINGS

- This study showed that sc dosing > 8 nmol/kg/day for 26 weeks resulted in test article related clinical signs and mortalities attributed to hypoglycaemia.
- Increased body weight and decreased food consumption remains were observed.
- Serum glucose levels were affected in a dose dependent manner in both genders.
- None of the test-article treated animals developed antibodies.
- Several main study animals had red discoloration at the injection sites attributed to dosing procedure.
- Minimal to moderate diffuse decreased rarefaction was seen in the liver in the 12/10/8 group, most likely attributed to hepatic glycogen depletion (recoverable).
- The NOAEL was established at 8 nmol/kg/day by the sponsor *(note: due to clinical sign/mortality seen at 8 nmol/kg, the NOAEL should be 4 nmol/kg/day)*.
- TK analysis: exposure was reasonably dose proportional with no gender differences.

Methods

Doses: 0, 4, 8, 12/10/8 nmol/kg/day insulin 454 (vs. 8 nmol NPH nmol/kg/day)
Frequency of dosing: Daily
Route of administration: Sc injection
Dose volume: 0.01 ml/kg (vs. 0.05 mg/kg for insulin NPH)
Formulation/Vehicle:
- 1200 nmol/ml Insulin 454 in isotonic solution of glycerol (b) (4) mg/mL, (b) (4) mg/ml, phenol 1.5 mg/mL, m-Cresol 1.72 mg/mL, with pH ~ 7.4
- 240 nmol/ml NPH in isotonic solution of: glycerol (b) (4) mg/mL, phenol (b) (4) mg/mL, m-Cresol (b) (4) mg/mL (b) (4) (b) (4) mg/mL (b) (4)) with pH ~7.3

Species/Strain: Beagle dogs
Number/Sex/Group: N=4/sex/group
Age: 7-8 months
Weight: 17.1-11.3 g (males); 6.6-10.4 g (females)
Satellite groups: Recovery (n=2)
Unique study design: n/a
Deviation from study protocol: Yes, but these did not affect the outcome of the study.

Group	Test item	Dose*	Dose concentration*	Animal Nos		Recovery animal Nos		Colour code
		(nmol/kg)	(nmol/ml)	Male	Female	Male	Female	
1	Vehicle	Control	0	101-104	105-108			White with a black frame
2	Insulin 454	4	400	109-112	113-116			Blue with a black frame
3	Insulin 454	8	800	117-120	121-124			Green with a black frame
4	Insulin 454	12/10**/ 8***	1200/1000**/ 800***	125-127	129-131	128,139-140	132,141-142	Red with a black frame
5	Insulin NPH	8	160	133-135	136-138			Yellow with a black frame

*Material as supplied
** From Day 48, Group 4 animals were treated with 10 nmol/kg. On Days 48 + 49, Group 4 animals were dosed with a formulation of 1200 nmol/ml at a dose volume of 0.0083 ml/kg to reach a dose of 10 nmol/kg.

***From Day 108, Group 4 animals were treated with 8 nmol/kg. On Days 108 to 112 Group 4 animals were dosed with a formulation of 1000 nmol/ml at a dose volume of 0.008 ml/kg to reach a dose of 8 nmol/kg

Observations and Results

- **Mortality/ Clinical Signs:** daily

There were treatment-related clinical signs of hypoglycaemia (Groups 3, 4, and 5). One Group 3 animal and 2 Group 4 animals were killed and 1 Group 4 was found dead due to severe clinical signs.

- **Body Weights/Food Consumption:** weekly/daily

There were non-statistically significant increases in body weight gain and food consumption observed in treated groups.

	Group 1	Group 2	Group 3	Group 4	Group 5
Body weight gain (kg), males	2.4	↑ 45.8	↑ 58.3	↑ 50.0	↑ 45.8
Body weight gain (kg), females	2.7	↓ -3.7	0.0	↑ 18.5	↑ 29.6

- **Ophthalmoscopy:** Weeks 13 and 26 and at the end of the recovery period

No treatment-related findings were seen.

- **ECG:** Weeks 13 and 26 and at the end of the recovery period

No treatment-related findings were seen.

- **Hematology:** Weeks 13 and 26, at the end of the recovery period

Parameter	Method/Equipment	Unit
Haemoglobin (Hb)	Direct measurement/ABX Pentra DX 120	mmol/l
Red blood cell count (RBC)	Direct measurement/ABX Pentra DX 120	10^{12}/l
Haematocrit (HT)	Direct measurement/ABX Pentra DX 120	ml/100 ml
Mean cell volume (MCV)	Calculated/ABX Pentra DX 120	fl
Mean cell haemoglobin (MCH)	Calculated/ABX Pentra DX 120	fmol
Mean cell haemoglobin concentration (MCHC)	Calculated/ABX Pentra DX 120	mmol/l
White blood cell count (WBC)	Direct measurement/ABX Pentra DX 120	10^{9}/l
Differential leucocyte count (NEUTRO, LYMPHO, EOS, BASO, MONO)	Direct measurement/ABX Pentra DX 120	% and 10^{9}/l
Platelet count (Plt)	Direct measurement/ABX Pentra DX 120	10^{9}/l
Activated partial thromboplastin time (APTT)	IL Test™/ACL™ (*)	sec.
Prothrombin time (Pt)	IL Test™/ACL™(*)	sec.
Fibrinogen (Fib)	IL Test™/ACL™(*)	g/l

(* (b) (4))

In Week 13, the Groups 2 and 3 males had a statistically significantly lower relative amount of monocytes and Group 2 males had a statistically significantly lower absolute amount of monocytes. The Group 4 females also had a statistically significantly lower mean cell hemoglobin concentration and Group 5 females had a statistically significantly higher relative amount of monocytes compared to Group 1.

	2	3	4	5
Monocytes, relative, Week 13, males	↓ -47.4	↓ -52.6		
Monocytes, absolute, Week 13, males	↓ -42.5			
Mean cell haemoglobin, Week 13 females			↓ -1.4	
Monocytes, relative, Week 13, females				↑ 43.5

- **Clinical Chemistry:** Weeks 13 and 26, at the end of the recovery period

Parameter	Method	Unit
Alanine aminotransferase (ALAT)	Hitachi 917	µkat/l
Aspartate aminotransferase (ASAT)	Hitachi 917	µkat/l
Alkaline phosphatase (ALKPH)	Hitachi 917	µkat/l
Bilirubin (total) (BILI)	Hitachi 917	µmol/l
Gamma-glutamyl transferase (GGT)	Hitachi 917	µkat/l
Cholesterol (CHOL)	Hitachi 917	mmol/l
Triglycerides (TRIG)	Hitachi 917	mmol/l
Carbamide (UREA)	Hitachi 917	mmol/l
Creatinine (CREAT)	Hitachi 917	µmol/l
Glucose (GLUC)	Hitachi 917	mmol/l
Sodium (Na)	Ion selective electrode/Hitachi 917	mmol/l
Potassium (K)	Ion selective electrode/Hitachi 917	mmol/l
Calcium (Ca)	Hitachi 917	mmol/l
Magnesium (Mg)	Hitachi 917	mmol/l
Inorganic phosphorus (P)	Hitachi 917	mmol/l
Chloride (Cl)	Ion selective electrode/Hitachi 917	mmol/l
Protein (total) (PROTEIN)	Hitachi 917	g/l
Albumin (ALB)	Hitachi 917	g/l
Globulin	Calculated	g/l
Albumin/Globulin (ALB/G) ratio	Calculated	No unit

Decreased group mean blood glucose on clinical chemistry was seen. Serum glucose levels were affected in both genders with a dose-related manner. This was the expected pharmacological effect of insulin 454 and insulin NPH.

	2	3	4	5
Cholesterol, males, Week 13 nmol/l				↑ 38.6
Magnesium, Week 13, males nmol/l				↓ -7.1
Triglycerides, Week 13, males nmol/l	↓ -38.5			
Glucose, males, Week 26, nmol/l	↓ -12.4	↓ -27.0	↓ -27.6	

- **Urinalysis:** Weeks 13 and 26, at the end of the recovery period

Parameter	Method/Equipment	Unit/Range
Sodium (Na)	Ion selective electrode/ Cobas Mira S	mmol/l
Potassium (K)	Ion selective electrode/ Cobas Mira S	mmol/l
Chloride (Cl)	Ion selective electrode/ Cobas Mira S	mmol/l
Specific gravity (SG)	Ames Multistix 10SG/Clinitek 500	No unit
pH	Ames Multistix 10SG/Clinitek 500	No unit
Colour (COLOUR)	Visual examination	No unit
Protein (PROTEIN)	Ames Multistix 10SG/Clinitek 500	g/l
Leucocytes (LEUC)	Ames Multistix 10SG/Clinitek 500	Cells/µl
Nitrite (NITRITE)	Ames Multistix 10SG/Clinitek 500	No unit
Blood (BLOOD)	Ames Multistix 10SG/Clinitek 500	Erythrocytes/µl
Glucose (GLUCOSE)	Ames Multistix 10SG/Clinitek 500	mmol/l
Ketones (KETONES)	Ames Multistix 10SG/Clinitek 500	mmol/l
Bilirubin (BILI)	Ames Multistix 10SG/Clinitek 500	No unit
Urobilinogen (UROBIL)	Ames Multistix 10SG/Clinitek 500	µmol/l

No treatment-related findings were seen.

- **Gross Pathology:** necropsy

Red discoloration was seen at the sc injection site in few animals in all main study dose groups. Oedema and hemorrhages were seen in 1 MD (8 nmol/kg insulin 454) female. These changes were likely related to the dosing procedure. No finding was seen at injection sites of the recovery animals.

- **Organ Weights:** necropsy

There was a statistically significant higher absolute weight of thymus in Group 3 males. A statistically significantly higher weight of the adrenal was seen in Group 5 females compared to Group 1. No other findings were noted.

	Group 3 (mg/kg/day)	Group 5 (mg/kg/day)
Thymus (absolute value), males	↑ (90.1)	
Adrenals (absolute value), females		↑ 27.2

- **Histopathology:** necropsy
  Adequate Battery: Yes
  Peer Review: Yes

Organs and tissues	Weigh	Fix	Micro	Organs and tissues	Weigh	Fix	Micro
Abnormalities (gross lesions)		x	x	Ovaries	x	x	x
Adrenals	x	x	x	Pancreas		x	x
Aorta (thoracic)		x	x	Pituitary	x	x	x
Brain	x	x	x	Prostate	x	x	x
Bone marrow smear		x		Salivary gland (right parotid, sublingual and submandibular)		x	x
Bones (medial condyl of right femur)		x	x	Sciatic nerve		x	x
Epididymides		x	x	Skeletal muscle		x	x
Eyes with lens/optic nerve		x	x	Skin		x	x
Gall bladder		x	x	Spinal cord (thoracic, lumbar)		x	x
Heart with aortic arch	x	x	x	Spleen	x	x	x
Injection site		x	x	Sternum (for bone marrow)		x	x
Intestine small (duodenum, jejunum, ileum)		x	x	Stomach		x	x
Intestine large (caecum, colon, rectum)		x	x	Testes	x	x	x
Joint (knee)		x	x	Thymus	x	x	x
Kidneys	x	x	x	Thyroid (incl. parathyroids)	x	x	x
Larynx		x	x	Tongue		x	x
Liver (all main lobes)	x	x	x	Trachea		x	x
Lungs (cranial and caudal lobes, both sides)		x	x	Ureters		x	x
Lymph nodes (right mandibular and mesenteric)		x	x	Urinary bladder		x	x
Mammary gland		x	x	Uterus (horn, cervix and oviducts)	x	x	x
Oesophagus		x	x	Vagina		x	x

Minimal to marked sc hemorrhage, minimal to moderate sc inflammation and/or minimal focal subcutaneous necrosis were seen in few animals in all main study control and insulin 454 treated dose group. There changes were considered to be related to the dosing procedure. Similar changes were seen in the insulin NPH treated group.

Minimal to moderate decreased diffuse rarefaction was seen in the liver of 3 HD animals (12/10/8 nmol/kg insulin 454) and 1 NPH insulin animal (8 nmol/kg). This was likely treatment-related effect due to pharmacological action of insulin, suggesting hepatic glycogen depletion. No such changes were seen in the liver of the recovery animals.

Table 3 Histopath Summary for Pivotal 6-month Toxicity Study in Dogs.

HISTOPATH SUMMARY REPORT

Main study animals:

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData@System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4	3	3
BRAIN :	4	4	4	4	4	4	4	4	3	3
- Vacuolation, focal :	-	-	-	-	-	1	-	1	-	-
Grade 1:	-	-	-	-	-	1	-	1	-	-
- Mononucl cells focal:	1	-	-	-	-	2	1	1	-	-
Grade 1:	1	-	-	-	-	1	1	1	-	-
Grade 2:	-	-	-	-	-	1	-	-	-	-
HEART :	4	4	4	4	4	4	4	4	3	3
- Hemorrhage, focal :	-	-	-	-	-	-	1	-	-	-
Grade 2:	-	-	-	-	-	-	1	-	-	-
- Myocarditis, focal :	-	-	-	-	-	-	1	-	-	-
Grade 2:	-	-	-	-	-	-	1	-	-	-
- Necrosis, myocytes :	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-
AORTA :	4	4	4	4	4	4	4	4	3	3
- Inflam cells focal :	-	-	-	-	1	-	-	-	-	-
Grade 1:	-	-	-	-	1	-	-	-	-	-
- Mineralization focal:	-	-	-	-	1	-	-	-	-	-
Grade 1:	-	-	-	-	1	-	-	-	-	-
LARYNX :	4	4	4	4	4	4	4	4	3	3
- Mononucl cells focal:	1	-	-	-	2	-	-	-	-	-
Grade 1:	-	-	-	-	2	-	-	-	-	-
Grade 2:	1	-	-	-	-	-	-	-	-	-

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4	3	3
LUNG :	4	4	4	4	4	4	4	4	3	3
- Pneumonia :	3	2	1	3	1	1	1	2	2	1
Grade 1:	3	1	1	2	1	1	1	1	2	-
Grade 2:	-	1	-	1	-	-	-	1	-	1
- Subpleural fibrosis :	2	2	-	1	1	1	-	-	-	-
Grade 1:	1	1	-	1	1	1	-	-	-	-
Grade 2:	-	1	-	-	-	-	-	-	-	-
Grade 3:	1	-	-	-	-	-	-	-	-	-
- Thrombus, focal :	1	-	-	-	-	-	-	-	-	-
Grade 1:	1	-	-	-	-	-	-	-	-	-
- Alveolar macrophages:	-	-	1	-	-	-	2	-	-	-
Grade 1:	-	-	1	-	-	-	2	-	-	-
- Osseous metaplasia :	-	-	-	1	-	-	-	-	-	-
TONGUE :	4	4	4	4	4	4	4	4	3	3
- Ulceration, focal :	-	-	-	-	-	-	1	-	-	-
Grade 2:	-	-	-	-	-	-	1	-	-	-
- Inflammation, focal :	-	-	-	-	-	-	1	-	-	-
Grade 2:	-	-	-	-	-	-	1	-	-	-
- Mononucl cells focal:	1	-	1	1	1	-	-	-	1	-
Grade 1:	1	-	1	1	1	-	-	-	1	-
ESOPHAGUS :	4	4	4	4	4	4	4	4	3	3
- Mononucl cells focal:	-	-	-	-	-	-	-	-	1	-
Grade 1:	-	-	-	-	-	-	-	-	1	-
STOMACH GLANDULAR :	4	4	4	4	4	4	4	4	3	3
- Mononucl cells focal:	1	-	-	-	-	-	-	-	1	-
Grade 1:	1	-	-	-	-	-	-	-	1	-
DUODENUM :	4	4	4	4	4	4	4	3	3	3
- Cystic dilat crypts :	2	2	2	2	1	-	-	-	-	-
Grade 1:	-	2	1	2	1	-	-	-	-	-
Grade 2:	2	-	1	-	-	-	-	-	-	-

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData@System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4	3	3
LIVER :	4	4	4	4	4	4	4	4	3	3
- Decr rarefaction dif:	-	-	-	-	-	-	2	1	1	-
Grade 1:	-	-	-	-	-	-	-	1	-	-
Grade 2:	-	-	-	-	-	-	-	-	1	-
Grade 3:	-	-	-	-	-	-	2	-	-	-
- Increas rarefaction :	-	-	-	-	-	-	-	-	-	1
Grade 2:	-	-	-	-	-	-	-	-	-	1
- Mononucl/EMH focal :	1	3	1	1	3	1	2	1	2	1
Grade 1:	1	3	1	1	3	1	2	1	2	1
- Inflam cells focal :	1	-	-	1	1	-	1	1	1	-
Grade 1:	1	-	-	1	1	-	1	1	1	-
- Fibrosis, focal :	1	1	-	-	-	1	-	1	1	-
Grade 1:	1	-	-	-	-	1	-	1	1	-
Grade 2:	-	1	-	-	-	-	-	-	-	-
- Bile duct hyperplas.:	-	1	-	-	-	-	-	-	-	-
Grade 2:	-	1	-	-	-	-	-	-	-	-
- Single cell necrosis:	-	-	-	-	-	-	-	-	-	1
Grade 1:	-	-	-	-	-	-	-	-	-	1
GALLBLADDER :	4	4	4	4	4	4	4	3	3	3
- Peri-/arteritis, foc:	-	-	-	-	-	1	-	-	-	-
Grade 1:	-	-	-	-	-	1	-	-	-	-

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData®System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4	3	3
KIDNEYS :	4	4	4	4	4	4	4	4	3	3
- Tubular vacuolation :	-	-	-	-	-	-	-	-	-	1
Grade 3:	-	-	-	-	-	-	-	-	-	1
- Tubular basophilia :	1	-	-	-	-	1	2	-	-	-
Grade 1:	1	-	-	-	-	1	2	-	-	-
- Tubular dilatation :	-	-	-	-	2	-	1	1	1	1
Grade 1:	-	-	-	-	2	-	-	1	1	-
Grade 2:	-	-	-	-	-	-	-	-	-	1
Grade 3:	-	-	-	-	-	-	1	-	-	-
- Mononucl cells focal:	2	-	2	-	2	2	1	1	-	-
Grade 1:	1	-	2	-	2	2	1	1	-	-
Grade 2:	1	-	-	-	-	-	-	-	-	-
- Mineralization, foc.:	4	4	4	4	4	3	4	3	3	3
Grade 1:	4	4	4	4	2	3	4	3	3	3
Grade 2:	-	-	-	-	2	-	-	-	-	-
- Congestion, focal :	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-
TESTES :	4	-	4	-	4	-	4	-	3	-
- Vacuolated tub epit :	1	-	1	-	1	-	2	-	-	-
Grade 1:	-	-	-	-	1	-	2	-	-	-
Grade 2:	1	-	-	-	-	-	-	-	-	-
Grade 3:	-	-	1	-	-	-	-	-	-	-
- Tubular atrophy/deg :	-	-	1	-	-	-	-	-	-	-
Grade 1:	-	-	1	-	-	-	-	-	-	-
EPIDIDYMIDES :	4	-	4	-	4	-	4	-	3	-
- Vacuolated tub epit :	3	-	1	-	1	-	1	-	2	-
Grade 1:	3	-	1	-	1	-	1	-	2	-
PROSTATE GLAND :	4	-	4	-	4	-	4	-	3	-
- Mononucl cells focal:	1	-	-	-	-	-	-	-	-	-
Grade 1:	1	-	-	-	-	-	-	-	-	-

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4	3	3
PITUITARY GLAND :	4	4	4	4	4	4	4	3	3	3
- Cyst(s), focal :	-	1	-	-	1	-	1	-	-	-
Grade 1:	-	1	-	-	1	-	-	-	-	-
Grade 2:	-	-	-	-	-	-	1	-	-	-
THYROID GLAND :	4	4	4	4	4	4	4	4	3	3
- Thyroiditis, diffuse:	-	-	-	-	-	-	-	-	1	-
Grade 2:	-	-	-	-	-	-	-	-	1	-
- C-cell hyperplasia :	4	2	-	2	2	3	1	2	-	3
Grade 1:	3	2	-	2	1	1	1	2	-	3
Grade 2:	1	-	-	-	-	2	-	-	-	-
Grade 3:	-	-	-	-	1	-	-	-	-	-
- Mononucl cells focal:	1	-	-	1	-	-	-	-	-	-
Grade 1:	1	-	-	1	-	-	-	-	-	-
- Cyst, focal :	-	-	-	1	-	2	-	-	-	-
Grade 1:	-	-	-	1	-	1	-	-	-	-
Grade 2:	-	-	-	-	-	1	-	-	-	-
- Peri-/arteritis foc.:	-	-	-	-	-	-	-	-	-	1
Grade 3:	-	-	-	-	-	-	-	-	-	1
PARATHYROID GLANDS :	4	3	3	3	3	4	4	4	2	3
- Cyst(s), focal :	1	-	-	-	1	1	-	1	-	-
Grade 1:	1	-	-	-	1	1	-	1	-	-
ADRENAL GLANDS :	4	4	4	4	4	4	4	4	3	3
- Vacuolation, focal :	1	2	3	2	2	1	2	-	2	1
Grade 1:	1	2	3	1	2	-	2	-	1	1
Grade 2:	-	-	-	1	-	1	-	-	-	-
Grade 3:	-	-	-	-	-	-	-	-	1	-
- Mononucl cells focal:	-	-	-	1	-	-	-	-	1	-
Grade 1:	-	-	-	1	-	-	-	-	1	-
- Inflam cells focal :	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-
- Dystrophic mineraliz:	1	-	-	-	-	-	-	-	-	-
Grade 1:	1	-	-	-	-	-	-	-	-	-

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4	3	3
THYMUS :	4	4	4	4	4	4	3	3	3	2
- Cortical atrophy :	1	-	1	-	2	1	1	1	1	-
Grade 1:	-	-	1	-	2	-	1	-	-	-
Grade 2:	1	-	-	-	-	1	-	-	1	-
Grade 4:	-	-	-	-	-	-	-	1	-	-
- Incr lymphocytolysis:	-	-	-	-	-	-	1	-	-	-
Grade 3:	-	-	-	-	-	-	1	-	-	-
- Dystrophic mineraliz:	-	-	-	-	-	-	-	1	-	-
Grade 1:	-	-	-	-	-	-	-	1	-	-
- Hemorrhage, focal :	1	-	-	-	-	-	-	-	-	-
Grade 1:	1	-	-	-	-	-	-	-	-	-
- Ultimobrachial cysts:	1	-	1	-	-	-	-	-	-	-
MESENT. LYMPH NODE :	4	4	4	4	4	4	4	4	3	3
- Hemorr/erythrophago.:	-	1	2	-	-	-	1	1	2	1
Grade 1:	-	1	2	-	-	-	1	1	1	1
Grade 2:	-	-	-	-	-	-	-	-	1	-
MANDIBULAR LN RIGHT :	4	4	4	4	3	4	4	3	3	2
- Hemorrhage, focal :	-	-	-	-	-	-	1	-	-	-
Grade 2:	-	-	-	-	-	-	1	-	-	-
PAROTID GLAND, RIGHT :	4	4	4	4	3	4	4	4	3	3
- Mononucl cells focal:	1	1	1	1	-	-	-	1	-	-
Grade 1:	1	1	1	1	-	-	-	1	-	-
SUBLING.GLAND, RIGHT :	3	3	3	4	4	4	4	3	3	3
- Mononucl cells focal:	1	1	-	-	1	-	1	-	-	1
Grade 1:	1	1	-	-	1	-	1	-	-	1
SUBMANDIB.GLD. RIGHT :	4	4	4	4	4	4	4	3	3	3
- Mononucl cells focal:	1	1	-	-	1	1	-	-	-	-
Grade 1:	1	1	-	-	1	1	-	-	-	-

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData@System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4	3	3
MAMMARY GLAND :	4	4	4	4	4	4	4	4	3	3
- Increased secretion :	-	-	-	1	-	-	-	-	-	-
Grade 2:	-	-	-	1	-	-	-	-	-	-
- Mononucl cells focal:	-	-	1	-	-	-	-	-	-	-
Grade 1:	-	-	1	-	-	-	-	-	-	-
SKIN/SUBCUTIS :	4	4	4	4	4	4	4	4	3	3
- Peri-/vasculitis :	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-
INJECTION SITE :	4	4	4	4	4	4	4	4	3	3
- Crust, focal :	-	-	-	-	-	-	1	-	2	-
Grade 1:	-	-	-	-	-	-	1	-	2	-
- Inflam focal s.c. :	2	2	1	1	-	3	-	2	3	3
Grade 1:	1	2	1	1	-	3	-	-	1	3
Grade 2:	1	-	-	-	-	-	-	1	1	-
Grade 3:	-	-	-	-	-	-	-	1	1	-
- Incr fibrosis s.c. :	-	-	-	-	-	-	-	1	2	1
Grade 1:	-	-	-	-	-	-	-	-	1	-
Grade 2:	-	-	-	-	-	-	-	1	1	1
- Necrosis, focal sc :	-	1	1	-	-	1	-	-	1	1
Grade 1:	-	1	1	-	-	1	-	-	1	1
- Hemorrhage focal sc :	-	3	1	-	-	2	2	-	3	1
Grade 1:	-	2	1	-	-	-	1	-	2	-
Grade 2:	-	1	-	-	-	1	1	-	1	1
Grade 4:	-	-	-	-	-	1	-	-	-	-
- Oedema, focal s.c. :	-	-	-	-	-	1	-	-	-	-
Grade 2:	-	-	-	-	-	1	-	-	-	-

Recovery animals:

Note: Only HD insulin 454 was assessed.

TEST ARTICLE : INSULIN 454 PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM : DOG, 6-MONTH, SUBCUTANEOUS INJ. DATE : 08-MAR-07
SPONSOR : Novo Nordisk A/S PathData@System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: R1

DOSE GROUP:	1		2		3		4		5	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	-	-	-	-	-	-	2	2	-	-
LARYNX :	-	-	-	-	-	-	2	2	-	-
- Mononucl cells focal:	-	-	-	-	-	-	-	1	-	-
Grade 1:	-	-	-	-	-	-	-	1	-	-
LUNG :	-	-	-	-	-	-	2	2	-	-
- Pneumonia :	-	-	-	-	-	-	-	1	-	-
Grade 1:	-	-	-	-	-	-	-	1	-	-
- Alveolar macrophages:	-	-	-	-	-	-	-	1	-	-
Grade 1:	-	-	-	-	-	-	-	1	-	-
TONGUE :	-	-	-	-	-	-	2	2	-	-
- Mononucl cells focal:	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-
LIVER :	-	-	-	-	-	-	2	2	-	-
- Mononucl/EMH focal :	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-
- Vacuolation, diffuse:	-	-	-	-	-	-	-	1	-	-
Grade 3:	-	-	-	-	-	-	-	1	-	-
KIDNEYS :	-	-	-	-	-	-	2	2	-	-
- Tubular basophilia :	-	-	-	-	-	-	-	1	-	-
Grade 1:	-	-	-	-	-	-	-	1	-	-
- Mononucl cells focal:	-	-	-	-	-	-	1	1	-	-
Grade 1:	-	-	-	-	-	-	-	1	-	-
Grade 2:	-	-	-	-	-	-	1	-	-	-
- Mineralization, foc.:	-	-	-	-	-	-	2	2	-	-
Grade 1:	-	-	-	-	-	-	2	2	-	-
TESTES :	-	-	-	-	-	-	2	-	-	-
- Vacuolated tub epit :	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-
- Tubular atrophy/deg :	-	-	-	-	-	-	1	-	-	-
Grade 1:	-	-	-	-	-	-	1	-	-	-

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------------------------------------------------------------------------------
TEST ARTICLE  : INSULIN 454                        PATHOL. NO.: 62800 (b) (6)
TEST SYSTEM   : DOG, 6-MONTH, SUBCUTANEOUS INJ.    DATE       : 08-MAR-07
SPONSOR       : Novo Nordisk A/S                   PathData@System V6.2a2
------------------------------------------------------------------------------
NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: R1
------------------------------------------------------------------------------
            DOSE GROUP:       1         2         3         4         5
            SEX       :    M    F    M    F    M    F    M    F    M    F
            NO.ANIMALS:    -    -    -    -    -    -    2    2    -    -
------------------------------------------------------------------------------
EPIDIDYMIDES          :    -    -    -    -    -    -    2    -    -    -
- Vacuolated tub epit :    -    -    -    -    -    -    2    -    -    -
               Grade 1:    -    -    -    -    -    -    2    -    -    -
------------------------------------------------------------------------------
PITUITARY GLAND       :    -    -    -    -    -    -    2    2    -    -
- Cyst(s), focal      :    -    -    -    -    -    -    1    -    -    -
               Grade 1:    -    -    -    -    -    -    1    -    -    -
------------------------------------------------------------------------------
THYROID GLAND         :    -    -    -    -    -    -    2    2    -    -
- C-cell hyperplasia  :    -    -    -    -    -    -    1    1    -    -
               Grade 2:    -    -    -    -    -    -    1    1    -    -
- Cyst, focal         :    -    -    -    -    -    -    -    1    -    -
               Grade 1:    -    -    -    -    -    -    -    1    -    -
------------------------------------------------------------------------------
ADRENAL GLANDS        :    -    -    -    -    -    -    2    2    -    -
- Vacuolation, focal  :    -    -    -    -    -    -    1    1    -    -
               Grade 1:    -    -    -    -    -    -    1    1    -    -
------------------------------------------------------------------------------
INJECTION SITE        :    -    -    -    -    -    -    2    2    -    -
- Needle canal, focal :    -    -    -    -    -    -    -    1    -    -
- Inflam focal s.c.   :    -    -    -    -    -    -    1    -    -    -
               Grade 1:    -    -    -    -    -    -    1    -    -    -
------------------------------------------------------------------------------
```

- **Special Evaluation**

1. Antibody formation: pretreatment and 24 hrs after last treatment in the main and recovery animals.

All 6 NPH insulin-treated dogs developed antibodies where as none of the insulin 454-treated dogs developed antibodies. The antibody measurement in samples taken from the main study animals 24 hr after last dose was interfered by the presence of insulin 454. The results from the 4 recovery animals were considered valid.

- **Toxicokinetics/Glucose Monitoring:** Day 1, during Weeks 13 and 26 at pre-treatment, 1, 3, 6, 9, and 24 hrs post-treatment. Glucose analysis: Weeks 2, 4, 6, and 16 at pre-treatment, 1, 3, 6, 9, and 24 hrs post-treatment

It was demonstrated that the dogs were treated with insulin 454. No gender differences in TK parameters were seen based on Cmax and AUC values. Linearity for insulin 343 was generally seen up to 10 nmol/kg. T ½ was not uniquely determined, however, the t ½ calculated were approx. ranging 7.53-8.09 hr. Serum concentrations were below LLOQ in most cases in the recovery animals. The mean actual accumulation ratio was approx. 1.45, 1.69, 2.29 in Groups 2, 3, and 4. Lastly, the TK analysis showed that exposure was reasonably dose proportional with no clear gender differences.

Summary of average toxicokinetic parameters of subcutaneous Insulin 454 to dogs

Group	Week	Gender	Dose (nmol/kg)	Rsq	C_{max} (pmol/L)	T_{max} (h)	$T_{½}$ (h)	AUC_{0-inf} (h*pmol/L)	AUC_{0-inf} dose normalized to 8 nmol/kg	$AUC_{\%Extrap}$ (%)	$AUC_{0-24\ h}$ (h*pmol/L)	$AUC_{0-24\ h}$ dose normalized to 8 nmol/kg	Cl/F (L/h/kg)	Vz/F (L/kg)	$R_{A(AUC\ 0-24\ h)}$	R_{acc}
2	1	female	4	0.99	6,670	6.75	-	80,568	161,135	4.1	77,417	154,834	0.050	0.329	-	-
2	1	male	4	0.99	6,829	5.25	4.42	73,906	147,812	3.5	71,344	142,688	0.055	0.345	-	-
2	13	female	4	0.99	9,799	6.00	-	108,641	217,282	6.8	101,478	202,958	0.040	0.317	1.32	1.05
2	13	male	4	1.00	8,679	4.00	5.26	94,108	188,215	6.0	88,266	176,533	0.046	0.362	1.28	1.05
2	26	female	4	1.00	11,094	4.33	6.04	139,923	279,847	7.0	130,108	260,216	0.032	0.256	1.63	1.06
2	26	male	4	1.00	8,707	4.75	7.73	128,896	257,793	13	112,093	224,186	0.036	0.395	1.58	1.12
3	1	female	8	1.00	9,666	6.75	-	141,250	141,250	12	118,076	118,076	0.063	0.542	-	-
3	1	male	8	0.99	11,984	6.00	-	142,577	142,577	8.6	129,791	129,791	0.057	0.466	-	-
3	13	female	8	0.99	13,920	2.25	7.58	192,497	192,497	12	168,750	168,750	0.049	0.533	1.46	1.13
3	13	male	8	1.00	14,670	6.75	-	223,840	223,840	11	197,169	197,169	0.041	0.385	1.53	1.18
3	26	female	8	0.98	14,080	6.00	16.71	242,836	242,836	4.9	194,931	194,931	0.043	0.853	1.72	1.40
3	26	male	8	0.95	22,793	6.00	-	338,985	338,985	16	277,975	277,975	0.029	0.375	2.06	1.18
4	1	female	12	0.99	11,803	8.00	-	223,214	148,809	18	181,835	121,223	0.062	0.662	-	-
4	1	male	12	0.99	10,908	6.00	18.75	178,383	118,922	16	139,083	92,722	0.071	0.800	-	-
4	13	female	10	0.99	20,990	2.75	8.09	284,869	227,895	8.8	262,105	209,684	0.039	0.408	1.82	1.12
4	13	male	10	1.00	18,030	5.33	-	-	-	-	282,409	225,927	0.040	1.253	2.63	1.55
4	26	female	8	1.00	15,863	6.75	-	251,399	251,399	16	224,474	224,474	0.036	0.459	1.87	1.16
4	26	male	8	0.99	13,557	5.00	18.77	251,222	251,222	17	230,511	230,512	0.035	0.808	2.82	1.59

Serum glucose levels were affected in both genders with a dose-dependent manner. The observed effects on lowering the serum glucose levels were the expected pharmacological effect of insulin 454 and insulin NPH.

- **Dosing Solution Analysis**

The results of the dose formulation analysis were generally in accordance with the expected levels.

Study No. 206315: 6 mo (4 wk recovery) SC toxicity study in rats

A 6-month study with a 4-week recovery period in rats with daily subcutaneous administration of insulin 454 [by (b) (4)]

Study design: This GLP study was to assess the toxicity and TK of NNC 0100-0000-0454 (batch 412-NO6140) administrated daily by sc injection to rats for 26 weeks following a recovery period of 4 weeks. The dosages were 0, 20, 50, 125 nmol/kg/day (vs. 80/50 nmol/kg/day NPH insulin). Endpoints included clinical signs, body weight, food consumption, ophthalmoscopy, ECG, clinical pathology, antibody analysis, organ weight, macroscopic/microscopic exanimation, and TK/serum glucose analysis.

Group	Compound	Dose	Dose concen-tration	Main Animal Nos		Satellite Animal Nos		Recovery Animal Nos		Colour code
		(nmol/kg)	(nmol/ml)	Male	Female	Male	Female	Male	Female	
1	Vehicle	Control	0	1-20	21- 40	201-209 ¤ 401-403	210-218 ¤ 404-406	291-300	301-310	White
2	Insulin 454	20	40	41-60	61- 80	219-227 ¤ 407-409	228-236 ¤ 410-412			Blue
3	Insulin 454	50	100	81-100	101-120	237-245 ¤ 413, 514, 415	246-254 ¤ 416-418			Green
4	Insulin 454	125	250	121-140	141-160	255-263 ¤ 419-421	264-272 ¤ 422-424	311-320	321-330	Red
5	Insulin NPH	80 / 50*	160 / 100*	161-180	181-200	273-276, 377, 278-281 ¤ 425-427	282-284, 385 286-290 ¤ 428-430			Yellow

*) From Day 130, Group 5 animals were treated with 50 nmol/kg. On Days 130, 131, 132 and 133 Group 5 animals were dosed with a formulation of 160 nmol/ml at a dose volume of 0.31 ml/kg to reach a dose of 50 nmol/kg.
¤) Animals used in Week 20 for kinetic blood sampling as a new Day 1.

Findings:

- This study showed that sc dosing > 50 nmol/kg/day for 26 weeks resulted in test article related clinical signs and mortalities attributed to hypoglycaemia and death.
- Body weight and food consumption as well as clinical pathology parameters (i.e. bilirubin, glucose, potassium and etc) were also affected.
- Serum glucose levels were affected in a dose dependent manner in both genders.
- Some of the test-article treated animals (7/51) developed antibodies (vs. 9/14 NHP-treated animals).
- There were some changes in haematologic (i.e. hematocrit) and urinalysis (i.e. specific gravity) parameters but considered as an expected pharmacological effect of insulin. Lower liver and spleen weights were observed in higher dose groups. Changes were seen at the injection sites (i.e. minimal to slight inflammation and fibrosis) in both treated and control animals.
- The NOAEL was established at 50 nmol/kg/day by the sponsor *(note: due to clinical sign/mortality seen at 50 nmol/kg, the NOAEL should be 20 nmol/kg/day)*.
- TK analysis: Rats were exposed (linearity was seen) with no gender differences. Tmax was approx. 3 hrs. There were accumulations in test-article treated animals.

Study No. 206538: 3 mo sc DFR study in rats

13-week DRF study in Spraque Dawley rats [by (b) (4)]

Study design: This GLP study was to assess the toxicity and TK of insulin 454 (batch 412-N06048) administrated daily by sc injection for 13 weeks. The dosages were 0, 100, 200 nmol/kg nmol/kg/day in the volume of 0.5 ml/kg. Endpoints included clinical signs, body weight, food consumption, ophthalmoscopy, ECG, clinical pathology, antibody analysis, organ weight, macroscopic/microscopic exanimation (only at injection sites, kidneys, liver, pancreas, spleen, and mammary gland), and TK/serum glucose analysis.

Group	Dose*	Dose concentration*	Animal Nos		Colour code
	(nmol /kg)	(nmol/ml)	Male	Female	
1	0	0	1 - 12	13 - 24	White
2	100	200	25, 27, 29, 31, 33, 34, 35, 36, 126, 128, 130, 132	37 - 48	Blue
3	200	400	49-51, 53-60, 152	61 - 72	Green

*Material as supplied

Findings:

- This study showed that sc dosing at 100 or 200 nmol/kg resulted in test article related clinical signs and mortalities (only at 200 nmol/kg) attributed to hypoglycaemia. Hypoglycaemia-related clinical signs were seen in both groups but most pronounced at 200 nmol/kg.
- Food consumption (but not body weight) was affected.
- Several changes were detected in hematological (i.e. hematocrit, hemoglobin, RBC counts and etc), clinical pathology (i.e. glucose, bilirubin, albumin, and etc), urinalysis (i.e. decreased volume and increased specific gravity, pH, and etc). These changes were likely as a result of the metabolic activity of insulin and a decreased hepatic activity. Decreased Liver weights were observed in treated groups with no test-article related macroscopic findings.
- No NOAEL was established.
- TK analysis: Rats were exposed to insulin 454. The AUC remained constant with the increase in dose, but increased from Day 1 to Week 11 at each dose level. No gender differences were observed.

Study No. 206539: 12 mo SC toxicity study in rats [PIVOTAL]

A 52-Week toxicity study in rats with daily subcutaneous administration of insulin 454

Study no.:	206539
Study report location:	Novo Nordisk, Denmark
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 3 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Insulin 454, batch number TLDP008; NPH insulin comparator, batch number TS60968, *Purity not demonstrated

KEY STUDY FINDINGS

- Daily sc injection to rats for 52 weeks with insulin 454 at dose 0, 20 (LD), 65/50/40 (MD), or 100/80/60 (HD) nmol/kg/day (vs. 65/50/40 nmol NPH insulin kg/day) resulted in dose-related clinical signs of hypoglycaemia (i.e. ataxia, lethargy, convulsions, and etc.) and mortality in MD/HD groups. Due to hypoglycemia-related deaths; middle and high doses were lowered twice mid-study.

- Increased in some RBC parameters, decreased liver enzymes levels, as well as reduction in liver and kidney weights were considered related to the exaggerated pharmacological effect of insulin.
- Insulin 454 did not show carcinogenetic potential, based on the histopathological examination and the absence of increase cell proliferation in the mammary gland.
- The NOAEL was established to be at 60 nmol/kg/day (due to hypoglycaemia-related clinical signs and mortality).
- TK analysis: The serum exposure to insulin 454 was similar for male and female rats. There was a slightly higherAUC0-24h in males but a somewhat higher Cmax in females in Weeks 25 and 52. The serum exposure increased with dose, and mostly in a dose-dependent manner. The accumulation for insulin 454 after 52 weeks was low which was in agreement with that expected from the observed terminal t ½ and the dose interval. Peak insulin 454 serum concentrations were observed at 3 hrs post dose and terminal serum t ½ was 3-5 hrs.

Methods

Doses:	0, 20, 65/50/40, 100/80/60 nmol/kg/day insulin 454 (vs. 65/50/40 nmol NPH nmol/kg/day)
Frequency of dosing:	Daily
Route of administration:	Sc injection
Dose volume:	0.5 ml/kg
Formulation/Vehicle:	▪ 600 nmol/ml Insulin 454 in isotonic solution of glycerol 19 (b)(4) mg/mL, phenol 1.5 mg/mL, m-Cresol 1.72 mg/mL, with pH ~ 7.6 ▪ 600 nmol/ml NPH in isotonic solution of: glycerol (b)(4) mg/mL, phenol (b)(4) mg/mL, m-Cresol (b)(4) mg/mL, (b)(4) with pH ~7.3
Species/Strain:	SD rats
Number/Sex/Group:	N=40/sex (vehicle control) and N=50/sex/group (test article/positive control)
Age:	7 weeks
Weight:	170.6-181.8 g (males); 164.2-166.6 g (females)
Satellite groups:	TK
Unique study design:	▪ Mammary tissue examination ▪ Antibody analysis
Deviation from study protocol:	Yes, but these did not affect the outcome of the study.

Group	Treatment	Initial number of animals	Dose (Day 1- 75) nmol/kg/day	Dose (Day 76- 224) nmol/kg/day	Dose (Day 225 - end) nmol/kg/day
1	Vehicle	40M + 40F	0	0	0
2	Insulin 454 - low	40M + 40F	20	20	20
3	Insulin 454 - mid	40M + 40F	65	50	40
4	Insulin 454 - high	51M* + 50F	100	80	60
5	NPH insulin	50M + 50F	65	50	40

*One animal was sent for necropsy on Day 1 due to signs of hypoglycaemia. As the animal was replaced, a total of 51 males was included in this group.

Observations and Results

- **Mortality/ Clinical Signs:** daily

Besides hypoglycaemia-related clinical signs, o other treatment related signs were observed.

Pre-sheduled deaths								
Group	Treatment	Other	Treatment-related hypoglycaemia			Sum of all pre-scheduled deaths		
		M+ F	M + F	M	F	M + F/initial number of animals	M	F
1	Vehicle	8	-	-	-	8/80	3	5
2	Insulin 454	11	-	-	-	11/80	4	7
3		2	7	-	7	9/80	2	7
4		2	26	4	22	28/101	6	22
5	NPH insulin	5	16	6	10	21/100	7	14

Other, refers to mortality or euthanasia due to clinical signs of e.g mass or injury.
Treatment-related hypoglycaemia, referes to mortality due to clinical signs of e.g piloerection, ataxia, lethargy, passivity, convulsions, repeated hypoglycamia. Furthermore, cases where hypoglycemia cannot be excluded, has been listed here e.g animals with no macro or microscopic findings.
Pre-schedule deaths define all animals that died or were sacrificed prior to schedule.
M: male, F: female

- **Body Weights/Food Consumption:** Day 1 and weekly thereafter

No treatment-related differences were seen in body weight and food consumption among groups.

- **Ophthalmoscopy:** pretreatment, Week 25/26, at study termination

No treatment-related adverse ophthalmoscopy changes were seen.

- **ECG:** n/a

- **Hematology:** Week 26/27 and the week prior to study termination

Parameter	Method/Equipment	Unit
Haemoglobin (Hb)	Direct measurement/ABX Pentra DX 120	mmol/l
Red blood cell count (RBC)	Direct measurement/ABX Pentra DX 120	10^{12}/l
Haematocrit (HT)	Direct measurement/ABX Pentra DX 120	ml/100 ml
Mean cell volume (MCV)	Calculated/ABX Pentra DX 120	fl
Mean cell haemoglobin (MCH)	Calculated/ABX Pentra DX 120	fmol
Mean cell haemoglobin concentration (MCHC)	Calculated/ABX Pentra DX 120	mmol/l
White blood cell count (WBC)	Direct measurement/ABX Pentra DX 120	10^9/l
Differential leucocyte count (NEUTRO, LYMPHO, EOS, BASO, MONO)	Direct measurement/ABX Pentra DX 120	% and 10^9/l
Platelet count (Plt)	Direct measurement/ABX Pentra DX 120	10^9/l
Activated partial thromboplastin time (APTT)	IL Test™/ACL™ (*)	sec.
Prothrombin time (Pt)	IL Test™/ACL™(*)	sec.
Fibrinogen (Fib)	IL Test™/ACL™(*)	g/l

(* (b) (4)

There were increases in the absolute and relative number of monocytes in Week 26 (but not in Week 52). The changes in the RBC parameters (I,e, increased hemoglobin, hematocrit, mean cell volume, and mean cell hemoglobin) were mainly observed in the males in Weeks 26 and 52. These changes were considered of limited toxicological significance. The table below showed the values where a significant difference was observed.

Parameter	Week	Sex	Group 2	Group 3	Group 4	Group 5
Haemoglobin (Hb)	26	M	-	-	↑ 2.2	-
Haematocrit (HT)	52	M	-	-	↑ 2.9	↑ 3.5
Mean cell volume (MCV)	26	M	-	-	↑ 3.5	↑ 3.5
	26	F	-	-	↑ 2.5	-
	52	M	-	-	↑ 2.9	↑ 3.6
Mean cell haemoglobin (MCH)	26	M	-	-	↑ 2.8	↑ 2.8
	52	M	-	-	↑ 2.7	↑ 2.7
Mean cell haemoglobin concentration (MCHC)	52	M	-	-	-	↓ -1.4
White blood cell count (WBC)	52	M	-	-	↓ -15.5	-
% Neutrophils	52	M	-	-	-	↓ -16.5
% Basophils	52	F	-	-	-	↑ #
Monocytes	26	M	-	-	↑ 37.9	-
	26	F	-	↑ 40.0	↑ 48.0	-
% Monocytes	26	F	-	-	↑ 38.5	↑ 38.5
Platelet count (Plt)	26	M	-	-	↓ -7.8	↓ -8.7
Prothrombin time (Pt)	26	F	-	↑ 3.0	↑ 2.4	↑ 2.4
Fibrinogen (Fib)	26	M	-	↓ -12.2	-	↓ -13.2

- No statistical significant difference observed
Not possible to calculate

- **Clinical Chemistry:** Week 26/27 and the week prior to study termination

Parameter	Method	Unit
Alanine aminotransferase (ALAT)	Hitachi 917	µkat/l
Aspartate aminotransferase (ASAT)	Hitachi 917	µkat/l
Alkaline phosphatase (ALKPH)	Hitachi 917	µkat/l
Bilirubin (total) (BILI)	Hitachi 917	µmol/l
Gamma-glutamyl transferase (GGT)	Hitachi 917	µkat/l
Cholesterol (CHOL)	Hitachi 917	mmol/l
Triglycerides (TRIG)	Hitachi 917	mmol/l
Carbamide (UREA)	Hitachi 917	mmol/l
Creatinine (CREAT)	Hitachi 917	µmol/l
Glucose (GLUC)	Hitachi 917	mmol/l
Sodium (Na)	Ion selective electrode/Hitachi 917	mmol/l
Potassium (K)	Ion selective electrode/Hitachi 917	mmol/l
Calcium (Ca)	Hitachi 917	mmol/l
Magnesium (Mg)	Hitachi 917	mmol/l
Inorganic phosphorus (P)	Hitachi 917	mmol/l
Chloride (Cl)	Ion selective electrode/Hitachi 917	mmol/l
Protein (total) (PROTEIN)	Hitachi 917	g/l
Albumin (ALB)	Hitachi 917	g/l
Globulin	Calculated	g/l
Albumin/Globulin (ALB/G) ratio	Calculated	No unit

A decrease in the liver enzymes (ALT and AST) was seen mainly in Groups 3, 4, and 5 in Weeks 26 and 52. An increase in the glucose level was observed in a dose-dependent manner at Weeks 26 and 52 (expected pharmacological effect of insulin). The table below showed the values where a significant difference was observed.

Parameter	Week	Sex	Group 2	Group 3	Group 4	Group 5
Alanine aminotransferase (ALAT)	26	M	-	↓ -19.3	↓ -28.0	↓ -29.8
	26	F	↓ -18.2	↓ -28.4	↓ -27.0	↓ -30.4
	52	M	-	↓ -22.3	↓ -28.2	↓ -36.9
	52	F	-	↓ -38.4	↓ -41.5	↓ -38.4
Aspartate aminotransferase (ASAT) (including ASAT log transformed for Week 26 ♂)	26	M	↓ -10.8 (-21.8)	↓ -19.9 (-38.9)	↓ -25.0 (-48.5)	↓ -23.9 (-46.7)
	26	F	↓ -26.8	↓ -34.7	↓ -29.6	↓ -37.1
	52	M	-	-	↓ -23.0	↓ -33.5
	52	F	-	↓ -42.2	↓ -45.0	↓ -46.2
Bilirubin (total) (BILI)	26	M	-	↑ 4.4	↑ 20.7	↑ 24.4
Triglycerides (TRIG)	26	M	-	↑ 23.7	-	-
	26	F	-	↓ -20.3	↓ -23.3	-
Carbamide (UREA)	26	M	-	↓ -11.8	↓ -17.7	↓ -13.0
	26	F	-	-	↓ -9.7	-
	52	M	-	↓ -12.7	↓ -11.9	↓ -13.2
	52	F	-	-	↓ -12.3	-
Creatinine (CREAT)	26	F	-	-	↑ 9.3	-
Glucose (GLUC)	26	M	-	↑ 16.3	↑ 40.2	↑ 37.4
	26	F	-	↑ 22.1	↑ 34.5	↑ 15.1
	52	M	↑ 7.9	↑ 11.3	↑ 18.5	↑ 21.1
	52	F	↑ 8.9	↑ 18.7	↑ 23.7	↑ 10.0
Sodium (Na)	26	M	-	↓ -1.7	↓ -2.0	↓ -1.3
	52	F	-	-	-	↓ -1.4
Calcium (Ca)	26	M	↑ 2.5	↑ 3.2	-	-
Inorganic phosphorus (P)	26	M	-	-	↑ 10.7	-
	26	F	-	-	↑ 8.7	-
	52	F	-	-	↑ 13.7	-
Chloride (Cl)	26	M	-	↓ -1.6	↓ -2.0	↓ -2.3
	26	F	-	-	↓ -2.9	-
Protein (total) (PROTEIN)	26	M	↓ -2.3	↓ -2.3	↓ -5.5	↓ -5.1
	26	F	-	↓ -4.4	↓ -6.1	↓ -3.4
	52	M	-	-	↓ -2.9	↓ -4.0
	52	F	-	-	↓ -5.5	-
Albumin (ALB)	26	M	-	-	↓ -4.3	↓ -2.9
	26	F	-	↓ -6.3	↓ -7.0	↓ -4.5
Globulin	26	M	-	-	↓ -7.3	↓ -8.0
	52	M	-	-	↓ -4.9	↓ -7.1
Albumin/Globulin (ALB/G) ratio	26	M	-	-	-	↑ 6.1

- No statistical significant difference observed

- **Urinalysis:** Week 26/27 and the week prior to study termination

Parameter	Method/Equipment	Unit/Range
Sodium (Na)	Ion selective electrode/ Cobas Mira S	mmol/l
Potassium (K)	Ion selective electrode/ Cobas Mira S	mmol/l
Chloride (Cl)	Ion selective electrode/ Cobas Mira S	mmol/l
Specific gravity (SG)	Ames Multistix 10SG/Clinitek 500	No unit
pH	Ames Multistix 10SG/Clinitek 500	No unit
Colour (COLOUR)	Visual examination	No unit
Protein (PROTEIN)	Ames Multistix 10SG/Clinitek 500	g/l
Leucocytes (LEUC)	Ames Multistix 10SG/Clinitek 500	Cells/µl
Nitrite (NITRITE)	Ames Multistix 10SG/Clinitek 500	No unit
Blood (BLOOD)	Ames Multistix 10SG/Clinitek 500	Erythrocytes/µl
Glucose (GLUCOSE)	Ames Multistix 10SG/Clinitek 500	mmol/l
Ketones (KETONES)	Ames Multistix 10SG/Clinitek 500	mmol/l
Bilirubin (BILI)	Ames Multistix 10SG/Clinitek 500	No unit
Urobilinogen (UROBIL)	Ames Multistix 10SG/Clinitek 500	µmol/l

An increase in ketones was observed in Groups 3 and 4 females in a dose dependent manner (secondary to the pharmacological effect of insulin). The table below showed the values where a significant difference was observed. In addition, there was an increased number of crystals in the urine Group 4 females (with no histological correlation).

Parameter	Week	Sex	Group 2	Group 3	Group 4	Group 5
Potassium (K)	26	F	-	-	-	↑ 58.2 mmol/l
Specific gravity (SG)	26	F	-	↓ **	-	↓ **
Leucocytes (LEUC)	26	M	-	-	↓ *	↓ **
	26	F	-	-	↓ *	-
	52	M	-	-	-	↓ *
Blood (BLOOD)	52	F	-	-	↑ ***	-
Ketones (KETONES)	26	F	-	↑ *	↑ **	↑ **
Bilirubin (BILI)	52	M	-	-	↑ *	-
Urobilinogen (UROBIL)	52	M	-	↑ **	↑ ***	-

* means 0.01<p<0.05, versus control group
** means 0.001<p<0.01, versus control group
*** means p<0.001, versus control group

- **Gross Pathology:** necropsy

Macroscopic evaluation of the masses was done during the in-life phase. The red discoloration was seen in the subcutis at the injection sites with no difference in incidence between groups 1-5. No other test article related findings were seen.

Incidence table: Macroscopic evaluation of masses (males)

Dose group	Group 1	Group 2	Group 3	Group 4	Group 5
Treatment	Control		Insulin 454		NPH insulin
Dose (nmol/kg/day)	0	20	65/50/40	100/80/60	65/50/40
Sex	M	M	M	M	M
Number of animals examined	40	40	40	51	50
Rectum					
Abcess, black-brown, pale, friable, soft, surface: irregular, cut surface: fatty	-	-	1	-	-
Thymus					
Nodule	-	-	1	-	1
Mammary Gland					
Nodule	1	1	-	-	-
Skin/subcutis					
Nodule	3	4	1	3	1
Tail					
Nodule	1	-	1	-	-

M = males

Incidence table: Macroscopic evaluation of masses (females)

Dose group	Group 1	Group 2	Group 3	Group 4	Group 5
Treatment	Control		Insulin 454		NPH insulin
Dose (nmol/kg/day)	0	20	65/50/40	100/80/60	0
Sex	F	F	F	F	F
Number of animals examined	40	40	40	50	50
Stomach					
Nodule	-	-	1	-	-
Kidneys					
Nodule	-	-	-	-	1
Ovaries					
Nodule	1	-	-	-	-
Uterus					
Nodule	1	-	1	-	-
Cervix					
Nodule	-	-	-	-	1
Vagina					
Nodule	1	-	-	-	-
Mammary Gland					
Nodule	-	3	-	-	2
Skin/subcutis					
Nodule	2	2	1	-	7
Tail					
Nodule	-	1	-	-	-

F = females

- **Organ Weights:** necropsy

A decrease in liver weight (absolute and relative) was seen for males in a dose-dependent manner, but only the relative liver weight was seen for females in Group 4. This might associated with decreased ALT and AST activities and depletion of stored glycogen. In addition, a decrease in absolute kidney weight was observed in Groups 3-5 males. This might related to the pharmacological action of insulin causing glycogen depletion in healthy rat with insulin. No correlating histopathological changes were observed.

- **Histopathology:** necropsy
  Adequate Battery: Yes
  Peer Review: Yes

Organs and tissues	Weigh	Fix	Micro	Organs and tissues	Weigh	Fix	Micro
Abnormalities (gross lesions)		x	x	Pancreas		x	x
Adrenals	x	x	x	Pituitary	x	x	x
Aorta (thoracic)		x	x	Prostate	x	x	x
Brain	x	x	x	Salivary glands (right parotid, sublingual and submandibular)		x	x
Bone marrow smear[1)]		x		Sciatic nerve		x	x
Bones (right femur)		x	x	Seminal vesicles		x	x
Epididymides	x	x	x	Skeletal muscle		x	x
Eyes with lens/optic nerve/Harderian gland[5)]		x	x	Skin		x	x
Heart	x	x	x	Spinal cord (cervical, thoracic, lumbar)		x	x
Injection site		x	x	Spleen	x	x	x
Intestine small (duodenum, jejunum, ileum)[3)]		x	x	Sternum (for bone marrow)		x	x
Intestine large (caecum, colon, rectum)		x	x	Stomach (glandular, non glandular)		x	x
Joint (knee)		x	x	Testes	x	x	x
Kidneys	x	x	x	Thymus	x	x	x
Larynx		x	x	Thyroids (incl. parathyroid)		x	x
Liver	x	x	x	Tongue		x	x
Lungs		x	x	Trachea		x	x
Lymph nodes (mesenteric, right mandibular and inguinal)[2)]		x	x	Ureters		x	x
Mammary gland[2)]		x	x	Urinary bladder		x	x
Oesophagus		x	x	Uterus (horn, cervix and oviducts)[4)]	x	x	x
Ovaries	x	x	x	Vagina[4)]		x	x

1. A bone marrow smear was taken from the femur of all animals. The smears were fixed and stained with May-Grünwald and Giemsa stain, but not examined as no treatment-related findings were seen in the periferal blood or haematopoietic tissues.
2. The right inguinal mammary gland (no. 4), incl. the inguinal lymph node, was processed for Haematoxylin and Eosin staining and histological examination at (b) (4) The left abdominal mammary glands (No 4, 5 and 6) inclusive the inguinal lymph node were sampled, fixed and used for assessment of BrdU cell proliferation labelling index as described in 2.25.3.
3. Approximately 1 cm of the duodenum was sampled, fixed and used for assessment of BrdU cell proliferation labelling index as described in 2.25.3.
4. As part of the standard evaluation of the female reproductive system, the status of the female oestrus cycle is listed under uterus in the path data report Appendix A, individual animal data.
5. Only one eye was examined.

Overall, no treatment-related neoplastic or non-neoplastic findings were seen at the microscopic examination. Special attention was paid to the female mammary glands (no differences between treated and control animals).

Non-neoplastic findings:

- In males: 1) A minimal increased incidence of extramedullary haemopoiesis and haemosiderin deposition in the spleen in some treated groups (Groups 3, 4, and 5)

when compared to controls. 2) A minimal increased incidence of lymphoid atrophy in the thymus in some treated groups (Groups 2, 4 and 5) when compared to controls. 3) A slight increase in cortical basophilic tubules in the kidneys was reported in Group 4. All these findings mentioned above were within histological background range or related to stress. 4) There was a spectrum of changes associated with minimal/slight inflammation in subcutis at the injection sites from all groups including controls (related to the injection procedure).

- In females: 1) The incidence of the various cyclic stages (by assessing the histological appearance at all levels of the reproductive tract i.e. ovary, cervix, and vagina) was considered to be comparable across all groups (Groups 1-5) and as such not affected by treatment. 2) There was also a spectrum of changes associated with minimal/slight inflammation in subcutis at the injection sites from all groups including controls (related to the injection procedure).

Neoplastic findings:

- A few tumors were observed, however, they were not considered test article related (within background findings). **Note: It is rare to see tumors in chronic toxicity studies.**
- Insulin 454 did not cause an increase in mammary or other tumors compared to controls (vehicle-only and NPH-treated rats).
- There was not a significant increase in mammary hyperplasia in males or females.
- There were also no test article-related increases in palpable masses among male or female study animals.

Group	Number of palpable masses in the mammary region	Number of palpable masses outside the mammary region	Total number of animals sent for necropsy due to size of mass or due to rupture.*
1	4 (1 male, 3 females)	4 (3 males, 1 female)	7 (3 males, 4 females)
2	6 (2 males, 4 females)	5 (3 males, 2 females)	8 (3 males, 5 females)
3	2 (2 males)	0	0
4	1 (1 male)	4 (4 males)	2 (2 males)
5	5 (4 males, 1 female)	6 (2 males, 4 females)	5 (1 male, 4 females)

* For further details see table in section 3.2.

Incidence table: Microscopic evaluation of masses (males)

Dose group	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	20/20/20 nmol insulin 454/kg/day	65/50/40 nmol insulin 454/kg/day	100/80/60 nmol insulin 454/kg/day	65/50/40 nmol insulin NPH/kg/day
Sex	M	M	M	M	M
*Brain**	40	40	40	50	50
Astrocytoma (malignant)	-	-	-	1	1
*Rectum**	40	40	40	50	50
Fibrosarcoma	-	-	1	-	-
*Pancreas**	39	40	40	50	50
Acinar Adenoma	-	2	-	-	-
*Urinary bladder**	40	39	38	50	50
Papilloma, transitional cell	-	-	-	1	-
*Pituitary gland**	39	40	38	50	50
Adenoma:P.Distalis	-	-	-	-	1
*Thyroid gland**	40	40	40	50	50
C-cell Adenoma	6	2	1	4	2
C-cell Carcinoma	-	1	-	1	1
*Adrenal glands**	40	40	40	50	50
Cortical adenoma	-	-	1	-	-
*Thymus**	39	39	38	47	47
Thymoma	-	-	1	-	1
*Mammary gland**	37	37	38	46	47
Fibroadenoma	2	-	-	1	-
Adenocarcinoma	-	1	-	-	-
*Skin/Subcutis**	40	40	39	49	50
Keratoacanthoma	1	1	1	-	-
Fibrosarcoma	1	2	-	2	-

* Number of organs examined

Incidence table: Microscopic evaluation of masses (females)

Dose group	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	20/20/20 nmol insulin 454/kg/day	65/50/40 nmol insulin 454/kg/day	100/80/60 nmol insulin 454/kg/day	65/50/40 nmol insulin NPH/kg/day
Sex	F	F	F	F	F
*Kidneys**	40	40	40	50	50
Nephroblastoma	-	-	-	-	1
*Ovaries**	40	40	40	50	50
Granulosa Cell Tumor (malignant)	1	-	-	-	-
*Uterus**	40	40	40	50	50
Endometrial polyp	2	2	3	3	1
*Cervix**	40	40	40	50	50
Endometrial polyp	-	-	-	-	2
*Vagina**	40	40	40	50	50
Leiomyoma	1	-	-	-	-
*Clitoral glands**	1	-	-	-	-
Keratoacanthoma	1	-	-	-	-
*Pituitary gland**	38	40	39	48	50
Adenoma:P.Distalis	1	1	-	-	3
*Thyroid gland**	40	40	40	50	50
Carcinoma:Follicular	-	-	-	-	1
C-cell Adenoma	-	-	2	-	2
C-cell Carcinoma	-	1	-	1	1
*Adrenal glands**	40	40	40	50	50
Phaeochromocytoma	-	1	1	-	1
Ganglioneuroma	-	-	-	1	-
*Mammary gland**	40	40	39	45	49
Fibroadenoma	1	3	-	-	4
Adenocarcinoma	4	2	-	-	3
Fibrosarcoma	-	1	-	-	-
Malignant mixed	-	1	-	-	-

* Number of organs examined

Note: 3 mammary gland nodules (in Group 2 females) noted on page 120 (macroscopic evaluation of masses (females)) corresponded to 3 fibroadenoma and 2 adenocarcinoma findings in Group 2 females.

Table 4 Histopath Summary for Pivotal 12-month Toxicity Study in Rats.

HISTOPATH SUMMARY REPORT (SELECTED)

[Note: only one set of summary tables was included here. For the remaining summary tables and individual data, please see the actual study report for details.]

MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX STATUS AT NECROPSY
MALE

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
BRAIN :	40	40	40	50	50
- Astrocytoma :	-	-	-	1	1
- Dystrophic min :	-	-	-	-	1
Grade 1:	-	-	-	-	1
SPINAL CORD, CERVIC. :	40	40	40	50	50
- Astrocytoma of brain:	-	-	-	-	1
HEART :	40	40	40	50	50
- Inflamm cell/myo fib:	6	9	7	8	2
Grade 1:	4	6	5	7	2
Grade 2:	2	3	2	1	-
- Myocard fib/degen :	13	6	7	9	8
Grade 1:	11	6	5	8	5
Grade 2:	2	-	2	1	3
- Vent hypertrophy :	1	-	-	-	-
Grade 1:	1	-	-	-	-
LARYNX :	39	40	40	48	50
- S'epith inflamm cell:	1	1	2	1	1
Grade 1:	1	1	1	1	1
Grade 2:	-	-	1	-	-
- S'epith gran inflamm:	-	1	-	1	-
Grade 1:	-	-	-	1	-
Grade 2:	-	1	-	-	-
- Inflamm vent pouch :	-	-	1	1	-
Grade 1:	-	-	1	-	-
Grade 2:	-	-	-	1	-
- Periarteritis :	-	-	-	1	-
Grade 3:	-	-	-	1	-
TRACHEA :	40	40	40	50	50
- S'epith inflamm cell:	1	-	-	-	-
Grade 1:	1	-	-	-	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
LUNG :	40	40	40	50	50
- Granulomatous inflam:	4	2	2	4	2
- Bronch/alv hyperpla :	-	1	-	-	1
Grade 1:	-	1	-	-	-
Grade 2:	-	-	-	-	1
- Alveolitis :	1	3	1	2	-
Grade 1:	1	3	1	2	-
- Alveolar macrophages:	-	-	-	-	2
Grade 1:	-	-	-	-	2
- Osteoid deposition :	1	1	-	1	-
Grade 1:	1	-	-	1	-
Grade 2:	-	1	-	-	-
TONGUE :	40	40	40	48	49
- S'epith inflamm cell:	2	-	3	2	1
Grade 1:	2	-	-	2	1
Grade 2:	-	-	3	-	-
- Inflamm cells/muscle:	13	9	10	13	14
Grade 1:	13	8	9	11	13
Grade 2:	-	1	1	2	1
- Periarteritis :	2	2	4	1	3
Grade 1:	2	-	1	-	3
Grade 2:	-	1	3	1	-
Grade 3:	-	1	-	-	-
- Inflamm lng sal gld:	-	-	2	-	-
Grade 1:	-	-	1	-	-
Grade 4:	-	-	1	-	-
STOMACH NONGLANDULAR :	39	40	40	50	50
- Hyperpl/hyperk epith:	1	-	1	3	1
Grade 1:	-	-	1	3	1
Grade 2:	1	-	-	-	-
Grade 4:	-	-	-	-	-
- S'epith inflam cells:	-	-	-	-	1
Grade 3:	-	-	-	-	1

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
STOMACH GLANDULAR :	39	40	40	50	50
- Ectopic nongld epith:	1	3	2	3	4
Grade 1:	1	1	2	2	4
Grade 2:	-	2	-	1	-
- S'muc lymphocytes :	-	1	2	3	-
Grade 1:	-	1	2	3	-
- Cong sup lam propria:	-	-	-	1	-
Grade 2:	-	-	-	1	-
Grade 3:	-	-	-	-	-
- Vacuolat chief cells:	-	-	-	1	-
Grade 2:	-	-	-	1	-
DUODENUM :	40	40	40	50	49
- Mucosal congestion :	-	-	-	1	-
Grade 2:	-	-	-	1	-
Grade 3:	-	-	-	-	-
JEJUNUM :	40	40	40	50	50
- Mucosal congestion :	-	1	-	2	-
Grade 1:	-	1	-	1	-
Grade 2:	-	-	-	1	-
ILEUM :	40	39	40	50	50
- Mucosal congestion :	-	-	-	1	-
Grade 1:	-	-	-	1	-
CECUM :	40	40	39	50	50
- Mucosal congestion :	-	-	-	1	-
Grade 1:	-	-	-	1	-
- S'mucosal inflamm :	-	-	1	-	-
Grade 1:	-	-	1	-	-
COLON :	40	40	40	50	50
- Mucosal congestion :	-	-	-	1	-
Grade 1:	-	-	-	1	-

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SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
RECTUM :	40	40	40	50	50
- Peritonitis :	-	-	1	-	-
Grade 2:	-	-	1	-	-
- S'mucos lymphocytes :	-	1	-	-	-
Grade 1:	-	1	-	-	-
- Fibrosarcoma :	-	-	1	-	-
LIVER :	40	40	40	50	50
- Bile duct hyperpl :	10	17	7	12	13
Grade 1:	10	15	3	12	12
Grade 2:	-	1	4	-	1
Grade 3:	-	1	-	-	-
- EMH/inflamm cells :	12	13	5	11	15
Grade 1:	12	11	5	11	15
Grade 2:	-	2	-	-	-
Grade 3:	-	-	-	-	-
- P'biliary infl cells:	-	2	-	-	-
Grade 1:	-	1	-	-	-
Grade 3:	-	1	-	-	-
- Hepatocyte necrosis :	1	2	-	2	1
Grade 1:	1	1	-	1	1
Grade 2:	-	1	-	1	-
- Hepatocyte vacuolat :	4	5	3	9	7
Grade 1:	3	4	3	9	7
Grade 2:	1	1	-	-	-
- Basophilic hepats :	1	-	-	-	-
Grade 1:	1	-	-	-	-
- Vasc/sinusoidal cong:	-	-	-	1	-
Grade 2:	-	-	-	1	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
PANCREAS :	39	40	40	50	50
- Acinar Adenoma :	-	2	-	-	-
- Exocrine atrophy :	2	1	5	3	-
Grade 1:	1	1	4	3	-
Grade 2:	1	-	1	-	-
- Exocrine nec/inflamm:	2	1	2	1	1
Grade 1:	2	1	1	1	1
Grade 2:	-	-	1	-	-
- Islet cell hyperpl :	21	8	8	13	24
Grade 1:	21	8	8	12	23
Grade 2:	-	-	-	1	1
- Red zymogen granules:	-	-	1	-	-
Grade 3:	-	-	1	-	-

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: Ko, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
KIDNEYS :	40	40	40	50	50
- CPN :	13	14	11	13	12
Grade 1:	8	10	6	10	6
Grade 2:	3	4	4	-	4
Grade 3:	2	-	1	2	1
Grade 4:	-	-	-	1	1
- Cort basophilic tubs:	11	15	17	24	12
Grade 1:	11	15	15	23	11
Grade 2:	-	-	2	1	1
- Cortical cyst(s) :	1	1	1	-	1
Grade 1:	1	1	1	-	1
- Dys mineralisation :	-	-	1	-	-
Grade 1:	-	-	-	-	-
Grade 2:	-	-	1	-	-
Grade 3:	-	-	-	-	-
- Hem/colloid pap tubs:	-	-	-	1	-
Grade 1:	-	-	-	1	-
- Inflamm cell pelvis :	-	-	-	1	-
Grade 1:	-	-	-	1	-
Grade 2:	-	-	-	-	-
- S'pelvic inflam cell:	-	-	-	-	1
Grade 2:	-	-	-	-	1
Grade 3:	-	-	-	-	-
- Pelvic dilation :	-	-	-	-	1
Grade 1:	-	-	-	-	1
Grade 2:	-	-	-	-	-
URETERS :	40	38	39	49	50
- Lumenal dilation :	-	-	-	-	1
Grade 1:	-	-	-	-	1

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
URINARY BLADDER :	40	39	38	50	50
- Peritonitis :	-	1	1	1	1
Grade 1:	-	1	-	-	-
Grade 2:	-	-	1	1	1
- S'epith inflam cells:	-	-	1	2	2
Grade 1:	-	-	1	2	2
- Papilloma trans cell:	-	-	-	1	-
- Ulcer epith/necrosis:	-	-	-	-	1
Grade 3:	-	-	-	-	1
TESTES :	40	39	40	50	50
- Interstitial oedema :	3	2	2	1	5
Grade 1:	1	1	1	-	-
Grade 2:	1	-	-	1	2
Grade 3:	-	-	1	-	2
Grade 4:	1	-	-	-	1
Grade 5:	-	1	-	-	-
- Interst acute inflam:	-	1	-	-	-
Grade 4:	-	1	-	-	-
- Semin tub atrophy :	3	2	5	5	7
Grade 1:	1	-	5	3	4
Grade 2:	-	1	-	-	1
Grade 3:	-	-	-	1	1
Grade 4:	1	1	-	1	1
Grade 5:	1	-	-	-	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
EPIDIDYMIDES :	40	39	40	50	50
- Lack spermatozoa :	-	-	-	1	-
Grade 5:	-	-	-	1	-
- Vac ductular epith :	4	10	10	15	12
Grade 1:	4	10	9	13	12
Grade 2:	-	-	1	2	-
- Oligospermia :	2	-	-	-	2
Grade 1:	-	-	-	-	1
Grade 2:	1	-	-	-	-
Grade 3:	-	-	-	-	1
Grade 5:	1	-	-	-	-
- Immat/ab s'gen cells:	-	1	-	2	1
Grade 1:	-	-	-	2	1
Grade 2:	-	1	-	-	-
- Spermatocele gran :	-	1	-	-	-
Grade 2:	-	1	-	-	-
- Gran inflammation :	-	-	-	2	-
Grade 1:	-	-	-	1	-
Grade 2:	-	-	-	1	-
- Interst inflammation:	-	-	-	1	-
Grade 1:	-	-	-	1	-
- Mesothelial prolif :	-	-	1	1	-
Grade 1:	-	-	-	1	-
Grade 3:	-	-	1	-	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
PROSTATE GLAND :	40	40	40	50	50
- Interst oedema :	-	-	2	2	-
Grade 1:	-	-	2	1	-
Grade 3:	-	-	-	1	-
- Interst inflammation:	-	4	3	4	6
Grade 1:	-	2	2	3	4
Grade 2:	-	2	1	-	2
Grade 3:	-	-	-	1	-
- Vac acinar epith :	-	-	1	-	1
Grade 1:	-	-	-	-	1
Grade 2:	-	-	1	-	-
- Secretion in acini :	-	-	-	-	1
Grade 2:	-	-	-	-	1
PREPUTIAL GLANDS :	2	-	-	-	-
- Abscess :	2	-	-	-	-
Grade 1:	1	-	-	-	-
Grade 2:	1	-	-	-	-
PITUITARY GLAND :	39	40	38	50	50
- Multiloc cyst p dist:	2	3	1	1	4
Grade 1:	1	2	1	1	3
Grade 2:	1	-	-	-	1
Grade 3:	-	1	-	-	-
- Cyst(s) :	1	-	1	1	1
Grade 1:	-	-	1	-	1
Grade 2:	-	-	-	1	-
Grade 3:	1	-	-	-	-
- Hyperplasia p dist :	-	-	-	-	1
Grade 1:	-	-	-	-	1
Grade 2:	-	-	-	-	-
- Adenoma:P.Distalis :	-	-	-	-	1

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
THYROID GLAND :	40	40	40	50	50
- Ultimobranchial cyst:	-	-	1	-	-
- C cell hyperplasia :	3	4	5	2	-
Grade 1:	3	3	4	2	-
Grade 2:	-	1	1	-	-
- C-cell Adenoma :	6	2	1	4	2
- C-cell Carcinoma :	-	1	-	1	1
- Interstitial inflamm:	1	-	-	-	-
Grade 1:	1	-	-	-	-
ADRENAL GLANDS :	40	40	40	50	50
- Cortical vacuolation:	8	10	6	12	13
Grade 1:	8	10	5	10	11
Grade 2:	-	-	1	2	2
- Cort cystic degen :	1	2	-	2	5
Grade 1:	1	1	-	2	5
Grade 2:	-	1	-	-	-
Grade 3:	-	-	-	-	-
- Cortical cyst :	-	1	-	-	-
Grade 1:	-	1	-	-	-
- Cortical hyperplasia:	2	1	4	4	7
Grade 1:	2	-	4	4	7
Grade 2:	-	1	-	-	-
- Medullary hyperpl :	1	-	1	2	3
Grade 1:	1	-	-	2	3
Grade 2:	-	-	1	-	-
- Cortical adenoma :	-	-	1	-	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						MALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	51	50	
SPLEEN :	40	40	40	50	50	
- Hemosiderin deposit :	9	7	14	25	18	
Grade 1:	9	7	13	17	12	
Grade 2:	-	-	1	7	5	
Grade 3:	-	-	-	1	1	
Grade 4:	-	-	-	-	-	
- EMH :	11	17	20	32	21	
Grade 1:	9	14	13	17	13	
Grade 2:	1	2	5	15	6	
Grade 3:	1	1	2	-	2	
Grade 4:	-	-	-	-	-	
- Prom marginal zone :	1	1	1	1	-	
Grade 1:	-	1	1	1	-	
Grade 2:	1	-	-	-	-	
THYMUS :	39	39	38	47	47	
- Lymphoid atrophy :	18	24	14	33	26	
Grade 1:	4	11	6	12	8	
Grade 2:	12	12	4	15	13	
Grade 3:	2	1	4	6	5	
Grade 4:	-	-	-	-	-	
- Epithelial cyst(s) :	6	2	2	4	6	
Grade 1:	5	2	-	4	3	
Grade 2:	1	-	2	-	3	
Grade 3:	-	-	-	-	-	
Grade 4:	-	-	-	-	-	
- Thymoma :	-	-	1	-	1	
LYMPH NODES :	-	-	-	-	1	
- Axillary LN WNL :	-	-	-	-	1	
MANDIBULAR LN RIGHT :	39	39	40	50	49	
- Sinusoidal congest :	7	6	8	12	10	
Grade 1:	4	4	8	8	9	
Grade 2:	3	2	-	4	1	
- Sinusoid histiocytes:	-	-	2	6	4	
Grade 1:	-	-	2	6	4	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
SUBLING.GLAND, RIGHT :	40	40	40	49	50
- Vacuolation serous :	-	1	-	-	-
Grade 1:	-	-	-	-	-
Grade 2:	-	1	-	-	-
- P'duct inflam cells :	-	-	-	-	1
Grade 1:	-	-	-	-	1
SUBMANDIB.GLD. RIGHT :	40	40	40	50	50
- Vacuolation serous :	-	1	-	-	-
Grade 2:	-	1	-	-	-
HARDERIAN GLANDS :	40	40	40	48	49
- Interst inflam cells:	-	-	1	-	-
Grade 1:	-	-	1	-	-
MAMMARY GLAND :	37	37	38	46	47
- Inflamm ass acini :	1	-	-	-	-
Grade 1:	1	-	-	-	-
- Fibroadenoma :	2	-	-	1	-
- Hyperplasia :	-	1	2	2	2
Grade 1:	-	1	1	-	-
Grade 2:	-	-	1	2	2
- Dist acin/secretion :	1	-	-	-	1
Grade 1:	1	-	-	-	1
Grade 2:	-	-	-	-	-
Grade 3:	-	-	-	-	-
- Adenocarcinoma :	-	1	-	-	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					MALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	51	50
SKIN/SUBCUTIS :	40	40	39	49	50
- Keratoacanthoma :	1	1	1	-	-
- Fibrosarcoma :	1	2	-	2	-
- Epiderm ulceration :	-	-	1	1	-
Grade 5:	-	-	1	1	-
- Epiderm hyperplasia :	-	-	1	1	1
Grade 1:	-	-	-	1	-
Grade 2:	-	-	1	-	1
- Scab :	-	-	-	1	1
Grade 1:	-	-	-	-	1
Grade 2:	-	-	-	1	-
- Hemorrhage subcutis :	2	2	-	-	1
Grade 1:	-	-	-	-	1
Grade 2:	-	1	-	-	-
Grade 3:	1	1	-	-	-
Grade 4:	1	-	-	-	-
- Acute infl/necrosis :	1	1	1	-	-
Grade 1:	1	-	-	-	-
Grade 2:	-	1	-	-	-
Grade 3:	-	-	1	-	-
- Baso/squamous hyperp:	-	1	-	-	-
Grade 2:	-	1	-	-	-
BONE, FEMUR :	40	40	40	50	50
- P'osteal thickening :	4	1	-	-	1
Grade 1:	4	-	-	-	1
Grade 2:	-	1	-	-	-
- Prom hemopoiesis :	-	-	1	-	-
Grade 3:	-	-	1	-	-
JOINT,KNEE,RIGHT :	40	40	40	50	50
- Osteoarthrosis :	6	6	4	4	4
Grade 1:	6	6	4	4	4

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						MALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	51	50	
STERNUM :	40	40	40	50	50	
- Prom hemopoiesis :	-	-	1	-	-	
Grade 3:	-	-	1	-	-	
- Cartilage prolif :	-	-	-	-	1	
Grade 3:	-	-	-	-	1	
TAIL :	1	-	1	-	-	
- Prom blood vessels :	1	-	-	-	-	
Grade 2:	1	-	-	-	-	
- Prom adnexal element:	-	-	1	-	-	
Grade 1:	-	-	-	-	-	
Grade 2:	-	-	1	-	-	

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						MALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	51	50	
INJECTION SITE :	39	40	40	49	50	
- Scab(s) :	-	1	2	1	-	
Grade 1:	-	1	-	-	-	
Grade 2:	-	-	2	-	-	
Grade 3:	-	-	-	1	-	
- Hemorrhage :	22	16	15	9	14	
Grade 1:	12	7	10	7	6	
Grade 2:	5	6	4	1	6	
Grade 3:	5	3	1	1	2	
Grade 4:	-	-	-	-	-	
- Inflamm/eosin debris:	12	3	-	1	1	
Grade 1:	1	1	-	-	-	
Grade 2:	7	2	-	1	1	
Grade 3:	4	-	-	-	-	
- Necrosis :	6	4	3	-	4	
Grade 1:	3	1	2	-	2	
Grade 2:	3	2	1	-	2	
Grade 3:	-	1	-	-	-	
- Chronic inflammation:	28	37	40	42	41	
Grade 1:	7	19	28	31	23	
Grade 2:	21	17	12	11	17	
Grade 3:	-	1	-	-	1	
- Cystic space :	8	10	9	9	13	
Grade 1:	6	7	9	9	7	
Grade 2:	2	3	-	-	6	
- Dermal fibrosis :	-	-	5	-	-	
Grade 1:	-	-	5	-	-	

FEMALE

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
LUNG :	40	40	40	50	50	
- Granulomatous inflam:	3	2	4	2	5	
- Alveolitis :	-	1	1	1	1	
Grade 1:	-	1	1	1	1	
- Alveolar macrophages:	1	1	3	1	3	
Grade 1:	1	1	3	1	3	
- Congestion :	-	-	-	5	-	
Grade 3:	-	-	-	4	-	
Grade 4:	-	-	-	1	-	
TONGUE :	40	40	38	47	48	
- S'epith inflamm cell:	5	1	-	2	2	
Grade 1:	5	1	-	2	2	
Grade 2:	-	-	-	-	-	
- Inflamm cells/muscle:	7	17	14	13	11	
Grade 1:	7	17	14	12	10	
Grade 2:	-	-	-	1	1	
- Periarteritis :	4	2	2	-	3	
Grade 1:	2	2	2	-	2	
Grade 2:	2	-	-	-	1	
Grade 3:	-	-	-	-	-	
STOMACH NONGLANDULAR :	40	40	40	50	50	
- Hyperpl/hyperk epith:	-	-	-	2	5	
Grade 1:	-	-	-	1	3	
Grade 2:	-	-	-	-	2	
Grade 4:	-	-	-	1	-	
- S'epith inflam cells:	-	-	-	1	-	
Grade 3:	-	-	-	1	-	
- S'mucosal congestion:	-	-	-	-	1	
Grade 2:	-	-	-	-	1	
- S'mucosal cyst LR :	-	1	1	-	-	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
STOMACH GLANDULAR :	40	40	40	50	50	
- Ectopic nongld epith:	3	-	5	4	1	
Grade 1:	3	-	5	4	1	
Grade 2:	-	-	-	-	-	
- Cong sup lam propria:	-	-	-	-	1	
Grade 2:	-	-	-	-	-	
Grade 3:	-	-	-	-	1	
- Peritonitis :	1	-	1	-	-	
Grade 2:	1	-	-	-	-	
Grade 3:	-	-	1	-	-	
DUODENUM :	40	40	39	50	50	
- Mucosal congestion :	-	-	-	-	1	
Grade 2:	-	-	-	-	-	
Grade 3:	-	-	-	-	1	
- Autolytic changes :	1	-	1	2	-	
Grade 2:	1	-	-	1	-	
Grade 5:	-	-	1	1	-	
JEJUNUM :	40	40	39	50	50	
- Mucosal congestion :	-	1	-	-	2	
Grade 1:	-	1	-	-	1	
Grade 2:	-	-	-	-	1	
- Autolytic changes :	1	-	2	8	1	
Grade 1:	-	-	-	1	-	
Grade 2:	1	-	1	1	-	
Grade 3:	-	-	-	2	-	
Grade 4:	-	-	-	1	-	
Grade 5:	-	-	1	3	1	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	50	50
ILEUM :	40	40	39	50	50
- Mucosal congestion :	-	-	-	-	1
Grade 1:	-	-	-	-	1
- Autolytic changes :	1	-	1	3	-
Grade 1:	-	-	-	1	-
Grade 2:	1	-	1	-	-
Grade 4:	-	-	-	1	-
Grade 5:	-	-	-	1	-
CECUM :	40	40	39	50	50
- S'mucosal congestion:	-	-	-	-	1
Grade 1:	-	-	-	-	1
- S'mucosal inflamm :	-	-	-	1	-
Grade 1:	-	-	-	1	-
- Autolytic changes :	1	-	-	1	-
Grade 2:	1	-	-	-	-
Grade 4:	-	-	-	1	-
COLON :	40	40	39	50	50
- Autolytic changes :	1	-	-	1	-
Grade 2:	1	-	-	-	-
Grade 4:	-	-	-	1	-
RECTUM :	40	40	38	50	50
- S'mucosal congestion:	-	-	-	-	1
Grade 2:	-	-	-	-	1
- Autolytic changes :	-	-	-	1	-
Grade 4:	-	-	-	1	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
LIVER :	40	40	40	50	50	
- Bile duct hyperpl :	12	14	7	3	5	
Grade 1:	8	8	1	2	2	
Grade 2:	4	2	4	1	2	
Grade 3:	-	4	2	-	1	
- EMH/inflamm cells :	13	13	8	2	10	
Grade 1:	8	9	7	1	6	
Grade 2:	4	3	1	-	2	
Grade 3:	1	1	-	1	2	
- Hepatocyte necrosis :	1	2	1	-	-	
Grade 1:	1	2	1	-	-	
Grade 2:	-	-	-	-	-	
- Hepatocyte vacuolat :	-	-	1	-	1	
Grade 1:	-	-	1	-	1	
Grade 2:	-	-	-	-	-	
- Basophilic hepats :	-	-	1	-	1	
Grade 1:	-	-	1	-	1	
- Clear Cell Focus :	-	1	-	-	1	
Grade 1:	-	1	-	-	1	
- Peritonitis :	1	-	-	-	-	
Grade 2:	1	-	-	-	-	
- Autolytic changes :	-	-	-	1	-	
Grade 1:	-	-	-	1	-	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
PANCREAS :	40	39	40	49	50	
- Autolytic changes :	-	-	1	1	-	
Grade 2:	-	-	1	1	-	
- Exocrine nec/inflamm:	-	-	2	2	1	
Grade 1:	-	-	2	2	1	
Grade 2:	-	-	-	-	-	
- Islet cell hyperpl :	9	7	12	12	16	
Grade 1:	9	7	12	12	16	
Grade 2:	-	-	-	-	-	
- Periarteritis :	1	-	-	-	-	
Grade 2:	1	-	-	-	-	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	50	50
KIDNEYS :	40	40	40	50	50
- Nephroblastoma :	-	-	-	-	1
- CPN :	1	2	1	-	-
Grade 1:	-	2	1	-	-
Grade 2:	1	-	-	-	-
Grade 3:	-	-	-	-	-
Grade 4:	-	-	-	-	-
- Cort basophilic tubs:	10	16	11	9	10
Grade 1:	8	16	10	9	9
Grade 2:	2	-	1	-	1
- Cortical cyst(s) :	-	-	1	1	1
Grade 1:	-	-	1	1	1
- Dil cortical tubules:	-	-	1	2	2
Grade 1:	-	-	1	1	1
Grade 2:	-	-	-	1	1
- Dys mineralisation :	10	15	9	12	12
Grade 1:	10	12	8	9	10
Grade 2:	-	3	-	3	2
Grade 3:	-	-	1	-	-
- Inflamm cell pelvis :	2	-	2	1	-
Grade 1:	2	-	1	-	-
Grade 2:	-	-	1	1	-
- S'pelvic inflam cell:	2	-	2	1	-
Grade 2:	2	-	1	1	-
Grade 3:	-	-	1	-	-
- Pelvic dilation :	-	1	-	1	1
Grade 1:	-	1	-	-	1
Grade 2:	-	-	-	1	-
- Hyperpl pelvic uroth:	-	-	-	1	-
Grade 2:	-	-	-	1	-
- Papillary cyst :	1	-	-	-	-
Grade 1:	1	-	-	-	-
- Autolytic changes :	-	-	-	1	-
Grade 2:	-	-	-	1	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
URETERS :	40	40	40	50	50	
- Lumenal dilation :	-	-	-	1	-	
Grade 1:	-	-	-	1	-	
URINARY BLADDER :	38	39	39	47	49	
- S'epith inflam cells:	-	-	3	4	2	
Grade 1:	-	-	3	4	2	
OVARIES :	40	40	40	50	50	
- Granulosa C.Tumor/M :	1	-	-	-	-	
- Reduced activity :	14	12	11	7	9	
Grade 1:	3	2	1	1	1	
Grade 2:	6	8	8	5	2	
Grade 3:	4	2	2	1	6	
Grade 4:	1	-	-	-	-	
- Cysts fat necrosis :	1	-	-	-	-	
Grade 3:	1	-	-	-	-	
- Cyst :	4	3	-	3	1	
Grade 1:	2	1	-	2	1	
Grade 2:	2	1	-	1	-	
Grade 3:	-	1	-	-	-	
- Corpora lutea absent:	12	11	11	7	9	
OVIDUCTS :	40	40	40	50	50	
- Ovum in lumen :	1	2	-	1	2	
- Dilated lumen :	-	1	-	-	-	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	50	50
UTERUS :	40	40	40	50	50
- Diestrus :	6	4	8	11	14
- Proestrus :	3	3	3	15	4
- Estrus :	1	3	1	3	4
- Metestrus :	-	4	5	5	2
- Anestrus :	30	26	23	16	26
- Decidual reaction :	-	-	1	-	-
Grade 3:	-	-	1	-	-
- Lumenal dilation :	5	4	7	15	6
Grade 1:	2	2	-	-	3
Grade 2:	1	-	4	7	-
Grade 3:	1	1	2	7	1
Grade 4:	1	-	1	1	2
Grade 5:	-	1	-	-	-
- Cyst endomet hyperpl:	4	2	2	3	3
Grade 1:	-	-	-	-	1
Grade 2:	2	2	2	1	-
Grade 3:	2	-	-	2	2
- Endometrial hyperpl :	1	1	2	-	-
Grade 1:	1	1	-	-	-
Grade 2:	-	-	1	-	-
Grade 3:	-	-	1	-	-
- Squamous metaplasia :	7	4	7	3	4
Grade 1:	1	1	-	-	1
Grade 2:	4	1	6	2	-
Grade 3:	2	1	1	1	3
Grade 4:	-	1	-	-	-
- Cystic endomet gland:	-	-	1	-	-
Grade 2:	-	-	1	-	-
- Endometrial polyp :	2	2	3	3	1

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
CERVIX :	40	40	40	50	50	
- Cystic gld/sq metapl:	1	-	1	1	2	
Grade 3:	1	-	1	-	1	
Grade 4:	-	-	-	1	1	
- Endometrial polyp :	-	-	-	-	2	
- Lumenal dilation :	1	-	-	3	-	
Grade 3:	-	-	-	3	-	
Grade 4:	1	-	-	-	-	
- Squamous metaplasia :	4	2	4	2	4	
Grade 1:	-	1	-	1	-	
Grade 2:	2	-	3	1	1	
Grade 3:	2	1	1	-	2	
Grade 4:	-	-	-	-	1	
- Endometrial hyperpl :	1	-	-	-	2	
Grade 1:	1	-	-	-	1	
Grade 2:	-	-	-	-	1	
VAGINA :	40	40	40	50	50	
- Mucification epith :	16	16	11	11	17	
Grade 1:	8	7	6	3	7	
Grade 2:	5	8	5	5	5	
Grade 3:	3	1	-	3	5	
- Keratinisation epith:	9	7	5	5	7	
Grade 1:	4	6	3	-	2	
Grade 2:	4	1	2	4	4	
Grade 3:	1	-	-	1	1	
- Debris in the lumen :	3	12	11	11	10	
Grade 1:	2	9	3	2	5	
Grade 2:	-	3	7	7	5	
Grade 3:	1	-	1	2	-	
- Leiomyoma :	1	-	-	-	-	
CLITORAL GLANDS :	1	-	-	-	-	
- Keratoacanthoma :	1	-	-	-	-	
- Abscess :	1	-	-	-	-	
Grade 3:	1	-	-	-	-	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
PITUITARY GLAND :	38	40	39	48	50	
- Multiloc cyst p dist:	-	-	1	-	1	
Grade 1:	-	-	1	-	1	
Grade 2:	-	-	-	-	-	
Grade 3:	-	-	-	-	-	
- Cyst(s) :	-	2	1	1	-	
Grade 1:	-	2	1	1	-	
Grade 2:	-	-	-	-	-	
Grade 3:	-	-	-	-	-	
- Hyperplasia p dist :	1	-	1	-	1	
Grade 1:	-	-	-	-	1	
Grade 2:	1	-	1	-	-	
- Vacuolation par nerv:	2	-	-	-	-	
Grade 2:	2	-	-	-	-	
- Adenoma:P.Distalis :	1	1	-	-	3	
- Degen/vac p distalis:	1	-	-	-	-	
Grade 2:	1	-	-	-	-	
THYROID GLAND :	40	40	40	50	50	
- Carcinoma:Follicular:	-	-	-	-	1	
- Ultimobranchial cyst:	1	-	-	-	1	
- C cell hyperplasia :	3	1	6	2	1	
Grade 1:	3	1	6	2	1	
Grade 2:	-	-	-	-	-	
- C-cell Adenoma :	-	-	2	-	2	
- C-cell Carcinoma :	-	1	-	1	1	
- Cystic follicle(s) :	1	-	-	-	-	
Grade 1:	1	-	-	-	-	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: Ko, INCL. DEATHS

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	50	50
ADRENAL GLANDS :	40	40	40	50	50
- Cortical vacuolation:	-	1	1	-	-
Grade 1:	-	1	1	-	-
Grade 2:	-	-	-	-	-
- Cort cystic degen :	5	6	7	13	9
Grade 1:	2	6	7	11	7
Grade 2:	3	-	-	2	1
Grade 3:	-	-	-	-	1
- Cortical hyperplasia:	3	2	3	3	5
Grade 1:	3	2	3	2	5
Grade 2:	-	-	-	1	-
- Medullary hyperpl :	2	1	1	-	-
Grade 1:	2	1	1	-	-
Grade 2:	-	-	-	-	-
- Phaeochromocytoma :	-	1	1	-	1
- Ganglioneuroma :	-	-	-	1	-
- Osteoid deposition :	-	-	1	-	-
Grade 3:	-	-	1	-	-
- Peritonitis :	-	-	-	-	1
Grade 2:	-	-	-	-	1
SPLEEN :	40	40	40	50	50
- Hemosiderin deposit :	24	31	28	30	29
Grade 1:	12	19	16	19	8
Grade 2:	11	10	7	8	21
Grade 3:	1	2	5	2	-
Grade 4:	-	-	-	1	-
- EMH :	33	33	22	23	31
Grade 1:	5	13	6	7	7
Grade 2:	24	18	12	11	21
Grade 3:	1	2	4	5	2
Grade 4:	3	-	-	-	1
- Prom marginal zone :	3	1	2	-	1
Grade 1:	2	1	2	-	1
Grade 2:	1	-	-	-	-
- Lymphoid atrophy :	1	-	-	-	1
Grade 4:	1	-	-	-	1

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
THYMUS :	40	38	36	49	48	
- Lymphoid atrophy :	27	31	25	20	26	
Grade 1:	11	12	7	10	10	
Grade 2:	12	13	13	9	13	
Grade 3:	4	5	5	1	3	
Grade 4:	-	1	-	-	-	
- Red medullary tissue:	-	-	-	1	-	
Grade 2:	-	-	-	1	-	
- Epithelial cyst(s) :	15	16	14	17	24	
Grade 1:	7	15	10	11	8	
Grade 2:	5	1	3	4	13	
Grade 3:	2	-	1	2	3	
Grade 4:	1	-	-	-	-	
- Hemorrhage/congest :	-	1	1	8	-	
Grade 1:	-	1	1	3	-	
Grade 2:	-	-	-	5	-	
MANDIBULAR LN RIGHT :	40	39	40	49	50	
- Sinusoidal congest :	6	3	1	5	3	
Grade 1:	5	3	-	5	3	
Grade 2:	1	-	1	-	-	
- Sinusoid histiocytes:	2	3	3	-	-	
Grade 1:	2	3	3	-	-	
PAROTID GLAND, RIGHT :	39	39	40	49	50	
- Ductular hyperplasia:	-	-	1	-	1	
Grade 2:	-	-	1	-	-	
Grade 3:	-	-	-	-	1	
SUBLING.GLAND, RIGHT :	40	39	40	50	50	
- Vacuolation serous :	1	-	-	-	-	
Grade 1:	1	-	-	-	-	
Grade 2:	-	-	-	-	-	

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	05
NO.ANIMALS:	40	40	40	50	50
HARDERIAN GLANDS :	38	39	37	48	50
- Granulomatous inflam:	-	1	-	-	-
Grade 3:	-	1	-	-	-
MAMMARY GLAND :	40	40	39	45	49
- Fibroadenoma :	1	3	-	-	4
- Hyperplasia :	1	1	3	-	4
Grade 1:	-	-	2	-	-
Grade 2:	1	1	1	-	4
- Dist acin/secretion :	4	-	3	1	1
Grade 1:	3	-	2	1	-
Grade 2:	-	-	1	-	-
Grade 3:	1	-	-	-	1
- Galactocele(s) :	-	-	1	-	-
Grade 1:	-	-	1	-	-
- Adenocarcinoma :	4	2	-	-	3
- Fibrosarcoma :	-	1	-	-	-
- Malignant mixed :	-	1	-	-	-
SKIN/SUBCUTIS :	38	37	39	46	49
- Epiderm hyperplasia :	1	-	-	-	-
Grade 1:	-	-	-	-	-
Grade 2:	1	-	-	-	-
- Squamous/seb hyperpl:	-	1	-	1	-
Grade 2:	-	1	-	1	-
- Epidermoid cyst :	-	1	-	-	-
JOINT,KNEE,RIGHT :	39	40	39	50	50
- Osteoarthrosis :	6	2	3	2	2
Grade 1:	6	2	3	2	2
EYES :	39	40	40	50	49
- Autolytic changes :	-	-	-	1	-
Grade 3:	-	-	-	1	-

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
TAIL :	-	1	-	-	-	
- Prom adnexal element:	-	1	-	-	-	
Grade 1:	-	1	-	-	-	
Grade 2:	-	-	-	-	-	
INJECTION SITE :	39	39	38	44	46	
- Scab(s) :	1	-	-	1	-	
Grade 1:	1	-	-	-	-	
Grade 2:	-	-	-	-	-	
Grade 3:	-	-	-	1	-	
- Hemorrhage :	9	6	12	7	4	
Grade 1:	5	4	10	2	2	
Grade 2:	3	1	1	2	2	
Grade 3:	-	1	1	2	-	
Grade 4:	1	-	-	1	-	
- Necrosis :	-	-	2	1	1	
Grade 1:	-	-	2	1	1	
Grade 2:	-	-	-	-	-	
Grade 3:	-	-	-	-	-	
- Chronic inflammation:	34	33	33	34	40	
Grade 1:	33	32	23	23	31	
Grade 2:	1	1	10	10	9	
Grade 3:	-	-	-	1	-	
- Cystic space :	5	8	11	9	4	
Grade 1:	4	8	10	8	4	
Grade 2:	1	-	1	1	-	

NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX STATUS AT NECROPSY
MALE

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						MALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	51	50	
BRAIN :	40	40	40	50	50	
- Astrocytoma :	-	-	-	1	1	
RECTUM :	40	40	40	50	50	
- Fibrosarcoma :	-	-	1	-	-	
PANCREAS :	39	40	40	50	50	
- Acinar Adenoma :	-	2	-	-	-	
URINARY BLADDER :	40	39	38	50	50	
- Papilloma trans cell:	-	-	-	1	-	
PITUITARY GLAND :	39	40	38	50	50	
- Adenoma:P.Distalis :	-	-	-	-	1	
THYROID GLAND :	40	40	40	50	50	
- C-cell Adenoma :	6	2	1	4	2	
- C-cell Carcinoma :	-	1	-	1	1	
ADRENAL GLANDS :	40	40	40	50	50	
- Cortical adenoma :	-	-	1	-	-	
THYMUS :	39	39	38	47	47	
- Thymoma :	-	-	1	-	1	
MAMMARY GLAND :	37	37	38	46	47	
- Fibroadenoma :	2	-	-	1	-	
- Adenocarcinoma :	-	1	-	-	-	
SKIN/SUBCUTIS :	40	40	39	49	50	
- Keratoacanthoma :	1	1	1	-	-	
- Fibrosarcoma :	1	2	-	2	-	

FEMALE

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: Ko, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	
NO.ANIMALS:	40	40	40	50	50	
KIDNEYS :	40	40	40	50	50	
- Nephroblastoma :	-	-	-	-	1	
OVARIES :	40	40	40	50	50	
- Granulosa C.Tumor/M :	1	-	-	-	-	
UTERUS :	40	40	40	50	50	
- Endometrial polyp :	2	2	3	3	1	
CERVIX :	40	40	40	50	50	
- Endometrial polyp :	-	-	-	-	2	
VAGINA :	40	40	40	50	50	
- Leiomyoma :	1	-	-	-	-	
CLITORAL GLANDS :	1	-	-	-	-	
- Keratoacanthoma :	1	-	-	-	-	
PITUITARY GLAND :	38	40	39	48	50	
- Adenoma:P.Distalis :	1	1	-	-	3	
THYROID GLAND :	40	40	40	50	50	
- Carcinoma:Follicular:	-	-	-	-	1	
- C-cell Adenoma :	-	-	2	-	2	
- C-cell Carcinoma :	-	1	-	1	1	
ADRENAL GLANDS :	40	40	40	50	50	
- Phaeochromocytoma :	-	1	1	-	1	
- Ganglioneuroma :	-	-	-	1	-	
MAMMARY GLAND :	40	40	39	45	49	
- Fibroadenoma :	1	3	-	-	4	
- Adenocarcinoma :	4	2	-	-	3	
- Fibrosarcoma :	-	1	-	-	-	
- Malignant mixed :	-	1	-	-	-	

NEOPLASTIC LESIONS STATUS AT NECROPSY

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

EVALUATION OF NEOPLASTIC LESIONS
STATUS AT NECROPSY: KO, INCL. DEATHS

NUMBER OF ANIMALS WITH NEOPLASMS:

DOSE GR:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.EXAM:	40	40	40	40	40	40	50	50	50	50
NO.AFF.:	10	10	9	12	5	6	10	5	6	17
% :	25.0	25.0	22.5	30.0	12.5	15.0	20.0	10.0	12.0	34.0

DOSE GR:	TOTAL	
SEX :	M	F
NO.EXAM:	220	220
NO.AFF.:	40	50
% :	18.2	22.7

NUMBER OF ANIMALS WITH MORE THAN ONE PRIMARY NEOPLASM:

DOSE GR:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.EXAM:	40	40	40	40	40	40	50	50	50	50
NO.AFF.:	1	1	0	0	0	0	0	0	0	2
% :	2.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0

TEST ARTICLE : Insulin 454-NNC 0100-0000-0454 PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM : RAT, 52-week, Subcutaneous DATE : 20-JAN-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

EVALUATION OF NEOPLASTIC LESIONS
STATUS AT NECROPSY: KO, INCL. DEATHS

DOSE GR:	TOTAL	
SEX :	M	F
NO.EXAM:	220	220
NO.AFF.:	1	3
% :	0.5	1.4

```
--------------------------------------------------------------------------------
TEST ARTICLE   : Insulin 454-NNC 0100-0000-0454     PATHOL. NO.: 65252 (b) (6)
TEST SYSTEM    : RAT, 52-week, Subcutaneous         DATE       : 20-JAN-10
SPONSOR        : Novo Nordisk A/S                   PathData©System V6.2a2
--------------------------------------------------------------------------------
EVALUATION OF NEOPLASTIC LESIONS
STATUS AT NECROPSY: KO, INCL. DEATHS
--------------------------------------------------------------------------------


NUMBER OF PRIMARY NEOPLASMS/GROUP/SEX:
................................................................................
DOSE GR:     01          02          03          04          05
SEX    :   M     F     M     F     M     F     M     F     M     F
................................................................................
PRIM.T.:  11    11     9    12     5     6    10     5     6    19


................................................................................

DOSE GR:    TOTAL
SEX    :   M     F
................................................................................
PRIM.T.:  41    53


................................................................................
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- **Special Evaluation**

1. Antibody formation: pretreatment and 48 hrs after last treatment

The antibody formation towards insulin 454 (1 out of 213 animals) and NPH insulin (1 out of 79 animals) was low.

2. Cell proliferation assessment: prior to study termination

The mammary gland cell proliferation BrdU labeling index (L.I.) from the vehicle and treated groups did not show a significant trend with increasing dose. Therefore cell proliferation was not increased by insulin 454-treatment and no significant dose-response relationship was seen.

Female rat mammary gland histopathological changes (incidences) and cell proliferation BrdU labelling index

Group	1 (control)	2	3	4	5
Treatment	**Vehicle**	**Insulin 454 – low**	**Insulin 454 – mid**	**Insulin 454 – high**	**NPH insulin**
Histopathology[a]					
Hyperplasia	1/40	1/40	3/39	0/45	4/49
Fibroadenoma	1/40	3/40	0/39	0/45	4/49
Adenocarcinoma	4/40	2/40	0/39	0/45	3/49
Fibrosarcoma	0/40	1/40	0/39	0/45	0/49
Malignant mixed	0/40	1/40	0/39	0/45	0/49
Cell proliferation[b]					
Obs. mean BrdU L.I (%)[c]	2.67	2.88	2.14	1.70	2.75
BrdU L.I Ratio[d]	1	1.12	0.92	0.96	N/A

a – Summary of proliferative/neoplastic lesions in mammary gland.
b – Epithelial cell proliferation (BrdU labelling index, L.I) of female mammary gland no.4 tissue
c – Observed arithmetric mean of the BrdU labelling index L.I
d – Estimated (oestrus adjusted) mammary gland BrdU labelling index (L.I) ratios. Treatment comparison with vehicle (e.g Group 2/Group1). Cell proliferation was not increased by treatment with insulin 454 and no significant dose-response relationship was observed (p = 0.68).

- **Toxicokinetics/Glucose Monitoring:** Day 1, Week 25, and Week 52

All animals dosed with insulin 454 appeared to have been systemically exposure to the test article. The effect on the serum glucose level was most pronounced 3 hrs after dosing. The serum exposure to insulin 454 was similar for males and females. However, there was a slight tendency of higher total exposure (AUC0-24h) in males but somewhat higher peak concentration (Cmax) in females in Weeks 25 and 52. Overall, the observed treatment and dose related effects on the lowering of serum glucose levels were the expected pharmacological effect of the test article and NHP. The duration of the serum glucose lowering effect of the prolonged acting insulin 454 and the NPH insulin was 1-9 and 1-3 hr, respectively.

Estimated toxicokinetic parameters for insulin 454 after once daily s.c. administration of insulin 454 to male and female rats.

Day/Week	Dose (nmol/kg/day)	Sex	C_{max} (nM)	t_{max} (h)	$AUC_{(0\text{-}9h)}$ (h*nM)	$AUC_{(0\text{-}24h)}$ (h*nM)	AUC (h*nM)	$\%AUC_{extra}$	$t_{½}$ (h)	Rac_{Obs}
Day 1	20	F	46.6	1	224	NA	241	6.9	NR[b]	NA
		M	56.6	1	224	248	249	0.18	2.6	NA
	65	F	178	1	886	974	976	0.24	2.6	NA
		M	179	3	778	859	861	0.24	2.6	NA
	100	F	241	3	1170	1310	1310	0.32	2.7	NA
		M	233	3	1140	1290	1300	0.41	2.9	NA
Week 25	20	F	71.3	1	369	434	440	1.2	3.6	1.6[a]
		M	50.5	3	323	465	481	3.2	4.6	1.9
	50	F	174	3.	883	1020	1030	0.65	3.1	1.4
		M	153	3	957	1300	1310	1.1	3.5	2.0
	80	F	240	3	1030	1210	1220	0.84	3.2	1.2
		M	197	3	1080	1450	1480	2.0	4.0	1.4
Week 52	20	F	61.6	3	307	372	377	1.3	3.6	1.4[a]
		M	36.5	3	260	399	418	4.7	5.3	1.6
	40	F	109	3	571	707	719	1.6	3.8	1.2
		M	72.0	3	507	768	803	4.3	5.2	1.5
	60	F	135	1	596	766	779	1.7	4.0	1.0
		M	111	3	694	1000	1040	3.4	4.7	1.3

NA – Not Applicable; NR – Not reported; a – $AUC_{(0\text{-}9h)}$ used; b – see Table 6.

- **Dosing Solution Analysis**

The results of the dose formulation analysis were generally in accordance with the expected levels (ranging 80-124% of the nominal concentration).

7 Genetic Toxicology

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

Not conducted.

7.2 *In Vitro* Assays in Mammalian Cells

Not conducted.

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

Not conducted.

7.4 Other Genetic Toxicity Studies

Not conducted.

8 Carcinogenicity

Not conducted.

9 Reproductive and Developmental Toxicology

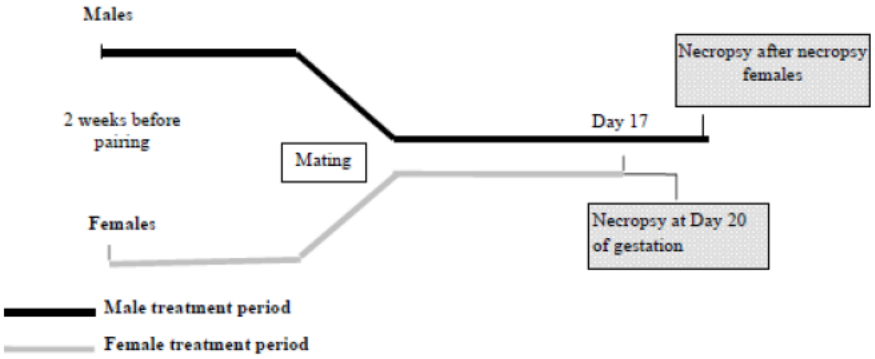
9.1 Fertility and Early Embryonic Development

Study No. NN206075: Seg I DRF rat

Preliminary combined fertility and embryo-fetal toxicity study by subcutaneous administration to Han Wistar rats [by (b) (4)]

Study design: This GLP study was 1) to assess the effect of insulin 454 (batch 412-N06048) on fertility and the progress and outcome of pregnancy in rats, 2) to assess exposure in pregnant females, and 3) to establish appropriate doses for main studies in rats. Three doses (25, 100, or 150 nmol/kg/day vs. 100 nmol/kg/day insulin NPH) were tested. Males were treated daily for 2 weeks prior to, and throughout pairing, until termination after necropsy of the females. Females were treated daily for two weeks prior to, and throughout pairing, until Day 17 after mating. Endpoints included clinical signs, body weight, food consumption, estrous cycles, mating procedure, organ weight, macroscopic/microscopic examination, and TK/glucose analyses.

Study design



Concentrations of formulations in terms of material as supplied were as follows:

Group	Treatment	Dose (nmol/kg/day)	Concentration (nmol/ml) @	Volume dosage (ml/kg)
1	control	0	0	0.5
2	insulin 454	25	50	0.5
3	insulin 454	100	200	0.5
4	insulin 454	150	300	0.5
5	insulin NPH	100	200	0.5

@ Expressed in terms of the test material as supplied

Findings:

- One MD, 1 HD, and 1 NPH females died. Clinical signs related to these deaths included unresponsive or vocalization behavior, slow respiration, convulsions, and/or body tremors.
- There were no effects on body weight gain and food consumption in males. On the other hand, body weight was not affected but higher food consumption was observed in MD or HD insulin 454 group from Days 1-7 and insulin NPH from Days 4-7 compared to the control group.
- Glucose analysis showed that lower serum glucose concentrations were measured in MD/HD insulin 454 or insulin NPH groups at 3 and 6 hrs (but returned to normal 9 hrs after dosing) after dosing on Day 17 after mating when compared to controls.
- In addition, insulin 454 or insulin NPH did not affect estrous cycles, pre-coital interval, mating performance, and fertility and corpora lutea counts. No conclusive adverse effect of insulin 454 or insulin NPH on post-implantation loss or the sex ratio. Lower mean fetal/placental weights in MD/HD insulin 454 and insulin NPH groups compared to the control (likely reflect the higher mean live litter sizes in these groups). No test article related macroscopic findings in dams or fetuses.
- Male reproductive organ weights were not affected by the treatment.
- TK analysis: The rate and systemic exposure to pregnant female rats to insulin 454 appeared to be dose-independent over the dose range 25-150 nmol/kg/day on Day 17 after mating.

Pharmacokinetic parameters of insulin 454 on Day 17 after mating following daily subcutaneous administration of insulin 454 to pregnant female rats

Dose level (nmol/kg/day)	C_{max} (pmol/L)	T_{max} (hours)	C_{24} (pmol/L)	$AUC_{(0-24)}$ (pmol.h/L)	Lambda z ($hours^{-1}$)	t½ (hours)
25	30920	3	-	146100[a]	0.2816	2.5
100	100700	3	1395	575100	0.1972	3.5
150	191800	3	1773	1156000	0.2211	3.1

[a] $AUC_{(0-9)}$

Insulin 454 did not affect fertility and mating performance, implantation, and number of live fetuses. In addition, there were no test article-related macroscopic fetal abnormalities during external examination. There was few deaths in both insulin 454 (MD/HD groups) and insulin NPH, likely attributed to hypoglycaemia. Based on this study, the doses for the pivotal study should be slightly reduced (i.e. max. of 125 nmol/kg/day insulin 454 and 90 nmol/kg/day insulin NPH).

Study No. NN206076: Seg I/II rat [PIVOTAL]

Combined fertility and embryo-fetal toxicity study in the Han Wistar rat by subcutaneous administration

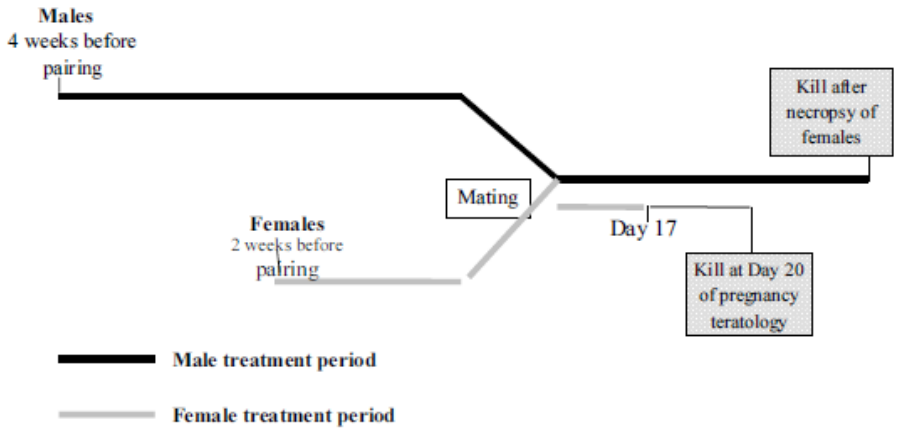
Study no.:	NN206067
Study report location:	Novo Nordisk
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Sept 6 2006 (protocol approval)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	412-N06048

KEY STUDY FINDINGS

- There were no clinical signs observed in rats receiving insulin 454 at 0, 20, 80, and 125 nmol/kg/day (vs. 80 nmol/kg/day insulin NPH).
- No adverse effects on body weight and food consumption were noted.
- No insulin 454 related macroscopic findings were observed.
- Insulin 454 did not have any effects on estrous cycles, pre-coital interval, male reproductive organ weights, fertility and mating performance, implantation and number of live fetuses.
- There were higher than expected incidence of major fetal abnormalities in insulin NPH group. There were also increases in minor skeletal abnormalities in the insulin NPH and insulin HD group (to a limit extend at LD group). There were minor differences from normal that were detected relatively frequently.
- NOAEL for fertility in rats was 125 nmol/kg/day.
- NOAEL for embryo-fetal development was < 20 nmol/kg/day (based on skeletal malformation at 80 and 125 nmol/kg/day groups and variations at 20, 80, and 125 nmol/kg/day groups).

Methods

Doses:	0, 20, 80, 125 nmol/kg/day (vs. 80 nmol/kg/day NPH)
Frequency of dosing:	Once daily
Dose volume:	0.5 ml/kg
Route of administration:	Sc injection
Formulation/Vehicle:	Phenol 1.50 mg/ml, m-Cresol 1.72 mg/ml, glycerol (b) (4) mg/ml in water for injection (pH 7.4)
Species/Strain:	Han Wistar rats
Number/Sex/Group:	22/sex/group
Satellite groups:	n/a
Study design:	Based on NN206075 Seg I DRF rat study
Deviation from study protocol:	There were no deviations from protocol.



Observations and Results

- **Mortality/Clinical Signs:** twice daily

No clinical signs were observed. Slight erythema and eschar formation were seen on a few occasions in 1 HD female.

- **Body Weight:** twice weekly

Insulin 454 had no effect on body weight in both males and females.

- **Feed Consumption:** twice weekly

Higher food consumption was observed in insulin NPH males (Days 1-3, 11-14, 18-21, and 25-28 prior to paring) and in insulin 454 HD females (Days 1-3 and 4-7 prior to paring). On the other hand, lower food consumption was recorded in insulin 454 HD and insulin NPH females during Days 18-19.

- **Toxicokinetics**

- **Dosing Solution Analysis**

All were within the estimated analytical concentrations.

- **Necropsy:** Day 20

No test-article related macroscopic findings were observed. Bruising at injection site was observed across all groups.

- **Organ Weights:** at Termination (including epididymides, prostate, seminal vesicles, testes)

Insulin 454 or insulin NPH had no effect on male reproductive organ weights.

Organ weights - group mean unadjusted and adjusted values (g) for males

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

Group /Sex		Terminal bodyweight	Epididymides	Prostate	Seminal Vesicles	Testis
Unadjusted Means						
1M	Mean	353.4	1.016	0.664	1.280	3.37
	SD	36.04	0.0790	0.1284	0.1962	0.254
	N	22	22	22	22	22
2M	Mean	346.1	1.008	0.704	1.240	3.30
	SD	26.85	0.0674	0.1473	0.2471	0.222
	N	22	22	22	22	22
3M	Mean	339.4	0.975	0.645	1.275	3.32
	SD	35.51	0.0628	0.1074	0.1853	0.306
	N	22	22	22	22	22
4M	Mean	350.4	0.974	0.626	1.280	3.23
	SD	27.58	0.0836	0.1061	0.2067	0.301
	N	22	22	22	22	22
Adjusted Means						
1M	Mean			0.662	1.277	3.37
2M	Mean			0.707	1.245	3.31
3M	Mean			0.652	1.288	3.35
4M	Mean			0.626	1.279	3.23

p≥0.05, no statistical significance

Organ weights - group mean unadjusted and adjusted values (g) for males

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

Group /Sex		Terminal bodyweight	Epididymides	Prostate	Seminal Vesicles	Testis
Unadjusted Means						
1M	Mean	353.4	1.016	0.664	1.280	3.37
	SD	36.04	0.0790	0.1284	0.1962	0.254
	N	22	22	22	22	22
5M	Mean	361.7	1.000	0.628	1.264	3.40
	SD	28.63	0.1582	0.0954	0.1937	0.361
	N	22	22	22	22	22
Adjusted Means						
1M	Mean			0.662	1.277	3.37
5M	Mean			0.621	1.251	3.37

p≥0.05, no statistical significance

Note: No maternal toxicity noted at 125 nmol/kg/day but the DRF study (NN206075) had deaths at 100 and 150 nmol/kg/day.

- **Estrous Cycles:** 10 days prior to pairing, daily vaginal smears

Estrous cycles were not affected by insulin 454.

Oestrous cycles - group values

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

Group	Number of animals		Regular 4 or 5 day cycles	Irregular cycle λ	Acyclic ψ
1	22	n	22	0	0
		(%)	(100)		
2	22	n	22	0	0
		(%)	(100)		
3	22	n	22	0	0
		(%)	(100)		
4	22	n	22	0	0
		(%)	(100)		
5	22	n	22	0	0
		(%)	(100)		

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

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

Mating performance and fertility were not affected by insulin 454.

Mating performance and fertility - group values

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	22	22	21	100	95	95
2M	22	22	21	100	95	95
3M	22	22	22	100	100	100
4M	22	22	22	100	100	100
5M	22	22	21	100	95	95
1F	22	22	21	100	95	95
2F	22	22	21	100	95	95
3F	22	22	22	100	100	100
4F	22	22	22	100	100	100
5F	22	22	21	100	95	95

- **Litter Data:**

Insulin 454 did not have an effect on pregnancy outcome (1 control, 1 LD, 1 NPH females were not pregnant). In addition, mean fetal and placental weights were not affected by insulin 454 or insulin NPH.

Litter data - group mean values on Day 20 of gestation

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

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	13.6	12.5	0.9	0.0	0.9	5.9	5.7	11.6	50.6	9.8	7.1
	SD	1.82	2.37				1.87	1.75	2.48			
	N	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	13.9	12.9	0.7	0.0	0.7	5.6	6.6	12.2	46.5	6.8	5.5
	SD	1.41	1.26				1.28	1.72	1.50			
	N	21	21	21	21	21	21	21	21	21	21	21
3F	Mean	13.4	11.5	0.8	0.0	0.9	5.2	5.5	10.6	48.6	15.6	9.2
	SD	1.76	3.22				2.44	2.42	3.65			
	N	22	22	22	22	22	22	22	22	22	22	22
4F	Mean	14.1	12.4	0.7	0.0	0.7	5.6	6.1	11.7	48.6	12.1	5.9
	SD	1.46	2.06				2.01	2.31	2.31			
	N	22	22	22	22	22	22	22	22	22	22	22
5F	Mean	14.0	12.9	0.8	0.1	0.9	6.9	5.1	12.0	56.6	7.2	6.6
	SD	1.72	1.51				2.45	1.82	1.53			
	N	21	21	21	21	21	21	21	21	21	21	21

p≥0.05, no statistical significance

Placental, litter and fetal weights - group mean values (g) on Day 20 of gestation

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

Group /Sex		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
1F	Mean	0.51	40.69	11.55	3.58	3.46	3.53
	SD	0.035	8.875	2.481	0.193	0.232	0.195
	N	20	20	20	20	20	20
2F	Mean	0.49	43.14	12.19	3.66	3.44	3.54
	SD	0.047	5.326	1.504	0.199	0.204	0.188
	N	21	21	21	21	21	21
3F	Mean	0.51	37.46	10.64	3.64	3.45	3.54
	SD	0.066	12.975	3.646	0.306	0.235	0.264
	N	22	22	22	22	22	22
4F	Mean	0.50	41.27	11.73	3.63	3.45	3.53
	SD	0.048	8.244	2.313	0.296	0.320	0.308
	N	22	22	22	22	22	22
5F	Mean	0.51	41.67	12.05	3.53	3.38	3.46
	SD	0.039	5.511	1.532	0.203	0.234	0.196
	N	21	21	21	21	21	21

p≥0.05, no statistical significance

- **Fetal Pathology:** assessment of visceral/skeletal development

There were no test article related major fetal abnormalities. Slight higher incidence of fetuses/litters with the major abnormalities of short/bent/thickened humerus or bent scapula were seen in MD/HD (as well as NPH). The applicant stated that these findings were within historical range and historical control data was provided by the applicant (see tables below).

Higher incidence of fetuses/litters with the minor abnormalities (i.e. precocious ossification, ribs with costal cartilage and offset alignment of pelvic girdle) were also observed. Minor fetal abnormalities considered to be related to insulin 454 were confined to 1) higher than expected incidences of precocious ossification of the cervical vertebral centra in all groups and 2) 13/14 or 14/14 ribs and complete 14th rib(s) with costal cartilage in the 20, 80, and 125 nmol/kg/day groups. No visceral malformation or variations but skeletal malformation/variations were present at 20 nmol/kg/day. The applicant provided background incidences and stated that these findings were within the historical control range.

Fetal examinations - major abnormalities - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

	Fetuses					Litters				
Group	1	2	3	4	5	1	2	3	4	5
Number examined	231	256	234	258	253	20	21	22	22	21
Number affected	2	5	3	5	10	2	4	3	5	7
Folded retina	-	1	-	-	-	-	1	-	-	-
Microphthalmia: disorganised retina	-	-	-	-	1	-	-	-	-	1
Fused maxilla to jugal	1	-	-	1	-	1	-	-	1	-
Displaced nasal septum	-	2[b,c]	-	-	-	-	1	-	-	-
Cleft palate	-	3[a,b,c]	-	1[f]	-	-	2	-	1	-
Anophthalmia: absent buccal cavity: agnathia: absent thyroid gland: muscular ventricular septal defect	-	-	-	-	1[h]	-	-	-	-	1
Misshapen, shortened mandible: cervical, lumbar vertebrae kyphosis, widely spaced: distorted ribcage: misshapen, medially thickened/kinked, marked ribs: bent ulna, radius, ilium, ischium	-	-	-	1[f]	-	-	-	-	1	-
Double/narrow aorta: membranous, muscular ventricular septal defect: atrial septal defect: malrotated heart	-	-	1	-	-	-	-	1	-	-
Transposition of aorta, pulmonary trunk: dorsally displaced pulmonary trunk	-	1[a]	-	-	1[h]	-	1	-	-	1
Short, bent, thickened humerus	1	1[d]	2[e]	2[g]	8[i,j,k]	1	1	2	2	5
Bent scapula	-	1[d]	1[e]	3[f,g]	3[i,j,k]	-	1	1	3	3

Superscript denotes fetuses with more than one abnormality

Fetal examinations - minor skeletal abnormalities/variants - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		111	127	118	124	120	20	21	22	22	21
Skeletal abnormalities											
Cranial	sutural bone	-	-	1	-	-	-	-	1	-	-
	bridge of ossification/partially fused maxilla to jugal	6	7	10	14	7	5	7	8	9	5
Ribs	medially thickened/kinked	9	6	8	9	14	8	2	5	5	8
Sternebrae	offset alignment	-	2	1	-	2	-	2	1	-	2
	bipartite ossified	-	1	-	-	2	-	1	-	-	2
Costal cartilage	partially fused	-	-	-	-	2	-	-	-	-	1
	offset alignment	-	1	-	-	1	-	1	-	-	1
	7th not connected to sternum	-	-	-	-	1	-	-	-	-	1
Total affected by one or more of the above		15	15	19	22	24	12	10	12	12	12
Rib and vertebral configuration											
Cervical rib		5	15	3	11	10	3	10	3	7	6
Number with 13/14 or 14/14 ribs		49	54	62	75	70	19	20	19	20	21
Complete 14th rib(s)/with costal cartilage		1	3	11	8	12	1	2	6	5	5
20 thoracolumbar vertebrae		-	6	1	3	10	-	4	1	3	3
Offset alignment pelvic girdle		1	3	9	6	3	1	2	6	4	2

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded

Fetal examinations - minor skeletal abnormalities/variants - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		111	127	118	124	120	20	21	22	22	21
Incomplete ossification/unossified											
Cranial centres		25	28	14	14	17	13	11	4	8	7
Hyoid		-	1	-	-	2	-	1	-	-	1
Vertebrae	thoracic	2	1	-	2	1	2	1	-	2	1
	sacrocaudal	1	1	-	-	2	1	1	-	-	2
Sternebrae	5th and/or 6th	34	30	27	30	38	16	13	14	14	13
	other	1	2	4	3	1	1	2	2	3	1
	total	34	30	29	31	39	16	13	14	14	14
Pelvic bones		-	-	-	1	-	-	-	-	1	-
Metacarpals/metatarsals		1	2	-	2	-	1	2	-	2	-
Precocious ossification											
Cervical vertebral centra (>5 ossified)		20	37	44	43	50	9	15	16	20	18
Additional observations at necropsy											
Dilated renal pelvis		1	-	-	-	1	1	-	-	-	1
Oedema		-	-	-	-	2	-	-	-	-	1
Shiny skin		1	-	-	2	-	1	-	-	2	-
Left umbilical artery		4	6	4	5	2	4	5	4	4	2

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded

Fetal examinations - minor visceral abnormalities - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	20	80	125	80

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		118	124	113	129	123	20	21	22	22	21
Number affected		23	20	24	30	26	16	15	15	19	13
Visceral abnormalities											
Thyroid	small	-	-	1	-	-	-	-	1	-	-
Thymus	partially undescended	3	1	1	1	-	3	1	1	1	-
Caudal vena cava	anomalous confluence with left hepatic vein	-	-	1	-	-	-	-	1	-	-
Diaphragm	thinning with protruding liver	3	1	4	3	5	3	1	3	3	5
Liver	additional lobe	-	1	-	-	-	-	1	-	-	-
	misshapen posterior caudate lobe	1	-	-	-	-	1	-	-	-	-
Kidney(s)	rudimentary renal papilla	-	-	-	1	-	-	-	-	1	-
Testis(es)	displaced	4	4	5	4	1	3	4	4	3	1
Umbilical artery	left sided	7	11	7	9	14	6	10	5	8	9
Haemorrhages	brain/spinal cord	6	2	4	6	3	5	2	3	5	3
	eye/surrounding tissue	1	-	1	-	-	1	-	1	-	-
	thymus	-	-	-	1	-	-	-	-	1	-
	thoracic cavity	-	-	-	-	1	-	-	-	-	1
	abdominal cavity	1	-	3	1	3	1	-	3	1	2
	hepatic	-	1	-	4	-	-	1	-	4	-
	subcutaneous	-	-	-	1	1	-	-	-	1	1
Additional observations at necropsy											
	shiny skin	1	-	-	-	1	1	-	-	-	1

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

HISTORICAL DATA PROVIDED BY THE APPLICANT

Historical Control data

Comment [KDB1]: Ok to leave in but please note the historical control data uses different route and vehicle than used in this study

Control incidence - major abnormalities - Wistar rats

Study number	1	2	3	4	5	6	7	8
Treatment - vehicle	MCL	lactose	MCL	water	MCL	R.O	RO	saline
Treatment period - days of gestation	6 to 17	-2 to 17	6 to 17	6 to17	6 to 19	6 to 17	6 to 17	6 to 17
Route	gavage	inhalation	gavage	gavage	gavage	gavage	gavage	IV
Necropsy start date	2.02	4.02	6.02	12.02	12.02	8.03	8.03	10.03
Number fetuses(litters) examined #	222(20)	223(20)	265(22)	262(21)	251(22)	235(22)	180(19)	261(24)
Anophthalmia/microphthalmia	-	-	-	-	-	-	-	-
Cleft palate	-	1(1)	2(2)b	2(1)ab	-	1(1)a	-	-
Curved/bent scapula/bent/short/thickened long bones	1(1)	-	1(1)b	1(1)b	-	-	-	2(2)

9	10	11	12	13	14	15	16	17
MCL	MCL	MCL	MCL	MCL, Tween 80	HPCD, Polysorbate	MCL, Tween 80	Glycerol, Gelatine, +	saline
6 to 19	6 to 17	6 to 17	6 to 17	6 to 17	0 to 17	6 to 17	0 to 17	-8, -1, 6, 15
gavage	gavage	gavage	gavage	gavage	gavage	gavage	gavage	intramuscular
7.04	3.05	7.05	9.05	12.05	4.06	6.06	7.06	8.06
233(21)	99(8)	255(22)	232(21)	57(5)	250(21)	246(22)	265(22)	247(21)
-	-	-	-	-	-	-	-	1(1)
-	-	-	-	-	-	-	-	-
3(2)a	1(1)	4(3)	2(2)	-	-	2(2)	2(2)	-

An individual fetus/litter may occur in more than one category, linked by superscript

R.O: reverse osmosis water
MCL: methylcellulose
HPCD: Hydroxy-propyl-b-CycloDextrin, polysorbate 80 in purified water
+: sodium carboxymethyl cellulose in purified water

Control incidence - minor skeletal and visceral abnormalities - Wistar rats

Study number		1		2		3		4	
In house/Supplier mated		IM		IM		IM		IM	
Treatment - vehicle		15% HPCD, 0.25%Polysorbate		025% MCL containing 0.05% Tween 80		4.0% glycerol, 0.4% gelatine, 0.6% sodium +		saline	
Treatment period - days of gestation		0 to 17		6 to 17		0 to 17		-8,-1,6,15	
Route		gavage		gavage		gavage		intramuscular	
Necropsy start date		4.06		6.06		7.06		8.06	
Skeletal abnormalities									
Number fetuses/litters examined #		126	21	122	22	129	22	122	21
Cranial	partially fused/bridge of ossification maxilla to jugal	15	8	8	5	5	5	17	11
Ribs	medially thickened/kinked	3	3	14	8	7	4	2	2
Rib and vertebral configuration									
Cervical rib		12	9	5	5	6	5	18	11
Number with 13/14, 14/14 ribs		46	18	54	17	67	21	60	18
Mean % fetuses per litter with 13/14, 14/14		38		45.9		51.9		49.4	
Complete 14th rib (with assoc. costal cartilage)		1	1	5	3	5	3	7	4
20 thoracolumbar vertebrae		3	3	1	1	1	1	4	2
Offset alignment pelvic girdle		2	1	3	3	5	4	2	2
Precocious ossification									
Cervical vertebral elements (>5 ossified)		16	8	22	13	26	9	20	11
Visceral abnormalities									
Number fetuses/litters examined #		124	21	118	22	132	22	124	21
Diaphragm	thinning with protrusion liver	4	4	6	6	-	-	4	4

Individual fetuses/litters may occur in more than one category, excludes fetuses with major abnormalities
IM In house mated
\+ carboxymethyl cellulose in purified water

9.2 Embryonic Fetal Development

Study No. NN206073: Seg II DRF rabbit

Preliminary embryo-fetal toxicity study by subcutaneous administration to rabbits [by (b) (4)]

Study design: This GLP study was 1) to assess the effect of insulin 454 (batch 412-N06048) on the progress and outcome of pregnancy in rats, and 2) to establish appropriate doses for main studies in rabbits. Three doses (5, 15, or 25 nmol/kg/day vs. 10, 20, or 25 nmol/kg/day insulin NPH) were given to animals from GDs 6-19 (14 days). Endpoints included clinical signs, body weight, food consumption, macroscopic examination, TK/glucose analyses, and reproductive assessment and fetus examination.

Study design

Group	Treatment	Dose (nmol/kg/day) @	Concentration (nmol/ml) @	Volume dosage (ml/kg/day)
1	Control	0	0	0.25
2	insulin 454	5	20	0.25
3	insulin 454	15	60	0.25
4	insulin 454	25	100	0.25
5	insulin NPH	25	100	0.25
6	insulin NPH	10	40	0.25
7	insulin NPH	20	80	0.25

@ Expressed in terms of the test material as supplied

Findings:

- Clinical signs (fast breathing, underactivity and etc – 4/6 animals died) were seen in 25 nmol/kg/day insulin NPH, therefore, two lower doses (10 and 20 nmol/kg/day) were added.
- No clinical signs were noted in insulin 454 treated animals. Insulin 454 had no effect on body weight, food consumption, and macroscopic findings.
- There was an indication of adverse effect on pre- and post-implantation survival of the conceptus (lower number of implantations and live fetuses was noted but interpretation was difficult due to small sample size). Mean fetal and placental weights were not affected by insulin 454.
- TK analysis: The 25 nmol/kg/day (less extent at 15 noml/kg/day) insulin 454 resulted in low mean serum glucose concentration at 3, 6, 9, hrs after dosing on GDs 6 and 19. The rate and exposure appeared to be dose-independent linear kinetics.

Dose level (nmol/kg/day)	C_{max} (pmol/L)		$AUC_{(0-24)}$ (pmol.h/L)	
	Day 6	Day 19	Day 6	Day 19
5	20090 (6810)	29080 (4390)	300800 (57900)	429800 (80700)
15	78180 (10490)	95210 (14680)	1009000 (318000)	1241000 (438000)
25	122300 (33000)	154800 (25700)	1423000 (399000)	2075000 (872000)

Based on this study, the doses for the pivotal embryo-fetal rabbit toxicity study should be limited to 20 nmol/kg/day of insulin 454 and insulin NPH.

Study No. NN206074: Seg II rabbit [PIVOTAL]

Embryo-fetal toxicity study in the rabbit by subcutaneous administration

Study no.:	NN206074
Study report location:	Novo Nordisk
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 17 2006 (protocol approval)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	412-N06048

KEY STUDY FINDINGS

- Insulin 454 at 0, 5, 10, and 20 mg/kg/day (daily from GDs 6-19) did not have any effect on maternal body weight, food consumption, macroscopic findings.
- There were no effects on implantation in the insulin 454 treated groups but in the insulin NPH treated groups, there was an increase in mean post implantation loss per litter, resulting in a marginally lower litter size when compared to controls.
- No fetal abnormalities were considered to be related to insulin 454 or insulin NPH. The incidence of fetuses/litters with minor skeletal abnormalities/variants was slightly higher in the insulin 454 and NPH insulin treated groups compared to the control group (but within background control range).
- NOAEL for maternal and embryo-fetal development toxicity is 20 mg/kg/day for insulin 454.

Methods

Doses:	0, 5, 10, 20 nmol/kg/day (vs 20 nmol/kg/day)
Frequency of dosing:	daily
Dose volume:	0.25 ml/kg/day
Route of administration:	Sc injection
Formulation/Vehicle:	Insulin 454: 1.50 mg/ml Phenol, 1.72 mg/ml m-Cresol, (b) (4) mg/ml Glycerol, (b) (4) in water for injection at a pH of 7.4 Insulin NPH: 0.65 mg/ml Phenol, 1.50 mg/ml m-Cresol, (b) (4) mg/ml Glycerol, (b) (4)

	(b) (4) in water for injection at a pH of 7.3
Species/Strain:	New Zealand white rabbits
Number/Sex/Group:	22/sex/group
Satellite groups:	n/a
Study design:	Based on NN206073 (Seg II DRF rabbit)
Deviation from study protocol:	Minor deviations

Study design

Group	Treatment	Dose (nmol/kg/day) @	Concentration (nmol/ml) @	Volume dosage (ml/kg/day)
1	Control	0	0	0.25
2	insulin 454	5	20	0.25
3	insulin 454	10	40	0.25
4	insulin 454	20	80	0.25
5	insulin NPH	20	80	0.25

@ Expressed in terms of the test material as supplied

Observations and Results

- **Mortality/Clinical Signs:** twice daily

One insulin NPH female gave birth prematurely on GD 29 (necropsy revealed no finding). No clinical signs were noted (except erythema at injection sites across all groups).

- **Body Weight:** weekly until mating and Days 0, 3, 6-19, 23, 26, and 29 after mating

Higher body weight gain was seen in insulin NPH females from GDs 6-19 than controls. No effect was noted in insulin 454 treated females.

- **Feed Consumption:** daily from Days 1 to 29 after mating

Higher food consumption was observed in insulin NPH females on GDs 6 and 10. No effect was noted in insulin 454 treated females.

- **Toxicokinetics:**

- **Dosing Solution Analysis**

All were within the expected values.

- **Necropsy/Macroscopic Findings:** GD 29

No macroscopic findings were observed with either insulin 454 or insulin NPH.

Macropathology - group distribution of observations for females

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

Tissue and finding	Group/sex:	1F	2F	3F	4F	5F
	Number Examined:	22	22	22	22	22
General comments						
Animal thin		0	0	0	1	0
Fetuses found in undertray		0	0	0	0	1
Fur stained		0	3	3	2	3
Hairloss		3	0	0	0	1
Scab		0	0	0	1	0
Paws						
Absent digit(s)		1	0	0	0	0
Mass(es)		1	0	0	0	0
Partially absent digit(s)		0	1	0	0	0
Injection site						
Subcutaneous bruising		5	0	1	4	9
Spleen						
Enlarged		0	0	0	0	1
Small		0	0	1	0	0
Swollen		2	0	0	0	1
Oviducts						
Cyst(s)		3	0	0	0	0

Note: fetuses found in undertray – it is not clear if this suggests spontaneous abortion (dead) or found after normal delivery (alive).

Macropathology - group distribution of observations for females

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

Tissue and finding	Group/sex:	1F	2F	3F	4F	5F
	Number Examined:	22	22	22	22	22
Head						
Snout misshapen		0	1	0	0	0
Kidneys						
Cyst(s)		0	0	0	0	1
Pale		0	1	0	0	1
Liver						
Enlarged		0	0	0	0	1
Firm		0	0	0	0	1
Lobular pattern accentuated		0	0	0	1	0
Pale		0	1	0	1	1
Ovaries						
Adhesion(s)		0	1	0	0	0
Lungs + bronchi						
Dark area(s)		0	0	1	1	1
Gall bladder						
Distended		0	0	0	1	0
Misshapen		0	0	0	1	0

Macropathology - group distribution of observations for females

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

Tissue and finding	Group/sex:	1F	2F	3F	4F	5F
	Number Examined:	22	22	22	22	22
Pancreas						
Splenulus		0	0	0	0	1

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

There were 6 non-pregnant animals (2 Control, 2 MD, 1 HD, 1 NPH females). In NPH treated females, there were higher mean numbers of early, late, and total embryo-fetal resorptions per litter, and the mean percentage of post-implantation loss per litter, when compared to the control (hence lower mean live litter size). However, the mean numbers of corpora lutea and implantations and the sex ratio were not affected by insulin 454 or insulin NPH. In addition, litter data as well as metal fetal and placental weights were not affected by either insulin 454 or insulin NPH.

Table 5 Seg II Study in Rabbits

C-SECTION DATA

Summary of female disposition

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

Group	Initial group size	Number of females - Premature Delivery	Number of females - Not pregnant	Number of females on Day 29 of gestation with live young
1	22	0	2	20
2	22	0	0	22
3	22	0	2	20
4	22	0	1	21
5	22	1	1	20

Litter data - group mean values on Day 29 of gestation

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

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	10.4	9.3	0.4	0.2	0.5	4.5	4.3	8.8	51.0	10.7	5.6
	SD	2.25	2.36				1.79	2.03	2.36			
	N	20	20	20	20	20	20	20	20	20	20	20
2F	Mean	9.7	8.6	0.3	0.3	0.5	4.5	3.5	8.0	55.7	10.4	5.5
	SD	2.00	2.04				1.74	1.84	1.76			
	N	22	22	22	22	22	22	22	22	22	22	22
3F	Mean	10.6	10.0	0.4	0.2	0.6	4.7	4.8	9.4	49.1	7.7	5.7
	SD	1.93	2.36				2.16	1.97	2.26			
	N	20	20	20	20	20	20	20	20	20	20	20
4F	Mean	10.5	9.5	0.6	0.3	1.0	4.8	3.8	8.5	56.2	12.2	9.1
	SD	1.54	2.54				2.17	2.02	2.29			
	N	21	21	21	21	21	21	21	21	21	21	21
5F	Mean	9.7	9.3	1.1	0.4	1.5 A	3.9	4.0	7.8	47.3	5.3	15.1 A
	SD	1.95	1.77				2.37	1.70	2.19			
	N	20	20	20	20	20	20	20	20	20	20	20

Significant when compared with Group 1: A - $p<0.05$; B - $p<0.01$

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

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

Group /Sex		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
1F	Mean	5.4	393.2	8.8	46.1	45.8	45.7
	SD	0.73	88.43	2.36	5.14	5.06	4.26
	N	20	20	20	20	20	20
2F	Mean	5.6	364.4	8.0	46.4	45.1	45.8
	SD	0.52	67.80	1.76	4.49	3.88	3.81
	N	22	22	22	22	22	22
3F	Mean	5.2	397.8	9.4	44.2	42.7	43.2
	SD	1.04	69.41	2.26	5.97	5.36	4.97
	N	20	20	20	20	20	20
4F	Mean	5.4	370.2	8.5	44.7	44.0	44.2
	SD	0.79	84.36	2.29	4.38	5.60	4.73
	N	21	21	21	21	21	21
5F	Mean	5.5	356.0	7.8	45.5	46.6	46.3
	SD	0.55	87.21	2.19	4.31	4.66	3.72
	N	20	20	20	19	20	20

$p \geq 0.05$, no statistical significance

- **Fetal Examination (Malformations, Variations, etc.):**

The incidence of fetuses with major abnormalities was not affected by insulin 454 or insulin NPH. In HD group, there was a slightly higher incidence of fetuses/litters with 12/13 or 13/13 ribs compared with controls (however, within historical range). There were also skeletal malformations/variations affecting metacarpals/phalanges, ribs, vertebrae, sternebrae, fontanelle, and pelvic girdle at 5 nmol/kg/day and visceral variation of lung, liver, and gall bladder at 20 nmol/kg/day. In insulin NPH group, higher incidence of fetuses/litters with cranial fissures/extra sutures, thoracic vertebral abnormalities, and offset alignment/bipartite ossified sternebrae (however, only a small numbers and most were within the historical range).

Table 6 Seg II Study in Rabbits

FETAL EXAMINATION DATA

Fetal examinations - major abnormalities - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

	Fetuses					Litters				
Group	1	2	3	4	5	1	2	3	4	5
Number examined	175	177	188	179	156	20	22	20	21	20
Number affected	1	1	2	1	1	1	1	2	1	1
Domed cranium: hydrocephaly	-	-	1	-	-	-	-	1	-	-
Partially fused frontal, parietal	1	-	-	-	-	1	-	-	-	-
Dilated ascending aorta, aortic arch, ductus arteriosus: dilated and dorsally displaced pulmonary trunk: transposition of pulmonary trunk, ascending aorta	-	-	-	1	-	-	-	-	1	-
Thoracic scoliosis	-	-	1	-	-	-	-	1	-	-
Protruding supraoccipital: lumbar spina bifida	-	-	-	-	1	-	-	-	-	1
Brachyury	-	1	-	-	-	-	1	-	-	-

Fetal examinations - minor skeletal abnormalities/variants - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		174	176	186	178	155	20	22	20	21	20
Number intact		108	108	118	111	96	20	22	20	21	20
Skeletal abnormalities											
Cranial	sutural bone	-	3	-	1	1	-	3	-	1	1
	fissures/extra sutures	1	1	2	1	5	1	1	1	1	5
	unossified areas	-	-	-	1	1	-	-	-	1	1
	bridge of ossification parietal/parietal	-	-	-	1	-	-	-	-	1	-
	bipartite/misshapen/small interparietal	-	-	1	1	-	-	-	1	1	-
	partially fused maxilla to jugal	-	-	-	1	1	-	-	-	1	1
	hyoid cornua bent	3	2	2	3	2	3	1	2	3	1
Vertebral element abnormality											
	thoracic	-	-	-	-	2	-	-	-	-	2
	lumbar	-	-	1	-	-	-	-	1	-	-
	scoliosis minimal	-	-	1	-	-	-	-	1	-	-
Ribs	branched	-	-	-	-	1	-	-	-	-	1
	medially thickened	-	-	2	-	1	-	-	2	-	1
	absent	-	-	-	-	1	-	-	-	-	1
	interrupted 13th	-	1	2	3	1	-	1	2	2	1
Sternebrae	additional centre(s)	-	-	2	1	1	-	-	1	1	1
	absent hemicentre	-	-	1	-	-	-	-	1	-	-
	offset alignment/bipartite ossified	-	1	1	1	3	-	1	1	1	3
	bridge of ossification/partially fused	-	2	1	3	2	-	2	1	2	1
	bifurcated 6th	-	-	-	1	-	-	-	-	1	-
Xiphoid cartilage	perforated	-	-	-	1	-	-	-	-	1	-
Costal cartilage	offset alignment	-	-	1	-	1	-	-	1	-	1
	branched/partially fused/fused	-	1	-	1	2	-	1	-	1	2
	additional	-	-	-	-	2	-	-	-	-	2
	7th not connected to sternum	4	10	16	4	8	1	5	6	2	4
	8th connected to sternum	-	1	-	-	-	-	1	-	-	-
Total affected by one or more of the above		8	21	27	20	25	4	11	11	13	14

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

Fetal examinations - minor skeletal abnormalities/variants - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined		174	176	186	178	155	20	22	20	21	20
Number intact		108	108	118	111	96	20	22	20	21	20
Rib and vertebral configuration											
Cervical rib		3	7	1	3	3	3	6	1	2	2
Short 12th rib(s)		-	-	-	-	1	-	-	-	-	1
Number with 12/13 or 13/13 ribs		65	67	77	95	65	18	19	17	20	19
18 thoracolumbar vertebrae		-	-	1	-	-	-	-	1	-	-
20 thoracolumbar vertebrae		30	31	49	41	40	12	11	12	12	15
Offset alignment pelvic girdle		5	6	6	9	9	5	4	6	7	6
14rib(s)		-	1	-	-	-	-	1	-	-	-
Incomplete ossification/unossified											
Enlarged posterior fontanelle		-	-	1	2	-	-	-	1	2	-
Posterior supra-orbital notch		-	-	1	-	-	-	-	1	-	-
Presphenoid		-	-	-	1	-	-	-	-	1	-
Vertebrae	cervical	-	3	3	2	2	-	2	3	2	1
	thoracic	2	1	-	2	-	2	1	-	2	-
	caudal	-	-	-	1	-	-	-	-	1	-
Sternebrae	5th	33	21	23	22	16	13	9	10	10	9
	other	4	2	0	3	3	3	2	0	2	3
	total	35	21	23	25	19	13	9	10	11	10
Pubes		-	-	-	1	-	-	-	-	1	-
Astragalus		-	-	-	1	-	-	-	-	1	-
Epiphyses		1	2	1	3	-	1	2	1	3	-
Metacarpals/phalanges		2	7	7	7	4	2	4	5	4	3
Precocious ossification											
Small anterior fontanelle		-	-	-	-	1	-	-	-	-	1
Ossified olecranon process		3	7	2	1	2	2	6	2	1	2
Ossified omosternum		1	1	1	1	-	1	1	1	1	-

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

Fetal examinations - minor visceral abnormalities - group incidences

Group	:	1	2	3	4	5
Compound	:	Control	Insulin 454	Insulin 454	Insulin 454	Insulin NPH
Dose (nmol/kg/day)	:	0	5	10	20	20

		Fetuses					Litters				
Group		1	2	3	4	5	1	2	3	4	5
Number examined at necropsy		174	176	186	178	155	20	22	20	21	20
Number of heads examined at detail visceral		66	68	68	67	59	20	22	20	21	20
Head	folded retina	1	-	-	-	1	1	-	-	-	1
	subdural haemorrhage	1	2	3	4	3	1	2	2	4	3
Total affected by one or more of the above		2	2	3	4	4	2	2	2	4	3
Additional observations at necropsy											
Interparietal region	cyst	-	-	-	1	-	-	-	-	1	-
Thyroid	enlarged	2	-	-	2	-	2	-	-	2	-
Lungs	unexpanded	1	-	-	1	-	1	-	-	1	-
	absent accessory lobe	-	-	-	1	1	-	-	-	1	1
Abdomen	blood in	1	-	-	-	-	1	-	-	-	-
Liver	additional lobe	-	-	-	1	-	-	-	-	1	-
Gall bladder	haemorrhage(ic)	-	-	3	-	2	-	-	1	-	2
	small/absent	2	3	3	2	4	1	3	2	2	3
	bilobed	-	-	-	2	-	-	-	-	2	-
Forepaw	flexure	-	-	-	1	-	-	-	-	1	-
Total number affected by one or more of the above		6	3	6	11	7	4	3	3	9	5

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

HISTORICAL DATA PROVIDED BY THE APPLICANT

Background Control Data

Note : Historical control data uses different route and vehicle than in this study.

Study number		1		2		3		4		5		6		7		8	
Source		(b) (4)															
In house/Supplier mated		IM		SM		IM		SM		SM		IM		SM		IM	
Treatment - vehicle		gel		Lactose		1% MCL		Lactose		0.2%sodium MCL in purified water		HP-β-CD		Lactose		glycerol/gelatine sodium mel	
Treatment period - days of gestation		6 to 19		7 to 19		6 to 28		7 to 19		6 to 19		6 to 19		7 to 19		6 to 19	
Route		IV		Inhalation		gavage		Inhalation		gavage		gavage		Inhalation		gavage	
Necropsy start date		02.06		03.06		04.06		4.06		5.06		6.06		8.06		8.06	
Number fetuses/litters examined#		175	20	180	21	45	6	151	18	206	20	154	18	151	18	193	21
Number intact skeletons/litters examined		112	20	90	21	21	6	73	18	132	20	98	18	73	18	91	21
Cranial	fissure/suture within	-	-	1	1	1	1	-	-	2	2	5	2	1	1	3	2
Vertebral	thoracic/min	-	-	4	3	-	-	-	-	-	-	1	1	1	1	-	-
Sternebrae	bipartite ossified/offset alignment/fragmented (^ inc min)	1	1	3^	3	1	1	1	1	-	-	-	-	3	3	-	-
Ribs	number with 12/13, 13/13 ribs	73	20	113	21	14	6	50	13	82	17	82	18	60	15	98	20
12/13,	mean % fetuses per litter with 13/13	44.8		63.0		35.9		36.3		39.7		55.5		36.8		48.1	

Individual fetuses/litters may occur in more than one category, excludes fetuses with major abnormalities
MCL:carboxymethylcellulose
IM In house mated
SM Supplier mated
Hydroxypropyl-β-cyclodextrin (HP-β-CD)

9.3 Prenatal and Postnatal Development

Study No. NN208335: Seg III DRF rat

Preliminary pre- and post-natal development study by subcutaneous injection (once daily) administration to Han Wistar rats [by (b) (4)]

Study design: This GLP study was 1) to assess the effect of insulin 454 (batch TQ50434) on pre- and post-natal development in rats, and 2) to establish appropriate doses for main studies in rats. Three doses (20, 80, and 125 nmol/kg/day vs. 80 nmol/kg/day insulin NPH) were given to animals from GDs 6- LDs 20. Endpoints included clinical signs, body weight, food consumption, macroscopic examination, parturition observations, gestation length, litter data (clinical signs, litter size, sex ratio, body weight), necropsy, glucose analysis.

Group	Treatment	Dose # (nmol/kg/day)	No. of females	Animal number
1	Control	0	8	1-8
2	NNC 0100-0000-0454	20	8	9-16
3	NNC 0100-0000-0454	80	8	17-24
4	NNC 0100-0000-0454	125	8	25-32
5	NPH insulin	80	8	33-40

Expressed in terms of the test substance as supplied

Findings: One death occurred in the MD group at time of parturition (attributed to hypoglycaemia). Only sight effects upon body weight gain and food consumption were noted in MD/HD groups. There were no test article-related effects upon pregnancy, litter size, and survival of the offspring. TK analysis: Mean plasma concentration of insulin 454 (measured at 3 and 9 hrs on Day 20) increased in proportion with the increase in dose. No insulin 454 was measurable on Day 5 (pre-dosing).

Day	Time after dosing		Serum concentration (nmol/L) Group 1 0	Group 2 20 nmol/kg/day	Group 3 80 nmol/kg/day	Group 4 125 nmol/kg/day
5 #	3 hours	Mean	NA	NA	NA	NA
		SD	NA	NA	NA	NA
	9 hours	Mean	NA	NA	NA	NA
		SD	NA	NA	NA	NA
20 @	3 hours	Mean	NA	12.9	164	220
		SD	NA	14.7	16.1	9.80
	9 hours	Mean	NA	1.94	9.84	24.5
		SD	NA	0.251	2.77	8.10

Day 5 after mating (prior to commencement of treatment)
@ Day 20 of lactation
NA - Not applicable

Based on this study, the dose levels of 20, 80, and 125 nmol/kg/day insulin 454 would be appropriate doses for the pivotal pre- and post-natal development rat toxicity study.

Study No. NN208336: Seg III rat [PIVOTAL]

Pre- and post-natal development study in the Han Wistar rat by subcutaneous administration

Study no.:	NN208336
Study report location:	Novo Nordisk, Denmark
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Jan 30 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	412-N08704 with 99.2% purity

KEY STUDY FINDINGS

- Daily treatment with insulin 454 (80 or 125 nmol/kg/day) or insulin NPH (80 nmol/kg/day) at the time of parturition could lead to maternal hypoglycaemia and death.
- Treatment of insulin 454 at 125 nmol/kg/day affected F1 offspring slightly more than treatment of insulin NPH at 80 nmol/kg/day (i.e. lower live birth index and viability index shortly after birth, birth weight and body weight gain). These affects were considered to be related to pharmacological effect of insulin.
- Treatment of insulin 454 at 80 nmol/kg/day had similar effects on live birth index and offspring body weight gain when compared to treatment of insulin NPH at 80 nmo/kg/day. These affects were considered to be related to pharmacological effect of insulin.
- No adverse effects were observed upon long term offspring development (i.e. physical and behavioral) or reproductive performance.

- The NOAEL was 20 mg/kg/day due to deaths in pregnant dams at 80 and 125 mg/kg/day. **Note: the applicant established the NOAEL to be 125 nmol/kg/day and the NOEL to be 20 nmol/kg/day since all findings were secondary to the exaggerated pharmacological effect of insulin in the dams.**

Methods

Doses:	0, 20, 80, 125 nmol/kg/day (vs. 80 nmol/kg/dayNPH)
Frequency of dosing:	Once daily
Dose volume:	0.5 ml/kg
Route of administration:	Sc injection
Formulation/Vehicle:	A solution containing 1.5 mg/ml phenol, 1.72 mg/ml *m*-cresol, 19.6 mg/ml glycerol, pH of 7.6
Species/Strain:	Wistar
Number/Sex/Group:	22/sex/group
Satellite groups:	n/a
Study design:	Based on Study NN208335 (Seg III DRF rat study)
Deviation from study protocol:	Minor deviation

Group	Treatment	Dose # (nmol/kg/day)	No. of females	Animal numbers
1	Control	0	22	1-22
2	NNC 0100-0000-0454	20	22	23-44
3	NNC 0100-0000-0454	80	22	45-66
4	NNC 0100-0000-0454	125	22	67-88
5	NPH insulin	80	22	89-110

Expressed in terms of the test substance as supplied

Group	Treatment	Dose (nmol/kg/day)	Concentration (nmol/mL)	Volume dose (mL/kg)
1	Control	0	0	0.5
2	NNC 0100-0000-0454	20	40	0.5
3	NNC 0100-0000-0454	80	160	0.5
4	NNC 0100-0000-0454	125	250	0.5
5	NPH insulin	80	160	0.5

Observations and Results

F_0 Dams

Survival/Clinical signs:	1 control female killed on GD20 (blockage in the vaginal area), 3 MD females found dead (hypoglycemia), 1 HD female found dead and 1 HD female killed (hypoglycemia), 2 NPH females killed (welfare and total litter loss) and 2 found dead (hypoglycaemia)
Body weight (GDs 0, 3, 6, 10, 14, 18, and 20 after mating and LDs 1, 4, 7, 11, 14, 18 and 21):	No effect was seen in LD females. Lower mean body weight change was noted in HD group on GDs 6-20. Slightly body weight loss early in lactation and sig. reduced body weight gain in HD group throughout lactation. Reduced body weight gain was seen in the insulin 454 MD group during LDs 1-18 and the insulin NPH group during LDs 1-21.
Feed consumption:	No effect was noted in LD females. It was unaffected during gestation but lower than control in HD females during LDs 7-20 and in MD females during LDs 11-19, and in NPH females during LDs 11-17.
Uterine content (i.e. gestation length, post-implantation, litter size):	Gestation length was not affected. The mean gestation index was similar between insulin 454 and control groups (lower for NPH insulin group, reflecting the deaths during late gestation). Post-implantation survival, numbers of implantations and litter size on Day 1 and sex ratio were similar across all groups. In addition, liver birth index was slightly lower than control in MD/HD females/litter and NPH females. Viability index was marginally low at HD females. None of these parameters were affected in LD females.
Necropsy observation:	
Toxicokinetics:	Animals receiving insulin 454 were systemically exposed at all dose levels at 3 and 9 hrs post dosing on Day 20, with exposure increasing with increasing dose.
Dosing Solution Analysis	The accepted range was 78-101%.
Other:	n/a

Table 7 Seg III Study in Rats

F0/F1 (PREWEANING) GENERATION DATA

Gestation length and gestation index - group mean values (F0)

Group	Number of pregnant animals		Gestation length (days) 22	22.5	23	23.5	Number of live litters born	Gestation index (%)
Statistical test:				Lt				Ca
1	22A	n	8	6	6	1	21	95
		(%)	(38)	(29)	(29)	(5)		
2	22	n	2	11	3	6	22	100
		(%)	(9)	(50)	(14)	(27)		
3	22B	n	0	12	7	1	20	91
		(%)		(60)	(35)	(5)		
4	21C	n	1	16	2	2	20	95
		(%)	(5)	(76)	(10)	(10)		
5	22D	n	0	13	2	4	18	82
		(%)		(68)	(11)	(21)		

A Percentage distribution of gestation lengths calculated from 21 animals - one pregnant female killed for welfare reasons on Day 20 after mating
B Percentage distribution of gestation lengths calculated from 20 animals - two pregnant females found dead, one on Day 19 and one on Day 21 after mating
C Excludes one pregnant female killed for welfare reasons on Day 15 after mating
D Percentage distribution of gestation lengths calculated from 19 animals - one pregnant female killed for welfare reasons on Day 20 after mating, one female found dead on Day 21 after mating and one pregnant female found dead on Day 22 after mating

Litter size - group mean values (F1)

Group		Implantations	Total litter size	Live litter size on Day							
			Day	Before cull		After cull					
			1	1	4	4	7	11	14	18	21#
Statistical test:		Wi	Wi	Wi	Wi						
1	Mean	12.5	11.2	11.1	11.0	7.9	7.9	7.9	7.9	7.9	6.9
	SD	2.2	2.3	2.2	2.2	0.4	0.4	0.5	0.5	0.5	1.1
	N	21	21	21	21	21	21	21	21	21	21
2	Mean	11.6	10.5	10.5	10.3	7.8	7.8	7.8	7.8	7.8	6.9
	SD	2.1	2.3	2.4	2.4	0.5	0.5	0.5	0.5	0.5	1.0
	N	21	22	22	22	22	22	22	22	22	22
3	Mean	11.8	11.1	10.3	10.3	7.2	7.1	7.2	7.2	7.2	6.1
	SD	2.3	2.6	3.8	3.8	2.0	2.0	2.0	2.0	2.0	2.1
	N	19	19	19	19	19	19	19	19	19	19
4	Mean	12.6	12.1	11.1	10.7	7.5	7.5	7.5	7.5	7.4	6.3
	SD	2.0	2.0	3.0	3.1	1.3	1.3	1.3	1.3	1.5	1.3
	N	18	18	18	18	18	18	18	18	18	18
5	Mean	12.5	11.9	11.6	11.6	8.0	8.0	7.9	7.9	7.9	7.9
	SD	2.0	1.9	2.0	2.0	0.0	0.0	0.2	0.2	0.2	0.2
	N	18	18	18	18	18	18	18	18	18	18

Some offspring killed for blood sampling on Day 20 of age

Offspring survival indices - group mean values (F1)

Group		Post implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%) on Day 7	11	14	18	21#
Statistical test:		Wi	Fe	Fe					
1	Mean	90.3	98.9	99.6	100.0	99.4	99.4	99.4	87.5
	N	21	21	21	21	21	21	21	21
2	Mean	89.1	99.5	98.7	100.0	100.0	100.0	100.0	88.5
	N	21	22	22	22	22	22	22	22
3	Mean	92.0	92.2	99.5	99.3	100.0	100.0	100.0	83.8
	N	19	19	19	19	19	19	19	19
4	Mean	95.9	87.5	91.4	100.0	100.0	100.0	98.6	84.7
	N	19	19	19	18	18	18	18	18
5	Mean	94.3	92.5	100.0	100.0	99.3	99.3	99.3	99.3
	N	19	19	18	18	18	18	18	18

Some offspring killed for blood sampling on Day 20 of age

Sex ratio - group mean values (F1)

Group		Total on Day 1			Live (before cull) on Day 1			Live (before cull) on Day 4			Live (after cull) on Day 4			Live (after cull) on Day 21#		
		M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
Statistical test:				Wa			Wa			Wa						
1	Mean	5.7	5.5	50.3	5.7	5.4	50.4	5.6	5.4	50.4	4.0	4.0	50.0	3.4	3.5	49.1
	SD	2.3	2.0	15.7	2.4	1.9	15.5	2.2	1.9	15.5	0.9	0.9	11.2	1.2	0.9	12.4
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
2	Mean	5.2	5.3	48.6	5.2	5.3	48.8	5.1	5.2	49.0	3.8	4.0	48.5	3.3	3.5	48.1
	SD	2.0	1.6	14.1	2.0	1.5	13.9	2.1	1.6	15.6	1.0	1.0	12.7	1.0	1.0	13.5
	N	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
3	Mean	5.7	5.4	52.6	5.2	5.2	49.4	5.2	5.1	49.6	3.7	3.4	51.1	3.2	2.9	50.2
	SD	2.0	2.4	15.9	2.5	2.6	19.2	2.5	2.6	19.1	1.3	1.1	15.1	1.4	1.0	15.1
	N	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
4	Mean	6.3	5.8	51.9	5.7	5.3	51.6	5.6	5.2	51.3	3.7	3.8	49.7	3.1	3.2	48.8
	SD	1.9	1.8	12.8	2.2	2.0	12.6	2.4	1.9	12.5	0.8	0.8	5.5	0.8	0.8	8.1
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
5	Mean	6.4	5.5	52.5	6.3	5.3	52.4	6.3	5.3	52.4	3.9	4.1	48.6	3.8	4.1	48.2
	SD	2.6	1.7	17.9	2.7	1.6	19.0	2.7	1.6	19.0	1.1	1.1	14.1	1.2	1.1	14.2
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18

Some offspring killed for blood sampling on Day 20 of age

F_1 Generation

Survival/Clinical signs:	There was an increase in the number of offspring dying soon after birth in HD group. All dose levels of insulin 454 had a small number of litters which were cold to touch. LD or HD group had a small number of litters contained a high incidence of offspring with little milk in the stomach/apparently unfed.
Body weight:	Day 1 of age in HD group, body weights were sig. lower and offspring weight gain to Day 13 of age was sig. low. However, mean overall weight gain during Days 1-21 of age was only slightly less than the control group and sig, for males only. In MD group or NPH group, overall offspring body weight gain during Days 1-21 of age was slightly lower than control.
Feed consumption:	
Physical development (i.e. motor activity, accelerating rotarod):	Reflexes and motor activity were unaffected by maternal treatment
Neurological assessment (i.e. morris maze performance):	Learning tests were also unaffected by maternal treatment
Reproduction (i.e. sexual maturation):	Selected HD males showed low body weight gain in the 1st week after allocation and delayed balanopreputial separation. However, there was no evidence upon reproductive capacity when mated at sexual maturity.
Other (F2 generation):	Litter data was not affected by maternal treatment.

Table 8 Seg III Study in Rats

F1 (PRE/POSTWEANING)/F2 GENERATION DATA

Clinical signs - group distribution of observations for offspring prior to weaning (F1)

Observation	Total number of offspring (litters) affected in Group				
	1	2	3	4	5
Found dead	5 (4)	2 (2)	15 (3)	32 (7)	14 (4)
Killed for reasons of animal welfare @	-	-	-	2 (2)	1 (1)
Missing	-	2 (2)	-	5 (2)	-
Total deaths (found dead, missing or killed for welfare reasons)	5 (4)	4 (3)	15 (3)	39 (8)	15 (5)
Patchy coat	-	-	-	8 (1)	-
Appear unfed/little or no milk in stomach	-	11 (1)	-	12 (2)	-
Thin build	-	1 (1)	-	1 (1)	-
Cold to touch	11 (1)	26 (3)	24 (2)	25 (3)	1 (1)
Tail – tip black	-	-	1 (1)	-	-
Eye(s) absent	-	-	-	1 (1)	-
Skin - pale	-	1 (1)	-	-	-
Underactive	-	-	-	1 (1)	-
Swelling and dark colouration on head	-	-	-	1 (1)	-
Bruising on head/muzzle	-	2 (2)	-	1 (1)	-
Bruising on hindlimb	-	-	1 (1)	-	-
Bruising on ventral/dorsal body surface	-	-	1 (1)	-	-

@ Excludes offspring killed for welfare reasons as a result of demise of the dam

Pre-weaning development - group mean values for offspring (F1)

Group		Surface righting Day of age	Air righting Day of age		Pupil reflex (% pass)	Startle response (% pass)
Statistical test:		Wi	Wi			
1	Mean	3.8	17.2	Number of animals tested	165	165
	SD	0.88	0.66	Number of animals failed	0	0
	N	21	21	% of animals passed	100.0	100.0
2	Mean	3.6	17.1	Number of animals tested	170	171
	SD	0.79	0.55	Number of animals failed	0	0
	N	22	22	% of animals passed	100.0	100.0
3	Mean	3.9	17.1	Number of animals tested	136	136
	SD	0.89	0.67	Number of animals failed	0	0
	N	19	19	% of animals passed	100.0	100.0
4	Mean	4.1	17.4	Number of animals tested	134	134
	SD	0.59	0.92	Number of animals failed	0	0
	N	18	18	% of animals passed	100.0	100.0
5	Mean	3.8	17.2	Number of animals tested	143	143
	SD	0.66	0.56	Number of animals failed	0	0
	N	18	18	% of animals passed	100.0	100.0

Macropathology - group distribution of findings for offspring (F1)

Observation	Total number of offspring (litters) affected in Group				
	1	2	3	4	5
Offspring dying before scheduled termination					
No. offspring (litters) examined	3 (2)	2 (2)	14 (2)	45 (7)	12 (5)
Thin build	-	-	-	1 (1)	-
No milk in stomach	2 (1)	1 (1)	9 (2)	38 (6)	8 (3)
Portion of liver within thoracic cavity	2 (1)	-	-	-	-
Placenta attached	-	-	5 (1)	1 (1)	-
Covered in shavings/bedding material	-	-	6 (1)	18 (4)	6 (1)
Multiple pale foci on ventral abdomen	-	-	-	2 (1)	-
Dark raised swelling on right rear of head	-	-	-	1 (1)	-
Cut on right forelimb	-	-	-	-	1 (1)
Offspring killed at scheduled termination #					
No. offspring (litters) examined	117 (21)	123 (22)	88 (19)	86 (18)	95 (18)
Small build	-	1 (1)	-	-	-
Liver					
raised area on median lobe adhering to diaphragm	-	-	1 (1)	-	-
raised yellow area on right median lobe	-	-	-	-	1 (1)
Kidney(s)					
unilateral/bilateral dilated renal pelvis	2 (2)	-	-	-	-
Testes/epididymides					
left testis and epididymis small and dark	-	1 (1)	-	-	-
Eyes					
right eye absent	-	-	-	1 (1)	-

Excludes autolysed, missing and grossly normal culled offspring
\# Includes offspring killed on Day 20 of age for blood sampling

Sexual maturation - group mean values for selected offspring (F1)

Group		Time of completion for preputial separation Day of age	Bodyweight (g) at preputial separation	Time of completion for vaginal opening Day of age	Bodyweight (g) at vaginal opening
Statistical test:		lWi	Wi	Wi	Wi
1	Mean	45	175	35	99
	SD	2.0	16.2	2.6	11.1
	N	20	20	20	20
2	Mean	47	184	34	99
	SD	3.7	16.7	2.5	12.1
	N	20	20	20	20
3	Mean	46	176	34	94
	SD	2.3	14.7	2.8	16.5
	N	20	20	20	20
4	Mean	49**	183	34	94
	SD	4.3	21.7	2.9	10.2
	N	20	20	20	20
5	Mean	46	177	34	97
	SD	2.6	15.3	2.8	11.2
	N	20	20	20	20

Clinical signs - group distribution of observations for selected animals (F1)

Days 0 - 69			Number of animals affected									
		Group/sex:	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Category	Observation	Number in group:	20	20	20	20	20	20	20	20	20	20
Behaviour	Vocalisation		0	0	0	0	0	0	5	1	1	2
Build Deformity	Kinked Tail		1	0	0	2	1	0	0	1	0	0
	Partially absent appendage, Tail		0	0	1	0	0	0	0	0	0	0
	Swollen Area, Forelimb (Left)		0	0	0	1	0	0	0	0	0	0
	Swollen Area, Hindlimb (Left)		0	0	0	0	0	0	0	1	0	0
Coat	Hair loss, Dorsal Body Surface		0	0	0	0	0	0	2	0	1	3
	Hair loss, Forelimbs		0	0	0	0	0	0	0	0	2	4
	Hair loss, Head		0	0	0	0	0	0	0	1	1	2
	Hair loss, Hindlimbs		0	0	0	0	0	0	0	0	2	1
	Hair loss, Lower Dorsal Thorax		0	0	0	0	0	0	2	0	0	0
	Hair loss, Ventral Abdomen		0	0	0	0	0	0	0	0	2	1
Eye	Prominent, Eye (Left)		0	0	0	0	0	0	1	1	0	0

Clinical signs - group distribution of observations for selected animals (F1)

Days 0 - 69			Number of animals affected									
		Group/sex:	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Category	Observation	Number in group:	20	20	20	20	20	20	20	20	20	20
Skin	Encrustation, Dorsal Body Surface		0	0	0	0	0	0	0	0	1	0
	Encrustation, Forelimbs		0	0	0	0	0	0	0	0	1	0
	Encrustation, Head		0	0	0	0	0	0	0	1	1	3
	Encrustation, Lower Dorsal Thorax		0	0	0	0	0	0	1	1	0	0
	Encrustation, Lower Eyelid (Left)		0	1	0	0	0	0	0	0	0	0
	Encrustation, Lumbar		0	0	0	0	0	0	1	0	0	0
	Encrustation, Pinna (Right)		0	0	0	0	0	1	0	0	0	0
	Encrustation, Tail		2	0	0	0	0	0	0	0	0	0
	Encrustation, Upper Eyelid (Left)		0	2	0	0	0	0	0	0	0	0
	Encrustation, Upper Eyelid (Right)		0	1	0	0	0	0	0	0	0	0
	Encrustation, Ventral Abdomen		0	0	0	0	0	0	0	0	1	0
Staining	Abnormal Colour, Brown, Head		0	0	0	0	0	0	1	0	0	0
	Abnormal Colour, Brown, Lower Jaw		0	0	0	0	0	0	0	0	0	1
	Abnormal Colour, Brown, Muzzle		0	0	2	0	2	0	0	0	0	1
	Abnormal Colour, Brown, Upper Dorsal Thorax		0	0	0	0	0	1	1	0	1	0
	Abnormal Colour, Brown, Vagina		0	0	0	0	0	0	1	0	0	0
	Abnormal Colour, Head		0	0	0	0	0	0	1	0	0	0
	Abnormal Colour, Red, Vagina		0	0	0	0	0	0	1	0	0	0
Teeth	Malocclude		0	0	0	1	0	0	0	0	0	1

Mating performance and fertility - group values (F1)

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	20	20	20	100	100	100
2M	20	20	20	100	100	100
3M	20	20	20	100	100	100
4M	20	20	19	100	95	95
5M	20	20	20	100	100	100
1F	20	20	20	100	100	100
2F	20	20	20	100	100	100
3F	20	20	20	100	100	100
4F	20	20	19	100	95	95
5F	20	20	20	100	100	100

Litter data - group values on Day 14 of gestation (F1)

Group /Sex		Corpora Lutea	Implantations		Resorptions		Live Embryos	Implantation Loss (%)	
				Early	Late	Total		Pre-	Post-
Statistical test:		Wi	Wi	Wc	Wc	Wc	Wi	Wa	Wa
1F	Mean	11.6	11.1	0.8	0.0	0.8	10.4	4.6	7.5
	SD	2.52	2.61				2.70		
	N	20	20	20	20	20	20	20	20
2F	Mean	13.0	11.7	0.8	0.1	0.9	10.8	8.9	8.1
	SD	2.61	2.32				2.55		
	N	20	20	20	20	20	20	20	20
3F	Mean	12.7	12.1	0.6	0.0	0.6	11.6	6.0	4.1
	SD	2.05	2.73				2.52		
	N	20	20	20	20	20	20	20	20
4F	Mean	13.4*	12.9*	0.8	0.3	1.1	11.8	4.0	8.3
	SD	1.80	1.97				2.46		
	N	19	19	19	19	19	19	19	19
5F	Mean	11.9	10.8	1.2	0.0	1.2	9.7	8.0	10.1
	SD	3.70	3.14				3.12		
	N	20	20	20	20	20	20	20	20

Macropathology - group distribution of findings for selected males (F1)

Tissue and finding	Group/sex:	1M	2M	3M	4M	5M
	Number Examined:	20	20	20	20	20
Testes						
Absent - unilateral		0	0	0	0	1
Flaccid - unilateral		0	1	0	0	0
Small - unilateral		0	1	0	0	0
Epididymides						
Absent - unilateral		0	0	0	0	1
Small - unilateral		0	1	0	0	0
Kidneys						
Pelvic dilatation - unilateral		1	0	0	0	0
Tail						
Kinked		0	0	0	2	1
Partially absent		0	0	1	0	0
Scab(s)		2	0	0	0	0
Skin						
Scab(s)		0	1	0	0	0
Thymus						
Dark area(s)		0	2	0	0	0
Teeth						
Incisor(s) malocclude		0	0	0	1	0

Macropathology - group distribution of findings for selected females on Day 14 after mating (F1)

Tissue and finding	Group/sex: Number Examined:	1F 20	2F 20	3F 20	4F 20	5F 20
Tail						
Kinked		0	0	1	0	0
Skin						
Hair loss		0	3	1	3	4
Scab(s)		1	3	2	2	0
Thymus						
Dark		1	0	0	0	0
General comments						
Fur stained		1	1	0	1	0
Adipose tissue						
Pedunculate body		0	0	1	0	0
Eyes						
Misshapen lens		0	0	1	0	0
Small		0	0	1	0	0

10 Special Toxicology Studies

10.1 Local Toxicity

Study No. NN206131: local toxicity pig

Local toxicity 2 and 5 days after subcutaneous injections in pigs [by (b) (4)]

Study design: This GLP study was to evaluate the local reactions at the injection site 2 and 5 days after sc injection of insulin 454 (batch 412-N06073 or 412-N06048) compared to NPH, SIAM, vehicle, and 0.9% NaCl (as negative control). Endpoints included clinical signs, body weight, and histopath.

Animal No	1		2		3		4		5		6	
Injection Day	1	4	1	4	1	4	1	4	1	4	1	4
Localisation	L	R	L	R	L	R	L	R	L	R	L	R
Cranial	A	E	C	A	E	C	B	D	D	F	F	B
	B	F	D	B	F	D	A	C	C	E	E	A
Caudal	C	A	E	C	A	E	F	B	B	D	D	F
	D	B	F	D	B	F	E	A	A	C	C	E

L = Left
R = Right

Treatment A: Insulin 454 (6 Zn), 600 nmol/ml, 0.1 ml = 60 nmol (10 UI)
Treatment B: Insulin 454 (6 Zn), 1200 nmol/ml, 0.1 ml = 120 nmol (20 UI)
Treatment C: Saline
Treatment D: NPH, 600 nmol/ml, 0.1 ml = 60 nmol (10 UI)
Treatment E: SIAM 40/60 mix, 1200 nmol/ml, 0.1 ml =120 nmol (20 UI)
Treatment F: Vehicle
For all injections, the dose volume was 0.1 ml

Findings:

- Minimal swellings were seen for up to a few days after treatment with insulin 454 (600 and 1200 nmol/ml), NPH (Insulatard), SIAM 40/60 mix (except saline and vehicle).
- Minimal sub-acute (Day 2) and minimal to slight sub-chronic (Day 5) inflammatory reaction in the injection sites were observed.
- Microscopic findings: After 2 days, minimal to slight inflammatory cell infiltration was seen. After 5 days, minimal to slight inflammatory cell infiltration, edema, fat cell necrosis, and slight formation of collagen tissues was seen in a few injections sites.
- Such reactions, however, were comparable to those seen after injection of 0.9% saline, vehicle for insulin 454 and less pronounced than those seen after NPH insulin injection.

Two days after subcutaneous injection

Dose group	A: Insulin 454, 600 nmol	B: Insulin 454, 1200 nmol	C: Saline	D: NPH	E: SIAM 40/60 mix	F: Vehicle
Inj.sites/Numbers examined	4	4	4	4	4	4
Grade 0			2			1
Grade A	4	3		1	4	2
Grade B		1	2	2		1
Grade Ca				1		
Grade Da						
Grade Ea						
Needle canal	1	2	1	0	1	0

Five days after subcutaneous injection

Dose group	A: Insulin 454 (6 Zn), 600 nmol/ml	B: Insulin 454 (6 Zn), 1200 nmol/ml	C: Saline	D: NPH	E: SIAM 40/60 mix	F: Vehicle
Inj.sites/Numbers examined	4	4	4	4	4	4
Grade 0						
Grade A	2	1	1	1	2	
Grade B	1	2		1	1	3
Grade Cb	1	1	2	1	1	1
Grade Db			1	1		
Grade Eb						
Needle canal	1	1	2	1	1	2

Study No. NN210297: local toxicity rabbit

Local toxicity 4 days after intramuscular, intravenous, and intra-arterial injection in rabbits [by (b) (4)]

Study design: This GLP study was to assess the local reactions of the to be marketed formulation of IDeg (batch XCQ0044_N for 100 IU and batch XCQ0042 for 200 IU) and IDegAsp (batch XCQ0040_N) after a single dose administered by intramuscular (IM), intravenous (IV), and intra-arterial (IA) injection to rabbits. NPH insulin was used as comparator. Endpoints included clinical signs, body weight, and histopath.

Group	Animal Nos (Female)	Colour code	Route of treatment	Test item (right side)	Vehicle control (left side)	Dose volume (ml/injection site)	Dose concentration (nmol)
1	1-4	White	Intraarterial	insulin degludec 100U	Vehicle insulin degludec	0.1	60
2	5-8			insulin degludec 200U	Vehicle insulin degludec	0.05	60
3	9-12			IDegAsp	Vehicle IDegAsp	0.1	60 (42/18*)
4	13-16			NPH	Vehicle NPH	0.1	60
5	17-20	Blue	Intramuscular	insulin degludec 100U	Vehicle insulin degludec	0.1	60
6	21-24			insulin degludec 200U	Vehicle insulin degludec	0.05	60
7	25-28			IDegAsp	Vehicle IDegAsp	0.1	60 (42/18*)
8	29-32			NPH	Vehicle NPH	0.1	60
9	33-36	Green	Intravenous	insulin degludec 100U	Vehicle insulin degludec	0.1	60
10	37-40			insulin degludec 200U	Vehicle insulin degludec	0.05	60
11	41-44			IDegAsp	Vehicle IDegAsp	0.1	60 (42/18*)
12	45-48			NPH	Vehicle NPH	0.1	60

*42 nmol insulin degludec in combination with 18 nmol insulin aspart

Organs	Fix	Micro
Abnormalities	x	x
Ears, left and right side – injection site (Group 1-4)	x	x
Thigh, left and right side – injection site (Group 5-8)	x	x
Ears, left and right – injection site (Group 9-12)	x	x

Findings:

- One animal given 200 IU IDeg (via IM administration) was sacrificed due to intra-tracheal dosing of prophylactic glucose.
- A single dose of IDeg 100/200 IU, IDegAsp, or NPH caused local clinical reactions (hemorrhage/bruising and swelling) after IM, IA, and IV administrations.
- Macroscopic and microscopic findings were mainly mild changes at the injection sites. No systemic toxicity was observed.

Intra-arterial injection (Groups 1-4)

Clinical score of the injection sites - individual scores - Intraarterial treatment

Insulin Degludec (100U and 200U), IDegAsp and NPH Insulin
Local toxicity 4 days after intramuscular, intravenous and intra-arterial injection in rabbits

Animal No	Treat-ment	Test item	Dose route	Pre dose				Post dose*				Day 2				Day 3			
				H	B	E	S	H	B	E	S	H	B	E	S	H	B	E	S
1	RE	Insulin degludec 100U	IA	0	0	0	0	0	0	1	1	0	0	2	1	0	0	1	0
1	LE			0	0	0	0	1	0	0	0	0	1	0	2	0	1	0	1
2	RE			0	0	0	0	2	0	0	1	3	0	0	2	0	3	0	2
2	LE			0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	1
3	RE			0	0	0	0	0	0	1	0	0	0	1	0	0	0	2	1
3	LE			0	0	0	0	3	0	0	1	0	1	0	1	0	2	0	1
4	RE			0	0	0	0	0	0	2	0	0	0	1	0	0	0	1	0
4	LE			0	0	0	0	3	0	0	2	2	0	0	2	0	3	0	2
5	RE	Insulin degludec 200U		0	0	0	0	3	0	0	2	3	0	0	2	0	3	0	2
5	LE			0	0	0	0	3	0	0	1	3	0	0	2	0	3	0	2
6	RE			0	0	0	0	3	0	0	3	0	2	0	1	0	2	0	1
6	LE			0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
7	RE			0	0	0	0	4	0	0	3	0	3	0	2	0	3	0	1
7	LE			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	RE			0	0	0	0	3	0	0	2	3	0	0	2	0	3	0	2
8	LE			0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0
9	RE	IDegAsp		0	0	0	0	1	0	0	0	0	2	0	1	0	2	0	1
9	LE			0	0	0	0	3	0	0	1	0	3	0	1	0	3	0	1
10	RE			0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1
10	LE			0	0	0	0	2	0	1	2	0	3	0	1	0	2	0	1
11	RE			0	0	0	0	1	0	0	0	0	0	2	1	0	0	1	1
11	LE			0	0	0	0	2	0	0	1	0	0	2	0	0	1	2	1
12	RE			0	0	0	0	2	0	0	2	0	2	0	2	0	2	0	2
12	LE			0	0	0	0	4	0	0	3	0	3	0	2	0	3	0	2
13	RE	NPH		0	0	0	0	2	0	0	2	0	2	0	2	0	2	0	2
13	LE			0	0	0	0	3	0	0	3	0	3	0	2	0	3	0	2
14	RE			0	0	0	0	3	0	0	3	3	0	0	2	0	3	0	2
14	LE			0	0	0	0	3	0	0	3	0	3	0	2	0	3	0	2
15	RE			0	0	0	0	2	0	0	1	0	3	0	2	0	3	0	2
15	LE			0	0	0	0	2	0	0	2	0	2	0	2	0	2	0	2
16	RE			0	0	0	0	2	0	0	3	0	2	0	1	0	2	0	1
16	LE			0	0	0	0	2	0	0	2	0	2	0	2	0	2	0	2

RE = Right ear (test item) IA = Intraarterial H = Haemorrhage B = Bruising
LE = Left ear (vehicle) * = 3 h after treatment E = Erythema, S = Swelling

0 – not present	1 – minimal	2 – slight	3 – moderate	4 – marked

Insulin Degludec(100U and 200U), IDegAsp and NPH Insulin

Local toxicity 4 days after intramuscular, intravenous and intra-arterial injection in rabbits *(cont.)*

Animal No	Treat-ment	Test item	Dose route	Day 4				Day 5			
				H	B	E	S	H	B	E	S
1	RE	Insulin degludec 100U	IA	0	0	0	0	0	0	0	0
1	LE			0	1	0	1	0	2	0	2
2	RE			0	2	0	2	0	2	0	2
2	LE			0	0	0	1	0	0	0	1
3	RE			0	1	0	2	0	1	0	2
3	LE			0	2	0	2	0	2	0	2
4	RE			0	0	0	1	0	1	0	2
4	LE			0	3	0	2	0	3	0	2
5	RE	Insulin degludec 200U		0	3	0	2	0	3	0	2
5	LE			0	3	0	2	0	3	0	2
6	RE			0	1	0	1	0	0	0	1
6	LE			0	0	0	0	0	0	0	0
7	RE			0	4	0	2	0	3	0	2
7	LE			0	0	0	0	0	0	0	0
8	RE			0	2	0	2	0	3	0	2
8	LE			0	0	1	0	0	0	0	0
9	RE	IDegAsp		0	2	0	1	0	2	0	0
9	LE			0	2	0	2	0	2	0	2
10	RE			0	0	0	1	0	0	0	0
10	LE			0	2	0	1	0	1	0	1
11	RE			0	0	0	1	0	1	1	0
11	LE			0	1	2	1	0	1	2	1
12	RE			0	3	0	2	0	3	0	2
12	LE			0	3	0	2	0	3	0	2
13	RE	NPH		0	2	0	2	0	2	0	2
13	LE			0	2	0	2	0	3	0	3
14	RE			0	2	0	2	0	2	0	1
14	LE			0	3	0	2	0	3	0	2
15	RE			0	2	0	2	0	3	0	2
15	LE			0	2	0	2	0	2	0	2
16	RE			0	2	0	1	0	2	0	1
16	LE			0	2	0	2	0	2	0	2

RE = Right ear (test item) IA = Intraarterial H = Haemorrhage B = Bruising
LE = Left ear (vehicle) E = Erythema, S = Swelling

0 – not present	1 – minimal	2 – slight	3 – moderate	4 – marked

Intra-muscular injection (Groups 5-8)

Clinical score of the injection sites - individual scores - Intramuscular treatment

Insulin Degludec(100U and 200U), IDegAsp and NPH Insulin

Local toxicity 4 days after intramuscular, intravenous and intra-arterial injection in rabbits

Animal No	Treat-ment	Test item	Dose route	Pre dose				Post dose*				Day 2				Day 3			
				H	B	E	S	H	B	E	S	H	B	E	S	H	B	E	S
17	RT	Insulin degludec 100U	IM	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
17	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	RT			0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0
19	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	RT			0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
20	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	RT	Insulin degludec 200U		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	RT			0	0	0	0	0	0	0	0	·	·	·	·	·	·	·	·
22	LT			0	0	0	0	0	0	0	0	·	·	·	·	·	·	·	·
23	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	RT	IDegAsp		0	0	0	0	0	0	0	0	0	3	0	0	0	3	0	0
25	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	RT	NPH		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	RT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	LT			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

RT= Right thigh (test item), LT= Left thigh (vehicle)

IM = Intramuscular
* = 3 h after treatment
· = animal euthanised

H = Haemorrhage, B = Bruising
E = Erythema, S = Swelling

0 – not present	1 – minimal	2 – slight	3 – moderate	4 – marked

Insulin Degludec(100U and 200U), IDegAsp and NPH Insulin

Local toxicity 4 days after intramuscular, intravenous and intra-arterial injection in rabbits (

Animal No	Treat-ment	Test item	Dose route	Day 4				Day 5			
				H	B	E	S	H	B	E	S
17	RT	Insulin degludec 100U	IM	0	0	0	0	0	0	0	0
17	LT			0	0	0	0	0	0	0	0
18	RT			0	0	0	0	0	0	0	0
18	LT			0	0	0	0	0	0	0	0
19	RT			0	1	0	0	0	0	0	0
19	LT			0	0	0	0	0	0	0	0
20	RT			0	1	0	0	0	0	0	0
20	LT			0	0	0	0	0	0	0	0
21	RT	Insulin degludec 200U		0	0	0	1	0	0	0	0
21	LT			0	0	0	0	0	0	0	0
22	RT			·	·	·	·	·	·	·	·
22	LT			·	·	·	·	·	·	·	·
23	RT			0	0	0	0	0	0	0	0
23	LT			0	0	0	0	0	0	0	0
24	RT			0	0	0	0	0	0	0	0
24	LT			0	0	0	0	0	0	0	0
25	RT	IDegAsp		0	2	0	0	0	0	0	0
25	LT			0	0	0	0	0	0	0	0
26	RT			0	0	0	0	0	0	0	0
26	LT			0	0	0	0	0	0	0	0
27	RT			0	0	0	0	0	0	0	0
27	LT			0	0	0	0	0	0	0	0
28	RT			0	0	0	0	0	0	0	0
28	LT			0	0	0	0	0	0	0	0
29	RT	NPH		0	0	0	0	0	0	0	0
29	LT			0	0	0	0	0	0	0	0
30	RT			0	0	0	0	0	0	0	0
30	LT			0	0	0	0	0	0	0	0
31	RT			0	0	0	0	0	0	0	0
31	LT			0	0	0	0	0	0	0	0
32	RT			0	0	0	0	0	0	0	0
32	LT			0	0	0	0	0	0	0	0

RT= Right thigh (test item) IM = Intramuscular H = Haemorrhage B = Bruising
LT= Left thigh (vehicle) · = Animal euthanised E = Erythema, S = Swelling

0 – not present	1 – minimal	2 – slight	3 – moderate	4 – marked

Intravenous injection (Groups 9-12)

Local toxicity 4 days after intramuscular, intravenous and intra-arterial injection in rabbits

Animal No	Treat-ment	Test item	Dose route	Pre dose				Post dose*				Day 2				Day 3			
				H	B	E	S	H	B	E	S	H	B	E	S	H	B	E	S
33	RE	Insulin degludec 100U	IV	0	0	0	0	0	0	1	0	0	2	2	0	0	2	1	0
33	LE			0	0	0	0	0	0	1	0	0	0	2	0	0	0	1	0
34	RE			0	0	0	0	2	0	0	0	4•	0	0	1	0	4	0	1
34	LE			0	0	0	0	1	0	0	0	0	2	1	1	0	1	1	1
35	RE			0	0	0	0	2	0	0	0	0	3	2	0	0	3	2	0
35	LE			0	0	0	0	1	0	0	0	0	1	2	0	0	1	2	0
36	RE			0	0	0	0	1	0	0	2	0	2	2	0	0	2	2	0
36	LE			0	0	0	0	2	0	0	1	0	2	1	1	0	2	1	1
37	RE	Insulin degludec 200U		0	0	0	0	2	0	0	1	0	2	0	0	0	2	0	0
37	LE			0	0	0	0	3	0	0	1	0	2	0	2	0	2	0	1
38	RE			0	0	0	0	2	0	0	0	0	2	1	1	0	2	1	0
38	LE			0	0	0	0	3	0	0	1	0	3	0	2	0	3	0	2
39	RE			0	0	0	0	1	0	0	0	0	2	2	0	0	1	2	0
39	LE			0	0	0	0	3	0	0	0	0	2	2	0	0	2	2	1
40	RE			0	0	0	0	2	0	0	0	0	2	0	0	0	2	2	1
40	LE			0	0	0	0	1	0	0	1	0	2	0	0	0	1	2	0
41	RE	IDegAsp		0	0	0	0	1	0	0	0	0	2	1	0	0	1	1	0
41	LE			0	0	0	0	2	0	0	1	0	2	0	0	0	1	2	0
42	RE			0	0	0	0	1	0	0	0	0	1	0	0	0	2	2	0
42	LE			0	0	0	0	1	0	0	1	0	1	0	1	0	1	1	0
43	RE			0	0	0	0	3	0	0	1	0	2	0	0	0	2	0	0
43	LE			0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0
44	RE			0	0	0	0	1	0	0	1	0	2	0	1	0	2	0	1
44	LE			0	0	0	0	2	0	0	1	0	2	0	0	0	2	1	1
45	RE	NPH		0	0	0	0	1	0	0	1	0	2	0	0	0	2	0	0
45	LE			0	0	0	0	2	0	0	1	0	2	0	0	0	2	0	0
46	RE			0	0	0	0	1	0	0	1	0	2	0	0	0	2	0	0
46	LE			0	0	0	0	2	0	0	2	0	3	0	2	0	3	0	1
47	RE			0	0	0	0	1	0	0	1	0	3	0	0	0	3	0	1
47	LE			0	0	0	0	1	0	0	0	0	2	0	1	0	2	1	1
48	RE			0	0	0	0	3	0	0	1	0	3	1	0	0	3	1	1
48	LE			0	0	0	0	3	0	0	1	3	0	2	0	0	3	1	0

RE = Right ear (test item)
LE = Left ear (vehicle)

IV = Intravenous
* = 3 h after treatment
• = Bruising as on Day 2

H = Haemorrhage B = Bruising
E = Erythema, S = Swelling

0 – not present	1 – minimal	2 – slight	3 – moderate	4 – marked

Insulin Degludec(100U and 200U), IDegAsp and NPH Insulin

Local toxicity 4 days after intramuscular, intravenous and intra-arterial injection in rabbits *(cont.)*

Animal No	Treat-ment	Test item	Dose route	Day 4				Day 5			
				H	B	E	S	H	B	E	S
33	RE	Insulin degludec 100U	IV	0	2	1	0	0	1	0	0
33	LE			0	0	1	0	0	1	0	0
34	RE			0	4	0	1	0	4	0	2
34	LE			0	2	1	2	0	2	0	2
35	RE			0	3	2	0	0	2	2	0
35	LE			0	2	1	1	0	1	0	1
36	RE			0	2	2	1	0	2	1	1
36	LE			0	2	1	1	0	1	1	1
37	RE	Insulin degludec 200U		0	2	0	0	0	1	0	0
37	LE			0	2	0	1	0	2	0	1
38	RE			0	2	1	1	0	2	0	1
38	LE			0	2	0	2	0	2	0	2
39	RE			0	1	2	0	0	1	1	0
39	LE			0	2	0	1	0	2	2	1
40	RE			0	2	2	1	0	1	1	0
40	LE			0	2	0	1	0	2	1	0
41	RE	IDegAsp		0	1	0	0	0	1	0	0
41	LE			0	1	1	0	0	1	0	0
42	RE			0	2	2	0	0	2	2	0
42	LE			0	1	1	0	0	1	1	0
43	RE			0	2	0	0	0	2	0	1
43	LE			0	1	1	0	0	0	0	0
44	RE			0	2	0	2	0	1	0	1
44	LE			0	1	1	0	0	1	0	0
45	RE	NPH		0	2	0	0	0	2	0	0
45	LE			0	2	0	1	0	2	0	1
46	RE			0	2	0	1	0	2	0	0
46	LE			0	2	0	2	0	2	0	2
47	RE			0	2	0	2	0	2	0	1
47	LE			0	2	1	1	0	1	0	0
48	RE			0	3	1	1	0	2	1	1
48	LE			0	2	1	1	0	2	0	0

RE = Right ear (test item) IV = Intravenous H = Haemorrhage B = Bruising
LE = Left ear (vehicle) E = Erythema, S = Swelling

0 – not present	1 – minimal	2 – slight	3 – moderate	4 – marked

Study No. NN210455: local toxicity minipig

A local tolerance study 2 and 5 days after subcutaneous dosing in minipigs [by (b) (4)]

Study design: This GLP study was to assess the local reactions of the to be marketed formulation of IDeg (batch XCQ0044_N for 100 IU and batch XCQ0042 for 200 IU) and IDegAsp (batch XCQ0040_N) 2 and 5 days after a single sc dosing. NPH insulin was used as comparator. Endpoints included clinical signs, body weight, food consumption, and histopath.

Group	Animal Nos	Injection site No 1 Day 1 and No 2 Day 4		Injection site No 3 Day 1 and No 4 Day 4		Dose volume pr. injection site* (µl)
1	1-4	IDeg 100 U/mL		Vehicle IDeg		100
2	5-8	IDeg 200 U/mL		Vehicle IDeg		100
3	9-12	IDeg 200 U/mL		Vehicle IDeg		50
4	13-16	IDegAsp		Vehicle IDegAsp		100
5	17-20	NPH		0.9 % sodium chloride		100

*Material as supplied

Findings:

- One animal given 200 IU had hypoglycemic-related signs (i.e. passive, no appetite but recovered without any treatment).
- Single sc dosing of minipigs with IDeg (100 IU or 200 IU), IDegAsp, vehicles, the comparator NPH insulin, and 0.9% NaCl negative control resulted in mild inflammatory and hemorrhagic subcutaneous reactions 2 and 5 days after treatment.
- Microscopic findings: After 2 days, minimal to slight inflammatory cell infiltration and minimal to slight hemorrhage were seen. After 5 days, minimal to moderate inflammatory cell infiltration and minimal to slight hemorrhage were observed.
- There were no differences between two concentrations or volumes of IDeg applied and the reaction was comparable to that observed after injection of vehicle or IDeg, NPH insulin and saline.

Two days after subcutaneous injection

Dose group	Group 1		Group 2		Group 3		Group 4		Group 5	
Treatment	IDeg 100 U	Vehicle IDeg 100 U	IDeg 200 U	Vehicle IDeg 200 U	IDeg 200 U	Vehicle IDeg 200 U	IDeAspg	Vehicle IDegAsp	NPH	0.9 % sodium chloride
Dose volume µl	100	100	100	100	50	50	100	100	100	100
Number of Injection sites examined	4	4	4	4	4	4	4	4	4	4
Focal accumulation of inflammatory cells, subcutis										
Total	3	3	4	4	4	4	3	2	4	1
Grade 1	3	3	3	4	4	3	2	2	1	1
Grade 2	-	-	1	-	-	1	1	-	3	-
Focal haemorrhage, subcutis										
Total	0	4	2	2	2	4	2	3	3	0
Grade 1	-	3	2	2	2	4	2	3	3	-
Grade 2	-	1	-	-	-	-	-	-	-	-
Needle canal*	-	3	2	2	2	1	2	2	1	1

*Needle canal present

Five days after subcutaneous injection

Dose group	Group 1		Group 2		Group 3		Group 4		Group 5	
Treatment	IDeg 100 U	Vehicle IDeg 100 U	IDeg 200 U	Vehicle IDeg 200 U	IDeg 200 U	Vehicle IDeg 200 U	IDeAspg	Vehicle IDegAsp	NPH	0.9 % sodium chloride
Dose volume µl	100	100	100	100	50	50	100	100	100	100
Number of Injection sites examined	4	4	4	4	4	4	4	4	4	4
Focal accumulation of inflammatory cells, subcutis										
Total	3	4	4	4	4	4	4	3	4	4
Grade 1	2	1	1	2	2	-	-	-	1	1
Grade 2	1	2	2	1	2	4	3	3	2	1
Grade 3	-	1	1	1	-	-	1	-	1	2
Focal haemorrhage, subcutis										
Total	2	4	4	3	3	4	4	4	3	4
Grade 1	1	2	2	3	3	4	3	3	2	3
Grade 2	1	2	2	-	-	-	1	1	1	1
Needle canal*	1	2	3	2	1	2	3	4	2	1

*Needle canal present

10.2 Impurities

Study No. NN210227: 1 mo rat [impurity – aged vs. non-aged]

1-month subcutaneous toxicity study in rats (bridging study) [by (b) (4)]

Study design: This GLP study was to compare the potential toxicity of non-aged insulin 454 (XCQ0044_N) and aged insulin 454 (XCQ0044_G) at 75 or 100 nmol/kg/day administered daily by sc injection to rats for 4 week. Accelerated aging of insulin 100 U/ml was performed by placing the formulations in an oven at 37 degree for approx. 5 months. The aging of the formulation was terminated at the time when approx. 58% of the initial amount of IDeg remained in the formulations. The aged formulation contained approx (b) (4) % impurities and related substances (hydrophilic impurities, hydrophobic related substances, and hydrophobic impurities) after storage at 37 °C for 5 months to force degradation whereas non-aged formulations contained approx (b) (4) % impurities and related substances. Endpoints included clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, TK/serum glucose analyses, and organ weights, macroscopic and microscopic examinations.

Group	Aging	Compound	Dose* (nmol/kg/day	Dose concen-tration (nmol/ml)*	Animal Nos (Main study)		Colour code
					Male	Female	
1	-	Vehicle	0	0	1-12	13-24	White
2	Non-aged	XCQ0044_N	75	75	25-36	37-48	Blue
3	Non-aged	XCQ0044_N	150	150	49-60	61-72	Green
4	Aged	XCQ0044_G	75	75	73-84	85-96	Red
5	Aged	XCQ0044_G	150	150	97-108	109-120	Yellow

*Material as supplied

Observations and Results

- Dose formulation analysis: The results were within the ±20% acceptance criteria.
- Clinical signs: No test article related clinical signs were observed. A few animals (2 in Group 1 and 2 in Group 3 had wounds at injection sites.
- Body weights: There were no differences between treated and control groups or the between aged and non-aged IDeg groups.
- Food consumption: There were no differences between the 2 insulin batches were noted. There were higher food consumption (4-10% increase) in high dose animals compared to the control for both aged and non-aged IDeg for both sexes however overall food consumption was not statistical significance. When compared between the aged and non-aged groups, Group 4 females had lower food consumption on several days and also overall compared to Group 2. However, this was not observed in males or in higher dose groups (Group 5 vs. Group 3).
- Ophthalmoscopy: No test article related findings were observed.
- Hematology: No differences were seen between two 2 IDeg aged and non-aged batches. There were some minor changes (had been considered incidental) when compared to control.

Haematology changes compared to control

Parameter	Group 1 (control)		Group 2 (75 non - aged nmol/kg)		Group 3 (150 non-aged nmol/kg)		Group 4 (75 aged nmol/kg)		Group 5 (150 aged nmol/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
White blood cell count	10.05	8.53	9.42	7.47	8.69	6.13 (***)	9.74	7.24	9.51	7.45
% Neutrophils	9.3	9.4	12.0 (*)	11.6	12.8 (***)	11.1	12.0 (*)	11.3	12.7 (**)	12.8
Lymphocytes	8.96	7.53	8.05	6.46	7.38	5.30 (***)	8.38	6.28	8.13	6.34
% Lymphocytes	88.9	88.4	85.7 (**)	86.8	85.0 (**)	87.0	85.8 (*)	86.3	85.3 (**)	84.8
Eosinophils	0.10	0.13	0.13	0.08 (*)	0.13	0.08 (*)	0.12	0.13	0.13	0.10
Monocytes	0.1	0.09	0.13	0.06	0.09	0.02 (**)	0.1	0.08	0.09	0.10
Protrombin time	13.3	13.5	13.7	12.9	13.5	12.8	13.2	13.3	13.6	13.0

* means p<0.05 vs. control group
** means p<0.01 vs. control group
*** means p<0.001 vs. control group

- Clinical chemistry: Several parameters in clinical chemistry were changed due to the insulin treatment. However, no difference between the aged and non-aged IDeg was seen.

Clinical chemistry compared to control

Parameter	Group 1 (control)		Group 2 (75 non-aged nmol/kg)		Group 3 (150 non-aged nmol/kg)		Group 4 (75 aged nmol/kg)		Group 5 (150 aged nmol/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Glucose	7.63	8.70	2.39 (***)	3.33 (***)	1.73 (***)	3.06 (***)	2.20 (***)	2.87 (***)	1.94 (***)	2.17 (***)
Inorganic phosphorus	2.51	2.33	2.27	2.10	2.12 (**)	2.17	2.08 (***)	1.77 (**)	2.46	2.29
Protein	68.0	66.9	68.3	67.3	67.1	70.0	68.9	67.9	65.4 (*)	69.5
Triglyceride	1.91	1.12	1.10 (***)	0.88	0.76 (***)	0.98	1.18 (***)	1.09	0.71 (***)	0.81

Parameter	Group 1 (control)		Group 2 (75 non-aged nmol/kg)		Group 3 (150 non-aged nmol/kg)		Group 4 (75 aged nmol/kg)		Group 5 (150 aged nmol/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Chloride	101.4	103.6	102.8	105.9 (*)	105.2 (***)	107.7 (***)	103.4 (*)	106.4 (**)	105.2 (***)	106.4 (**)
Sodium	149.5	146.6	149.7	146.6	151.6	148.1	150.4	148.3	151.0	149.5 (**)
Calcium	3.04	2.99	3.00	3.01	2.93 (*)	3.08 (*)	3.03	3.00	2.92 (**)	2.99
Magnesium	1.21	1.23	1.24	1.27	1.21	1.32 (*)	1.21	1.21	1.17	1.25
Urea	9.40	9.99	10.42 (**)	12.57 (***)	11.69 (***)	13.34 (***)	10.31 (**)	11.73 (***)	11.42 (***)	12.51 (***)
Creatinine	23.3	22.9	20.7 (*)	21.3	19.9 (**)	21.4	21.3	20.8 (*)	19.7 (***)	19.2 (***)
Alkaline phosphatase	4.38	2.61	3.77	2.61	3.48 (*)	2.45	3.85	2.36	3.50 (*)	2.64

* means p<0.05 vs. control group
** means p <0.01 vs. control group
*** means p <0.001 vs. control group

- Toxicokinetic: All animals dosed with either non-aged or aged IDeg for 4 weeks were systemically exposed to IDeg. The TK of IDeg in rats was gender-independent and systemic exposure increased proportionally with dose between 75 and 150 nmol/kg/day. Accumulation in system exposure to IDeg was low. No apparent differences in the systemic exposure between two insulin batches.

Mean toxicokinetic parameters for insulin degludec in rats after once daily s.c. administration of 75 and 150 nmol/kg non-aged or aged insulin degludec for 4 weeks.

Day	Group	Dose (nmol/kg/day)	Sex	C_{max} (nM)	t_{max} (h)	$AUC_{(0\text{-}24h)}$ (h*nM)	AUC (h*nM)	$AUC_{\%Extra}$ (%)	Rac_{pred}	Rac_{obs}
1	2	75	F	147	3.0	699	700	0.21	1.0	NA
			M	141	1.0	677	679	0.27	1.0	NA
	3	150	F	360	3.0	1710	1710	0.19	1.0	NA
			M	335	3.0	1590	1590	0.33	1.0	NA
	4	75	F	151	1.0	670	672	0.27	1.0	NA
			M	141	1.0	740	743	0.39	1.0	NA
	5	150	F	295	3.0	1690	1690	0.21	1.0	NA
			M	311	3.0	1510	1510	0.44	1.0	NA
28	2	75	F	200	1.0	1010	1010	0.51	NA	1.5
			M	212	3.0	1040	1050	0.98	NA	1.4
	3	150	F	535	3.0	2680	NR	NR	NA	1.6
			M	397	3.0	2350	2370	1.1	NA	1.6
	4	75	F	194	3.0	1000	1010	0.85	NA	1.5
			M	172	3.0	980	995	1.5	NA	1.3
	5	150	F	379	3.0	1960	1970	0.60	NA	1.2
			M	336	3.0	1980	2020	2.1	NA	1.3

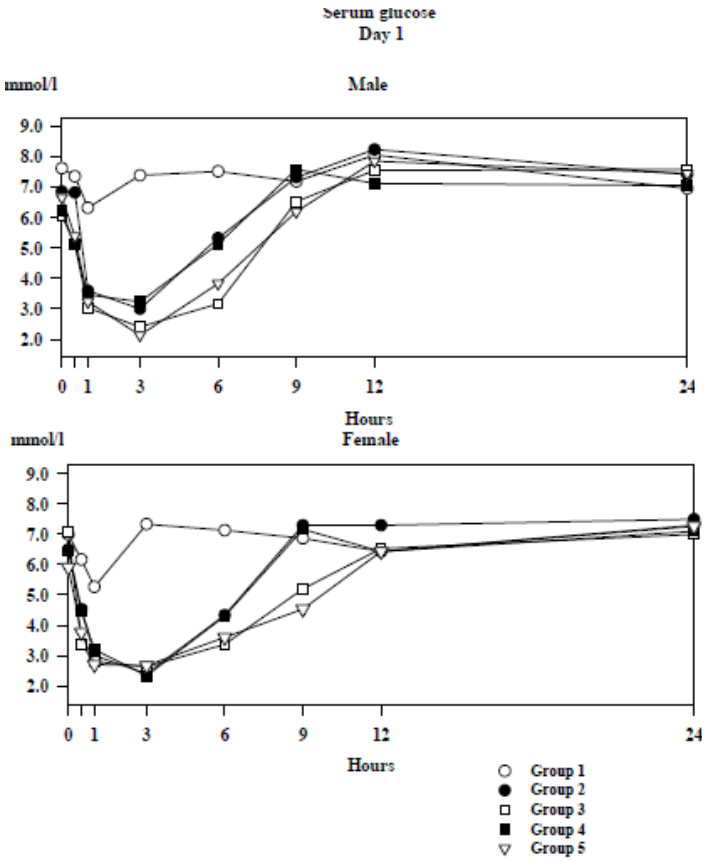
NA – Not applicable; NR – not reported as R^2 <0.85; F- Female; M – Male

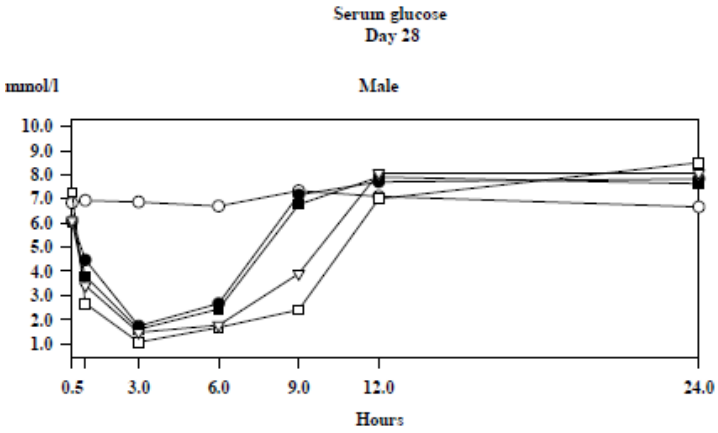
Comparison of systemic exposure for non-aged and aged insulin degludec after once daily s.c. administration of 75 and 150 nmol/kg insulin degludec to rats.

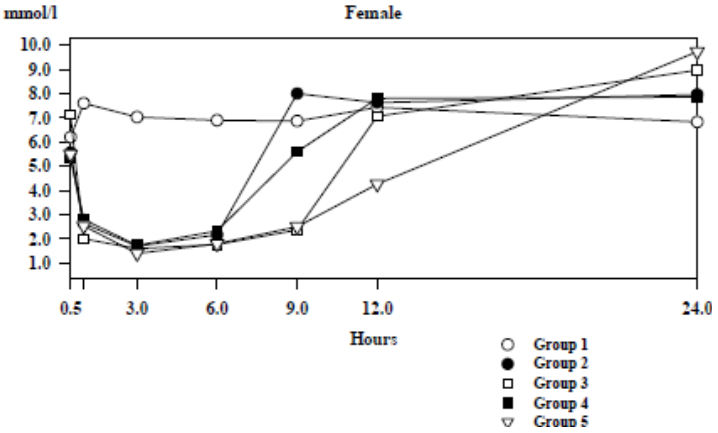
Dose (nmol/kg/day)	Day	C_{max} (nM)			$AUC_{(0\text{-}24h)}$ (h*nM)		
		Aged	Non-aged	A/N	Aged	Non-aged	A/N
75	1	146	144	1.01	705	688	1.02
	28	183	206	0.89	990	1025	0.97
150	1	303	348	0.87	1600	1650	0.97
	28	357.5	466	0.77	1970	2515	0.78

A – Aged, N – Non-aged

- Serum glucose level: Day 1: serum glucose level was reduced from 0.5 hr post-dosing, statistical significance at 1 hr post-dosing for all groups in both sexes, and returned to normal at 6 hr post-dosing (generally at normal levels by 9 hr post-dosing). Day 28: serum glucose level was reduced from 1 hr post-dosing, statistical significance at 3 hr post-dosing for all groups in both sexes, and returned to normal at 9 hr post-dosing for lower dosed groups and 12 hrs post-dosing for all groups (except high dose females). Prolonged effect of insulin treatment was seen on Day 28 compared to Day 1. In addition, minor differences were seen in the serum glucose level between the aged and non-aged test articles. These were considered incidental.


Serum glucose
Day 1
mmol/l
Male
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0 1 3 6 9 12 24
Hours
mmol/l
Female
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0 1 3 6 9 12 24
Hours
Group 1
Group 2
Group 3
Group 4
Group 5


Serum glucose
Day 28
mmol/l
Male
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.5 3.0 6.0 9.0 12.0 24.0
Hours



- Organ weights: Lower absolute and/or relative liver weights were seen in IDeg treated groups compared to controls. Higher testes weight was observed in some IDeg treated groups compared to controls. In addition, lower absolute and relative adrenal weight was seen in Group 4 (aged IDeg) females compared to Group 2 (non-aged IDeg) females. This was not seen in high dose animals. Lower relative heart weight was observed in Group 5 females (aged IDeg) compared to Group 4 females (non-aged IDeg). There findings were considered incidental.

Organ weights compared to control

Parameter	Group 1 (control)		Group 2 (75 non-aged nmol/kg)		Group 3 (150 non-aged nmol/kg)		Group 4 (75 aged nmol/kg)		Group 5 (150 aged nmol/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Liver	12791	7653	12026	7115	10769 (***)	7257	11863	7080	11644	6990
Liver as % of body weight	3.85	3.77	3.72	3.54 (*)	3.39 (***)	3.57	3.68	3.57	3.48 (***)	3.42 (***)
Testes	3182		3306		3272		3321		3277	
Testes as % of body weight	0.958		1.030 (*)		1.032 (*)		1.031 (*)		0.981	

* means p<0.05 vs. control group
** means p <0.01 vs. control group
*** means p <0.001 vs. control group

- Macroscopic findings: A red discoloration was seen at the injection sites in all groups (higher incidence in treated groups compared to the vehicle group).

Injection site reactions

Dose group	Group 1		Group 2		Group 3		Group 4		Group 5	
	Vehicle		75/ XCQ0044_N nmol/kg/day		150/ XCQ0044_N nmol/kg/day		75/ XCQ0044_G nmol/kg/day		150/ XCQ0044_G nmol/kg/day	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals examined	12	12	12	12	12	12	12	12	12	12
Injection site										
Discoloration: red										
Total	1	1	3	1	5	2	3	5	7	2
Slight degree	1	1	3	1	5	2	2	5	5	2
Moderate degree	-	-	-	-	-	-	1	-	1	-
Marked degree	-	-	-	-	-	-	-	-	1	-

- Microscopic findings: Liver and injection site findings were considered test-article related. The incidence of rarefaction (intracellular glycogen accumulation) of the hepatocytes had decreased in animals of both sexes in all treated groups (more pronounced in the females). No different was seen between the dose groups treated with aged or non-aged IDeg. Changes were seen in the sc injection sites of animals from all dose groups. The incidences and/or severity of the reaction seemed more pronounced in IDeg treated animals compared to vehicle control. However there was no dose-dependent for any of the two IDeg batches and no clear difference between those two batches.

Rarefaction of hepatocytes

Dose group	Group 1		Group 2		Group 3		Group 4		Group 5	
	Vehicle Control		75 nmol XCQ0044_N /kg/day		150 nmol XCQ0044_N /kg/day		75 nmol XCQ0044_G /kg/day		150 nmol XCQ0044_G /kg/day	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals examined	12	12	12	12	12	12	12	12	12	12
Liver										
Rarefaction										
Total	11	8	4	0	1	0	6	0	3	0
Minimal	10	8	4	-	1	-	5	-	3	-
Slight	1	-	-	-	-	-	1	-	-	-

Overview of subcutaneous injection site reactions

Dose group	Group 1		Group 2		Group 3		Group 4		Group 5	
	Vehicle Control		75 nmol XCQ0044_N /kg/day		150 nmol XCQ0044_N /kg/day		75 nmol XCQ0044_G /kg/day		150 nmol XCQ0044_G /kg/day	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals examined	12	12	12	12	12	12	12	12	12	12
Injection site										
Necrosis focal sc										
Total	2	1	6	2	6	2	9	3	8	2
Minimal	1	1	3	2	3	2	2	-	3	2
Slight	1	-	2	-	3	-	5	2	3	-
Moderate	-	-	1	-	-	-	2	1	2	-
Inflammatory cells focal sc										
Total	12	8	12	11	12	9	12	10	11	7
Minimal	9	8	3	7	4	6	2	5	3	5
Slight	2	-	7	4	8	3	6	4	3	2
Moderate	1	-	2	-	-	-	4	1	5	-

Overall Finding:

- This study showed that dosing with aged and non-aged IDeg at 75 and 150 nmol/kg/day for 28 days resulted in test-article related changes in body weight, food consumption, blood glucose, some clinical pathology parameters, liver weight, and liver rarefaction. However these changes were attributed to the pharmacological effect of IDeg.
- Minor differences between aged and non-aged IDeg were noted in food consumption, hematology, clinical chemistry, serum glucose, and organ weights. However, these findings were all minor, seen in only in one sex or considered incidental.
- In addition, there was no clear difference in injection site reactions between aged and non-aged IDeg.

10.3 Other Studies in Mice

Study No. NN209385: tolerability mouse

Preliminary tolerability study in CD-1 mice [by Novo Nordisk, Denmark]

Study design: This non-GLP study was to determine a tolerable dosage of insulin (batch VKOSHP002) and insulin NPH by treating mice daily for 4 days. Three doses of 75, 150, and 300 nmol/kg/day insulin 454 or NPH insulin were tested. Endpoints included clinical signs, body weight, food consumption, glucose monitoring, and histopath.

Group	Test Item	Concentration (nmol/ml)	Dose Volume (ml/kg)	Dose Level (nmol/kg/day)	Animals		Colour Code
					Males	Females	
1	Insulin 454	15	5	75	1-5	101-105	Pink
2		30	5	150	6-12 + 39	106-112	Lilac
3		60	5	300	13-19	113-119	Red
4	NPH insulin	15	5	75	20-24	120-124	Orange
5		30	5	150	25-31	125-131	Green
6		60	5	300	32-38	132-138	Blue
7	Spare animals				40	139-140	White

Findings:

- In insulin 454 treated animals, low blood glucose levels were found 1, 3, 6, hrs after dosing in insulin 454 treated animals and the levels were within normal ranges 12 hrs after dosing.
- In insulin NPH treated animals, low blood glucose levels were found 1 hr after dosing and the levels were within normal ranges 3-6 hrs after dosing.
- Clinical signs (i.e. subdued, piloerection) were considered consistent with hypoglycaemia. No mortalities occurred but three animals had to be treated with glucose (insulin 454: 1 MD male on Day 5, 1 HD female on Day 4; insulin NPH: 1 MD male on Day 2).
- The insulin 454 was well tolerated as only subtle changes in overall body weight gain and/or food consumption were seen in females.
- At necropsy, minimal or slight discoloration was seen at injection sites in 13/38 insulin 454-treated mice and 6/38 insulin NPH-treated mice. Small superficial ulcers were also seen also at the injection sites in 4/39 insulin 454-treated mice and 2/38 insulin NPH-treated mice.

Group Mean Body Weight Gain

Description	Sex	Insulin 454 (nmol/kg/day)			NPH Insulin (nmol/kg/day)		
		75 (Group 1)	150 (Group 2)	300 (Group 3)	75 (Group 4)	150 (Group 5)	300 (Group 6)
Body Weight Gain from First to Last Day of Dosing	Males	0.2 g	0.8 g	0.3 g	0.7 g	1.1 g	1.0 g
		101%	103%	101%	103%	104%	104%
	Females	0.5 g	0.4 g	-0.1 g	0.4 g	0.8 g	0.6 g
		102%	101%	99%	102%	103%	103%

%: Body weight Day 5 as percent of body weight Day 1

Food Consumption

Description	Sex	Insulin 454 (nmol/kg/day)			NPH Insulin (nmol/kg/day)		
		75 (Group 1)	150 (Group 2)	300 (Group 3)	75 (Group 4)	150 (Group 5)	300 (Group 6)
Food consumption (g/day/mouse)	Males	11.0	10.5	11.8	11.2	10.7	11.8
	Females	10.2	9.7	9.5	10.0	9.5	9.4

blood glucose levels

Day	Hours after Dosing	Insulin 454 (nmol/kg/day)			NPH Insulin (nmol/kg/day)		
		75 (Group 1)	150 (Group 2)	300 (Group 3)	75 (Group 4)	150 (Group 5)	300 (Group 6)
3	3 h	2.1	2	2.2	6.2	3.4	3.8
	6 h	5.8	2.6	2.2	6.6	8.6	9.9

Blood Glucose Levels as Percent of Value Measured 24 hours after Dosing

Sex	Sample Time (Hours after Dosing)	Insulin 454 (nmol/kg/day)			NPH Insulin (nmol/kg/day)		
		75 (Group 1)	150 (Group 2)	300 (Group 3)	75 (Group 4)	150 (Group 5)	300 (Group 6)
Male	1	42%	45%	63%	39%	32%	39%
	3	61%	34%	50%	93%	61%	52%
	6	80%	48%	58%	95%	110%	102%
	12	80%	71%	128%	94%	118%	99%
	24	100%	100%	100%	100%	100%	100%
Female	1	48%	36%	60%	85%	73%	35%
	3	48%	44%	74%	67%	74%	42%
	6	73%	45%	54%	127%	129%	101%
	12	81%	81%	127%	133%	108%	105%
	24	100%	100%	100%	100%	100%	100%

%: Percent of the level measured 24 hours post-dosing

Incidence of Macroscopic Findings at Injection Site

Sex	Macroscopic Findings at Injection Site	Insulin 454 (nmol/kg/day)			NPH Insulin (nmol/kg/day)		
		75 (Group 1)	150 (Group 2)	300 (Group 3)	75 (Group 4)	150 (Group 5)	300 (Group 6)
Male	No. examined	5	8	7	5	7	7
	NAD	1	6	5	5	6	6
	Subcutaneous discoloration, red, minimal	1				1	1
	Subcutaneous discoloration, red, slight	3	2	2			
	Superficial ulcer (2x2 mm in diameter)	1		1			
Female	No. examined	5	7	7	5	7	7
	NAD	5	4	5	3	6	6
	Subcutaneous discoloration, red, minimal			1			
	Subcutaneous discoloration, red, slight		3	1	2		
	Superficial ulcer (2x2 mm in diameter)		1	1		1	1

All in all, the effects on blood glucose, body weight gain, and food consumption were considered directly or indirectly related to the pharmacologic effect of insulin 454 or insulin NPH. Dose levels up to 300 nmol/kg/day were considered tolerable for treating mice daily for 4 days, but these animals were need to be monitored 1-6 hrs after dosing to prevent blood glucose levels become critical low.

Study No. NN209388: 28 day DRF mouse

28 day subcutaneous administration range-finding study in the mouse [by (b) (4)]

Study design: This GLP study was to assess the systemic toxic potential and TK of insulin 454 (VCQ0014), following daily sc administration to the CD-1 mouse for 28 days in order to provide the basis for the selection of dose levels for pivotal studies. Endpoints included mortality, clinical signs, body weight, food consumption, clinical pathology, macroscopic pathology, TK and serum glucose analyses.

Group Number	Group Description	Dose level (nmol/kg/day)	Dose concentration (nmol/mL)	Number of animals in group			
				Toxicity (Main study)		Toxicokinetics/serum glucose (TK study)	
				Male	Female	Male	Female
1	Control	0	0	6	6	14	14
2	Insulin 454 (Low)	25	5	6	6	14	14
3	Insulin 454 (Intermediate)	150/100#	30/20#	6	6	14	14
4	Insulin 454 (High)	250/200/150#	50/40/30#	6	6	14	14
5	NPH insulin (Low)	150	30	6	6	14	14
6	NPH insulin (High)	250/200#	50/40#	6	6	14	14

Volume dose = 5 mL/kg

dose level for Group 4 was reduced from 250 to 200 nmol/kg/day with effect from Day 9 of the toxicity study (Day 7 of the toxicokinetic/glucose study and from 200 to 150 nmol/kg/day with effect from Day 20 of the toxicity study (Day 18 of the toxicokinetic/glucose study). In addition, dose levels for Groups 3 and 6 were reduced from Day 20 of the toxicity study (Day 18 of the toxicokinetic/glucose study) from 150 to 100 nmol/kg/day and from 250 to 200 nmol/kg/day, respectively.

Findings:

- The daily sc administration of insulin 454 to CD-1 mice (at 25, 150/100 or 250/200/150 nmol/kg/day) for 28 days was well tolerated in most animals.
- There were treatment related decreases in serum glucose concentrations associated with hypoglycaemia-related clinical signs.
- There were also reduced liver glycogen vacuolization and hypoglycaemia-related mortality in a few animals given ≥ 150 nmol/kg/day.
- The administration of NPH insulin to CD-1 mice at doses of 150 or 250.200 nmol/kg/day for 28 days was well tolerated at 150 nmol/kg/day but caused hypoglycemia related mortality in 2 females given 250.200 nmol/kg/day.
- When compared to insulin NPH, insulin 454 had higher hypoglycemia-related mortality among females (9 vs. 2 at HD group and 2 vs. 0 at MD group) and greater hypoglycaemia-related clinical signs in animals. This could be related to the lower serum glucose concentrations noted in insulin 454-treated animals when compared with insulin NPH groups.
- There were minor inter-group differences in body weight gain between insulin 454 and NPH insulin treated animals. However, food consumption of insulin 454-treated was generally similar to or slightly lower than the corresponding NPH insulin group.
- The NOAEL was established 150 nmol/kg/day by the sponsor since changes were directly or indirectly related to the pharmacological action of insulin 454.

- TK analysis: TK (satellite) animals were systemically exposed to insulin 454. The TK of insulin 454 in mice was generally sex and dose-independent. Accumulation in systemic exposure to insulin 454 was low.

Mortality

Group	1F	2F	3F	4F	5F	6F
Test article	Control	---------- insulin 454 ----------			-- NPH insulin --	
Dose (nmol/kg/day)	0	25	150/100	250/200/150	150	250/200
Group size (Toxicity)	6	6	6	6	6	6
Number of toxicity study decedents	0	0	0	3	0	0
Group size (TK)	14	14	14	14	14	14
Number of TK decedents	0	0	2	6	0	2

Serum glucose profile parameters are as follows:

Occasion	Dose Level (nmol/kg/day)	Dose Group	Sex	$AUC_{(0-24)}$ (mmol/L.h)	C min (mmol/L)	T min (hours)	Ratio*
Day 1	25 insulin 454	2	Male	243.4	5.9	3	-
	150 insulin 454	3	Male	194.6	3.5	6	0.85
	250 insulin 454	4	Male	174.7	5.0	1	0.77
	150 NPH insulin	5	Male	228.8	5.6	1	-
	250 NPH insulin	6	Male	228.3	4.1	1	-
	25 insulin 454	2	Female	184.4	3.4	3	-
	150 insulin 454	3	Female	172.5	4.8	3	0.79
	250 insulin 454	4	Female	159.3	3.9	6	0.84
	150 NPH insulin	5	Female	219.5	5.5	1	-
	250 NPH insulin	6	Female	190.6	3.6	6	-
Day 28	25 insulin 454	2	Male	229.9	3.3	1	-
	150/100 insulin 454	3	Male	185.2	2.4	3	0.92
	250/200/150 insulin 454	4	Male	162.5	1.5	3	0.72
	150 NPH insulin	5	Male	202.1	3.3	3	-
	250/200 NPH insulin	6	Male	226.4	1.8	3	-
	25 insulin 454	2	Female	185.3	3.6	1	-
	150/100 insulin 454	3	Female	157.5	2.6	3	0.74
	250/200/150 insulin 454	4	Female	83.35	1.9	3	0.41
	150 NPH insulin	5	Female	213.7	3.2	3	-
	250/200 NPH insulin	6	Female	202.6	3.8	1	-

* Ratio: insulin 454 AUC(0-24)/NPH insulin AUC(0-24)

Microscopic findings

Incidence of glycogen vacuolation: liver – all animals, including decedents

Tissue and finding		Males 1M	2M	3M	4M	5M	6M	Females 1F	2F	3F	4F	5F	6F
		Insulin 454				NPH insulin		Insulin 454				NPH insulin	
Liver	No. examined:	6	6	6	6	6	6	6	6	6	6	6	6
glycogen vacuolation	Grade -	0	0	3	5	2	0	0	0	3	4	1	2
	1	6	5	2	1	4	5	4	4	3	2	5	3
	2	0	1	1	0	0	1	2	1	0	0	0	0
	3	0	0	0	0	0	0	0	1	0	0	0	1

Dose levels (nmol/kg/day): Gp 1 = 0, Gp 2 = 25, Gp 3 = 150/100, Gp 4 = 250/200/150
Gp 5 = 150, Gp 6 = 250/200

Grade: "-" = not remarkable, 1 = minimal, 2 = slight, 3 = moderate,4 = moderately severe, 5 = severe

Incidence of myopathy/myositis: injection sites – all animals, including decedents

Tissue and finding		Males 1M	2M	3M	4M	5M	6M	Females 1F	2F	3F	4F	5F	6F
		Insulin 454				NPH insulin		Insulin 454				NPH insulin	
Scruff (Injection site 1)	No. examined:	6	6	6	6	6	6	6	6	6	6	6	6
myopathy/myositis	Grade -	4	5	5	4	5	5	3	5	5	3	3	3
	1	2	1	0	0	1	0	0	1	1	2	2	3
	2	0	0	1	2	0	1	3	0	0	1	1	0
Left hip (Injection site 2)	No. examined:	6	6	6	6	6	6	6	5	6	6	6	6
myopathy/myositis	Grade -	2	3	3	1	5	5	4	4	4	4	4	5
	1	1	1	2	1	1	1	1	1	2	1	2	1
	2	1	2	1	2	0	0	0	0	0	0	0	0
	3	1	0	0	2	0	0	1	0	0	1	0	0
	4	1	0	0	0	0	0	0	0	0	0	0	0
Right hip (Injection site 3)	No. examined:	6	6	6	6	6	6	6	6	6	6	6	6
myopathy/myositis	Grade -	3	2	3	2	2	4	5	4	3	3	6	3
	1	1	2	2	2	3	2	0	0	1	1	0	1
	2	2	2	1	2	1	0	1	2	1	1	0	2
	3	0	0	0	0	0	0	0	0	1	1	0	0

Dose levels (nmol/kg/day): Gp 1 = 0, Gp 2 = 25, Gp 3 = 150/100, Gp 4 = 250/200/150
Gp 5 = 150, Gp 6 = 250/200

Grade: "-" = not remarkable, 1 = minimal, 2 = slight, 3 = moderate,4 = moderately severe, 5 = severe

Study No. NN209479: 13 week tox mouse

13 week subcutaneous administration toxicity study in the mouse [by (b) (4)]

Study design: This GLP study was to determine the tolerability and toxicity of the test article insulin 454 (XCG0036) at 15, 40, and 75 nmol/kg/day (vs. 75 nmol/kg/day insulin NPH), following daily subcutaneous administration to the mouse for 13 weeks. Endpoints included mortality, clinical signs, body weight, food consumption, clinical pathology, hematology, clinical chemistry, macroscopic and microscopic pathology, antibody analysis, TK and serum glucose analyses.

Group Number	Group Description	Dose level (nmol/kg/day)	Dose concentration (nmol/mL)	Number of animals in group			
				Toxicity phase (Main study)		Toxicokinetic/serum glucose phase (Satellites)	
				Male	Female	Male	Female
1	Control	0	0	12	12	14	14
2	Insulin 454 (Low)	15	6	12	12	14	14
3	Insulin 454 (Intermediate)	40	16	12	12	14	14
4	Insulin 454 (High	75	30	12	12	14	14
5	NPH insulin	75	30	12	12	14	14

Volume dose 2.5mL/kg

Findings:

- The daily sc administration of insulin 454 to CD-1 mice (at 15, 40, and 75 nmol/kg/day) for 13 weeks was well tolerated in most animals.
- There were hypoglycaemia related clinical signs and mortality in 4 females given insulin 454 ≥ 15 nmol/kg/day. These findings were the expected pharmacological actions of insulin 454. In addition, 1 male and 2 females given 75 nmol/kg/day insulin NPH were also euthanized following clinical signs of hypoglycaemia.
- The body weight gain and food consumption of main study and TK animals were quite variable between the two cohorts of animals and between the sexes.
- No inter-group differences in hematological parameters, serum glucose concentrations, organ weights, and macroscopic or microscopic findings were noted.
- The NOAEL for insulin 454 was established at 75 nmol/kg/day by the sponsor.
- TK analysis: these animals were shown to have systemically exposed to insulin 454 in a sex and dose independent manner.

Mortality

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Test article	Control	------- insulin 454 -------			NPH insulin	Control	------- insulin 454 -------			NPH insulin
Dose *	0	15	40	75	75	0	15	40	75	75
Main study animals										
Found dead	1	0	2	1	0	0	0	0	2	0
Euthanised	1	0	0	1	1	1	1	0	0	1
Total	2	0	2	2	1	1	1	0	2	1
Cause of demise										
Hypoglycaemia	0	0	0	0	1	0	1	0	0	1
Suspected hypoglycaemia	0	0	0	0	0	0	0	0	2	0
Other•	1	0	2	2	0	0	0	0	0	0
Unknown	1	0	0	0	0	1	0	0	0	0
Satellite animals										
Found dead	0	0	0	1	0	0	0	0	3	0
Euthanised	0	0	0	0	2	1	0	2	1	1
Total	0	0	0	1	2	1	0	2	4	1
Cause of demise										
Hypoglycaemia	0	0	0	0	0	0	0	2	1	1
Suspected hypoglycaemia	0	0	0	1	0	0	0	0	2	0
Other•	0	0	0	0	2	1	0	0	1	0

* Dose = nmol/kg/day
• Other = died after blood sampling or as consequence of bleed damaged eye, skin lesions or impaired mobility

Serum glucose profile parameters are as follows:

Occasion	Dose Level (nmol/kg/day)	Dose Group	Sex	$AUC_{(0-24)}$ (mmol/L.h)	C min (mmol/L)	T min (hours)
Day 1	Control	1	Male	212	6.2	24
	15 insulin 454	2	Male	186	5.8	3
	40 insulin 454	3	Male	191	5.2	1
	75 insulin 454	4	Male	208	5.7	6
	75 NPH insulin	5	Male	234	8.0	1
	Control	1	Female	194	6.9	Pre-dose
	15 insulin 454	2	Female	194	5.8	3
	40 insulin 454	3	Female	191	7.1	3
	75 insulin 454	4	Female	186	5.0	1
	75 NPH insulin	5	Female	189	6.7	1

Occasion	Dose Level (nmol/kg/day)	Dose Group	Sex	$AUC_{(0-24)}$ (mmol/L.h)	C min (mmol/L)	T min (hours)
Week 13	Control	1	Male	111 *	7.3	Pre-dose
	15 insulin 454	2	Male	98 *	4.7	3
	40 insulin 454	3	Male	84 *	3.0	1
	75 insulin 454	4	Male	89 *	3.3	1
	75 NPH insulin	5	Male	97 *	3.0	1
	Control	1	Female	194	6.2	Pre-dose
	15 insulin 454	2	Female	172	2.9	1
	40 insulin 454	3	Female	171	2.4	3
	75 insulin 454	4	Female	168	3.0	3
	75 NPH insulin	5	Female	226	4.7	1

* = $AUC_{(0-12)}$, due to insufficient samples for analysis 24 hours post-dose.

Group incidence microscopic data – terminal kill

(b) (4)

Test article Control Insulin 454 NPH Insulin .
Group 1 2 3 4 5 .
Level (mmol/kg/day) 0 15 40 75 75 .

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PAGE: 1

STUDY NUMBER: 6218754

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:		----------MALE----------					----------FEMALE----------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		10	12	10	10	11	11	11	12	10	11
** TOP OF LIST **											
EYE	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--RETINAL ATROPHY		0	0	0	0	0	1	0	0	0	0
OPTIC NERVE	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--NEUROPATHY		1	0	0	0	6	5	0	0	2	8
SKIN + SUBCUTIS	NUMBER EXAMINED:	10	2	2	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		1	0	0	1	0	0	0	0	0	0
--DERMATITIS		3	1	0	0	1	0	0	0	0	0
--MYOSITIS/MYOPATHY		1	0	2	0	0	0	0	0	0	0
MUSCLE	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		1	0	0	0	0	0	0	0	0	0
--MYOSITIS/MYOPATHY		0	0	0	2	1	0	0	0	0	0
FEMUR + MARROW	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--ARTHROPATHY		1	0	0	1	1	0	0	0	1	2
STERNUM + MARROW	NUMBER EXAMINED:	10	0	2	10	11	11	0	0	10	11
--DEFORMITY		0	0	2	0	1	0	0	0	0	0
--CHONDROPATHY		0	0	0	0	0	0	0	0	0	3
LIVER	NUMBER EXAMINED:	10	1	0	10	11	11	2	7	10	11
--INFLAMMATORY CELL FOCI		10	0	0	8	7	11	2	5	10	10
--AGONAL CONGESTION/HAEMORRHAGE		0	0	0	0	0	0	0	1	0	0
--FOCAL NECROSIS		0	1	0	0	1	0	0	1	0	0
--GLYCOGEN VACUOLATION		4	1	0	5	9	6	2	7	5	9

** CONTINUED ON NEXT PAGE **

Group incidence microscopic data - terminal kill

Test article Control Insulin 454 NPH Insulin .
Group 1 2 3 4 5 .
Level (mmol/kg/day) 0 15 40 75 75 .

(b) (4)

PRINTED: 15-DEC-10
PAGE: 2

STUDY NUMBER: 6218754

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:		----------MALE----------					----------FEMALE----------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:		10	12	10	10	11	11	11	12	10	11
** FROM PREVIOUS PAGE **											
LIVER	NUMBER EXAMINED:	10	1	0	10	11	11	2	7	10	11
--HAEMOPOIESIS		0	0	0	0	0	0	0	1	0	0
--INCREASED MITOTIC FIGURES		0	0	0	0	1	0	0	0	0	0
SPLEEN	NUMBER EXAMINED:	10	1	0	10	11	11	0	0	10	11
--HAEMOPOIESIS		10	1	0	10	11	11	0	0	10	11
--PIGMENT		0	0	0	0	0	0	0	0	1	0
PANCREAS	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--DEGENERATION		0	0	0	0	0	0	0	0	1	0
--INFLAMMATORY CELL FOCI		0	0	0	0	0	0	0	0	0	1
--FATTY ATROPHY		0	0	0	0	0	0	0	0	0	1
STOMACH	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--GLAND		0	0	0	0	0	0	0	0	1	0
--CHRONIC GASTRITIS		0	0	0	1	0	0	0	0	0	0
--HYPERPLASIA		0	0	0	1	1	0	0	0	0	0
--GLANDULAR STOMACH EROSION/ULCER		0	0	0	1	1	0	0	0	0	0
--INFLAMMATORY CELL FOCI		0	0	0	1	0	0	0	0	0	0
--JUNCTIONAL METAPLASIA		0	0	0	0	0	0	0	0	0	1
GALL BLADDER	NUMBER EXAMINED:	10	0	0	8	11	11	0	1	10	11
--INFLAMMATORY CELL FOCI		0	0	0	0	0	1	0	0	0	0
--DISTENSION		0	0	0	0	0	0	0	1	0	0
ADRENAL	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--SUBCAPSULAR CELL HYPERPLASIA		1	0	0	0	3	6	0	0	5	7

** CONTINUED ON NEXT PAGE **

Group incidence microscopic data - terminal kill

Test article Control Insulin 454 NPH Insulin .
GROUP 1 2 3 4 5 :
Level (nmol/kg/day) 0 15 40 75 75 :

(b) (4)

PRINTED: 15-DEC-1
PAGE: 3

STUDY NUMBER: 8218754

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:		----------MALE----------					---------FEMALE---------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	10	12	10	10	11	11	11	12	10	11
** FROM PREVIOUS PAGE **											
ADRENAL	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--CORTICOMEDULLARY VACUOLATION		0	0	0	0	0	5	0	0	2	4
--INFLAMMATORY CELL FOCI		0	0	0	0	0	1	0	0	0	0
--CORTICOMEDULLARY PIGMENT		0	0	0	0	0	0	0	0	1	0
KIDNEY	NUMBER EXAMINED:	10	1	1	10	11	11	0	0	10	11
--PAPILLARY MINERALISATION		6	0	1	4	7	4	0	0	2	3
--INFLAMMATORY CELL FOCI		6	1	0	7	7	7	0	0	7	8
--FOCAL NEPHROPATHY		4	1	0	4	1	0	0	0	1	3
--CYST		0	0	0	0	1	0	0	0	0	0
--CASTS		0	0	0	1	1	0	0	0	0	1
--TUBULAR DILATION		0	0	0	1	1	0	0	0	0	1
TESTIS	NUMBER EXAMINED:	10	0	0	10	11	0	0	0	0	0
--TUBULAR ATROPHY		0	0	0	0	1	0	0	0	0	0
EPIDIDYMIS	NUMBER EXAMINED:	10	0	1	10	11	0	0	0	0	0
--CELLULAR DEBRIS		0	0	1	0	0	0	0	0	0	0
--OLIGOSPERMIA		0	0	1	0	0	0	0	0	0	0
--SPERMATOCOELE		0	0	0	1	0	0	0	0	0	0
--INFLAMMATORY CELL FOCI		0	0	0	0	1	0	0	0	0	0
OVARY	NUMBER EXAMINED:	0	0	0	0	0	11	3	6	10	11
--CYST		0	0	0	0	0	2	2	4	2	6
--PIGMENT		0	0	0	0	0	0	0	0	1	0
--CYSTIC BURSA		0	0	0	0	0	0	0	0	2	0

Group incidence microscopic data - terminal kill

Test article Control Insulin 454 NPH Insulin .
GROUP 1 2 3 4 5 :
Level (nmol/kg/day) 0 15 40 75 75 :

(b) (4)

PRINTED: 15-DEC-10
PAGE: 4

STUDY NUMBER: 8218754

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:		----------MALE----------					---------FEMALE---------				
GROUP:		-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	10	12	10	10	11	11	11	12	10	11
URINARY BLADDER	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		1	0	0	0	0	1	0	0	2	6
PROSTATE	NUMBER EXAMINED:	10	0	0	10	11	0	0	0	0	0
--INFLAMMATORY CELL FOCI		0	0	0	0	2	0	0	0	0	0
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	11	3	9	10	11
--OESTRUS		0	0	0	0	0	4	0	4	3	2
--METOESTRUS		0	0	0	0	0	6	1	6	6	6
--DIOESTRUS		0	0	0	0	0	3	1	1	2	3
--PRO-OESTRUS		0	0	0	0	0	3	1	0	0	0
--INFLAMMATORY CELL FOCI		0	0	0	0	0	0	0	0	1	0
--CYSTIC GLANDS		0	0	0	0	0	0	0	1	0	0
--CERVICO-VAGINAL MUCIFICATION		0	0	0	0	0	0	0	1	0	3
VAGINA	NUMBER EXAMINED:	0	0	0	0	0	11	0	0	10	11
--SQUAMOUS CYST		0	0	0	0	0	0	0	0	1	0
--MUCIFICATION		0	0	0	0	0	0	0	0	0	1
SALIVARY GLAND	NUMBER EXAMINED:	10	0	1	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		8	0	0	1	3	2	0	0	2	3
PAROTID GLAND	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--LOBULAR ATROPHY		1	0	0	0	0	0	0	0	0	0
MANDIBULAR LN	NUMBER EXAMINED:	10	0	1	10	11	11	0	0	10	11
--LYMPHOID HYPERPLASIA		0	0	1	0	0	0	0	0	0	0

Table 21

Group incidence microscopic data - terminal kill

Test article	Control	Insulin 454			NPH Insulin	.
Group	1	2	3	4	5	:
Level (nmol/kg/day)	0	15	40	75	75	:

(b) (4)

PRINTED: 15-DEC-10
PAGE: 5

STUDY NUMBER: 6218754

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

		--- NUMBER-OF-ANIMALS-AFFECTED ---									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	10	12	10	10	11	11	11	12	10	11
THYMUS	NUMBER EXAMINED:	10	1	0	10	11	11	0	0	10	11
--CYST		1	0	0	0	0	1	0	0	1	1
--LYMPHOCYTOLYSIS		1	0	0	0	0	0	0	0	0	0
--LYMPHOID HYPERPLASIA		0	0	0	0	0	2	0	0	0	0
LUNG	NUMBER EXAMINED:	10	3	2	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		0	0	0	1	4	1	0	0	0	0
--PERIARTERITIS		0	0	0	1	0	0	0	0	0	0
--FOAMY MACROPHAGES		0	0	0	0	0	0	0	0	0	1
HEART	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--DEGENERATION/FATTY		0	0	0	0	0	0	0	0	0	0
--INFLAMMATORY CELL FOCI		0	0	0	1	0	0	0	0	0	0
--ARTERITIS		0	0	0	0	0	0	0	0	0	1
OESOPHAGUS	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--OESOPHAGITIS		0	0	0	0	0	0	0	0	0	0
TONGUE	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		0	0	0	1	2	1	0	0	0	0
--CYST		0	0	0	0	0	0	0	0	1	0
--EROSION/ULCER		0	0	0	0	0	0	0	0	0	1
THYROID	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--CYSTIC FOLLICLES		1	0	0	1	2	0	0	0	0	1
--ECTOPIC THYMUS		2	0	0	0	1	0	0	0	1	1

Group incidence microscopic data - terminal kill

Test article	Control	Insulin 454			NPH Insulin	.
Group	1	2	3	4	5	:
Level (nmol/kg/day)	0	15	40	75	75	:

(b) (4)

PRINTED: 15-DEC-10
PAGE: 6

STUDY NUMBER: 6218754

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

		--- NUMBER-OF-ANIMALS-AFFECTED ---									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	10	12	10	10	11	11	11	12	10	11
PITUITARY	NUMBER EXAMINED:	10	0	0	10	11	11	0	1	10	11
--CYST		0	0	0	0	0	0	0	0	2	0
SPINAL CORD	NUMBER EXAMINED:	10	0	0	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		0	0	0	0	0	1	0	0	0	0
POPLITEAL LN	NUMBER EXAMINED:	10	1	0	10	11	11	1	1	10	11
--LYMPHOID HYPERPLASIA		0	1	0	0	0	0	1	1	0	0
SCROTT	NUMBER EXAMINED:	10	1	0	10	11	11	0	1	10	11
--DEGENERATIVE		1	1	0	0	0	0	0	0	0	0
--GRANULOMA		1	0	0	0	0	0	0	0	0	1
--MYOSITIS/MYOPATHY		0	0	0	0	1	0	0	0	1	0
--INFLAMMATORY CELL FOCI		0	0	0	0	0	0	0	0	1	1
--FASCIITIS/FIBROSIS		0	0	0	0	0	0	0	0	1	2
LEFT HIP	NUMBER EXAMINED:	10	3	1	10	11	11	2	0	10	11
--MYOSITIS/MYOPATHY		2	1	1	2	3	4	1	0	0	0
--DERMATITIS		1	3	0	0	1	1	0	0	0	0
--INFLAMMATORY CELL FOCI		0	0	0	0	0	1	0	0	0	0
--FASCIITIS/FIBROSIS		0	0	0	0	0	1	0	0	0	0
--GRANULOMA		0	0	0	0	0	0	1	0	1	0
RIGHT HIP	NUMBER EXAMINED:	10	2	0	10	11	11	0	0	10	11
--INFLAMMATORY CELL FOCI		1	0	0	0	1	0	0	0	0	0
--MYOSITIS/MYOPATHY		4	2	0	1	4	3	0	0	0	0
--DERMATITIS		1	2	0	1	1	3	0	0	0	0

** CONTINUED ON NEXT PAGE **

Group incidence microscopic data - terminal kill

Test article	Control	Insulin 454			NPH Insulin	
Group	1	2	3	4	5	
Level (nmol/kg/day)	0	15	40	75	75	

(b) (4)

PRINTED: 16-DEC-10
PAGE: 7

STUDY NUMBER: 6218754

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

	--- NUMBER-OF-ANIMALS-AFFECTED ---									
SEX:	----------MALE---------					---------FEMALE--------				
GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION / NUMBER:	10	12	10	10	11	11	11	12	10	11
** FROM PREVIOUS PAGE **										
RIGHT HIP ... NUMBER EXAMINED:	10	2	0	10	11	11	0	0	10	11
--BAYMARK CAST	0	0	0	1	0	0	0	0	0	0
--HAEMORRHAGE	0	0	0	0	0	1	0	0	0	0
--FASCIITIS/FIBROSIS	0	1	0	0	0	0	0	0	0	1
--GRANULOMA	0	0	0	0	0	0	0	0	0	1
PREPUT/CLIT GL ... NUMBER EXAMINED:	0	0	0	0	1	0	0	0	0	0
--CYSTIC DISTENSION	0	0	0	0	1	0	0	0	0	0
--INFLAMMATORY CELL FOCI	0	0	0	0	1	0	0	0	0	0
LYMPH NODE ... NUMBER EXAMINED:	0	0	0	0	0	0	0	1	0	0
--SINUS HISTIOCYTOSIS	0	0	0	0	0	0	0	1	0	0
TAIL ... NUMBER EXAMINED:	0	0	0	0	1	0	1	1	0	1
--DERMATITIS	0	0	0	0	1	0	1	0	0	0
--FRACTURE/DISLOCATION	0	0	0	0	0	0	0	1	0	1
BONE MARROW ... NUMBER EXAMINED:	0	1	0	0	0	0	0	0	0	0
--INCREASED HAEMOPOIESIS	0	1	0	0	0	0	0	0	0	0

** END OF LIST **

11 Integrated Summary and Safety Evaluation

Drug Product: Insulin Degludec

The applicant submitted NDA 203314 for insulin degludec (IDeg), a long-acting basal insulin, for T1DM and T2DM. The structure of insulin degludec allows it to form soluble and stable multi-hexamers, resulting in a depot in the sc tissue after injection. The gradual separation of insulin degludec monomers from the multi-hexamers results in a slow and continuous delivery of insulin degludec from the sc injection site into the blood stream, leading to the observed longer PK/PD profiles. The drug product comes in two strengths: U100 (containing 600 nmol/ml insulin degludec) and U200 (containing 1200 nmol/ml insulin degludec). This will be given once-daily via sc administration at any time of the day, independent of meals.

Pharmacodynamic Profile of Insulin Degludec

Overview of Pharmacology studies

Study type	Administration	Species
Efficacy Pharmacology		
Insulin receptor binding	*in vitro*	rat, pig, dog, human
IGF-1 receptor binding	*in vitro*	rat, dog, human
Insulin receptor signalling and kinetics	*in vitro*	human
Metabolic effect (adipocyte, hepatocyte, muscle cell)	*in vitro*	rat, human
Mitogenic effect (COLO205, HMEC, L6-hIR, MCF-7 cells)	*in vitro*	human
Receptor selectivity	*in vitro*	various
Pharmacodynamic (normal and diabetic animals)	i.v.	rat
PK/PD	s.c.	pig
Safety Pharmacology		
Effect on action potential	*in vitro*	rabbit
Effect on CNS and respiration	s.c.	rat
Effect on cardiovascular system	s.c. and i.v.	dog

- **Receptor profiling**

These studies showed that IDeg could bind to the insulin receptor, both IR-A and IR-B, with similar affinities (with a lower affinity compared to human insulin). The IDeg could activate the same signaling cascade as human insulin and was a full-agonist to the insulin receptor, reaching the same maximum response as human insulin. In addition, IDeg could also bind to IGF-1 receptor with a lower affinity than for the insulin receptor.

- **Metabolic and mitogenic response**

The cellular metabolic response to IDeg relative to human insulin was determined in number of insulin target cells (i.e. adipocytes, hepatocytes, and myocytes). The mitogenic potency of IDeg relative to human insulin was determined in several cell types (i.e. COLO-25, HMEC, L6-hIR, MCF-7). These cell types represented 1) different organ tissues, 2) different insulin/IGF-1 receptor expression levels and 3) neoplastic and non-neoplastic tissues. The overall mitogenic/metabolic potency ratio was estimated by comparing the relative mitogenic potency (5-10%) with the relative metabolic potency (4-21%). These studies confirmed that the balance between the metabolic and proliferative actions of IDeg was similar to that of human insulin. As for an effect of albumin binding (both in vitro and in vivo), IDeg could bind strongly to albumin, resulting in a decrease in the apparent binding affinity or potency as a consequence of increasing the albumin concentration.

In addition, euglycemic clamp studies were done to evaluate the efficiacy of IDeg in rats and pigs. In rats, the estimated in vivo potencies of IDeg relative to human insulin were 65% in SD rats and 47% in Zucker-obese rats. These values were obtained with iv infusion to reach steady-state. In pigs, these studies confirmed the blood glucose lowering effect of IDeg. It was also demonstrated that in the presence of high zinc concentrations, the IDeg action profile (as determined by the glucose infusion rate) became flatter and more prolonged.

- **Safety Pharmacology**

IDeg was evaluated for its potential effects on the CNS, CV, and respiratory function in rats and/or dogs. In vitro CV studies on action potential and a hERG channel bindings assays were also conducted. The highest dose given (300 nmol/kg in rats and 24 nmol/kg in dogs) did not cause any unexpected adverse findings (except those associated with hypoglycaemia).

- There was no effect on CNS noted in rats up to 300 nmol/kg, using the Irwin's test.

- There was an increased heart rate (as a response to the significant decrease in blood glucose level observed) in female dogs after single sc administration of 24 nmol/kg IDeg (not significant compared to control). No effect on blood pressure and ECG were observed. In addition, there were no effect on ECG parameters in anaesthetized and glucose clamped male dogs after iv infusion of 4, 8, and 23 nmol/kg over 2 hrs. No effect on the action potential recorded from Rabbit Purkinje fibers was seen following incubation with 1000 nmol/L of IDeg. At 1000 nmol/L, there was also no significant binding affinity to hERG channels.

- There was no significant effect of IDeg at 2 and 30 nmol/kg on respiratory parameters in rats dosed and restrained for up to 6 hrs with no access to food. However, at 300 nmol/kg, IDeg resulted hypoglycaemia related clinical signs which led to premature sacrifice in 12/24 animals. In the remaining animals, there were significant decreases in the respiratory rate and increases in the tidal volume (attributable to pharmacological effect of IDeg and no access to food).

Pharmacokinetic Profile of Insulin Degludec

Overview of pharmacokinetic studies

Toxicokinetic		
DRF/MTD, 4-, 13-, 26- and 52-weeks	s.c.	mouse[b], rat, dog[a]
Fertility[d] and embryo-foetal development	s.c.	rat, rabbit
Pharmacokinetic		
Single dose (insulin degludec)	s.c. and i.v.	rat, rabbit, dog, pig
Multiple dose (7-days)	s.c. and i.v.	rat, dog
Single dose (radioactivity)	s.c. and i.v.	rat, dog
Distribution		
QWBA	s.c. and i.v.	rat
Placenta transfer	s.c.	rat
Protein binding	*in vitro*	rat, dog, rabbit, pig, human
Metabolism		
Hepatocytes, Cathepsin D	*in vitro*	rat, dog, human
Plasma, urine[c], bile[c], faeces[c], milk[c]	s.c.	rat, dog
Excretion		
Urine, bile, faeces	s.c.	rat
Milk	s.c.	rat

a – DRF/MTD, 4- and 26-week b – 4- and 13-week c – rat only

The PK studies showed that IDeg had prolonged PK profile after sc injection. This was based on protracted absorption process where the elimination of IDeg was dependent on the absorption rate. This was seen in all species (i.e. mouse, rat, rabbit, dog, pig, and human). Longer t ½ was observed after sc than after iv administration. IDeg was also highly protein bound in plasma and thus had a relatively low apparent volume of distribution. IDeg was extensively metabolized before excretion.

Anti-drug antibody was seen in a few rats but not in dogs after repeated dosing with IDeg. However, no difference in the plasma exposure was seen, indicating that the presence of IDeg antibodies did not affect the PK profile of IDeg.

Toxicological Profile of Insulin Degludec

Overview of pivotal toxicity studies

Study type and duration	Route of administration	Species
Single-dose toxicity	s.c.	Rat and dog[a]
Repeat-dose toxicity		
4 week	s.c.	Rat and dog
26 week	s.c	Rat and dog
52 week including carcinogenicity assessment	s.c.	Rat
Reproductive and developmental toxicity studies		
Fertility	s.c	Rat
Embryo-foetal development	s.c.	Rat and rabbit
Pre- and post-natal development	s.c.	Rat
Local tolerance		
Early development drug product and "to be marketed" drug product	s.c.	Pig/Minipig
"To be marketed" drug product	i.m., i.v., i.a.	Rabbit

a – integrated part of maximum tolerable dose study (204317); this study was conducted as a non-GLP activity

- **Single dose toxicity**

The MTD dose in rats was 24000 nmol/kg and in dogs was 30 nmol/kg.

- **Repeat dose toxicity** (up to 52 weeks in rats and up to 26 weeks in dogs)

In rats, 4, 26 (with 4 wk recovery), and 52 week (with carcinogenic assessment) studies were done. In dogs, 4 and 26 (with 4 wk recovery) weeks studies were conducted. Due to hypoglycaemia-related clinical signs and mortality in 26 week rat and dog studies and 52 week rat study, the dose levels of IDeg and/or the comparator NPH insulin were reduced during the course of these studies. Such need to reduce dose levels suggests that the initial dose levels were close to or exceeded the MTD (maximum tolerance dose). In both species, lowering blood glucose was dose-dependent. Between these two species, dogs were less tolerant and developed clinical signs at lower doses of IDeg compared to rats. The difference in tolerability to insulin between the rat and dog is also observed for other insulin analogs, such as insulin lispro, insulin detemir, insulin aspart, and insulin glargine.

In addition, due to the effect on blood glucose, these animals had adaptive compensatory responses including increased good consumption, body weight gain, changes in clinical pathology, decreased liver weight and depletion of liver glycogen.

- In rats, changes in clinical pathology parameters included decreased plasma liver enzymes/proteins/urea, increased urinary ketones and decreased pH (indicative of a compensatory metabolic response) in rats dosed with higher than 40 nmol/kg IDeg or NPH insulin. Increased hemoglobin and hematocrit values were also indicative of dehydration. Decreased liver weight was seen in rats given IDeg and NPH insulin (with glycogen depletion). Inflammation and haemorrhage were seen at the injection

sites (similar to vehicle dosed animals). All these changes were reversible after 4-week recovery period.

- In dogs, changes included decreased monocytes, decreased mean cell hemoglobin in females and decreased triglycerides in males. No effect on liver weight was seen in dogs (but with glycogen depletion). Inflammation and haemorrhage were also seen at the injection sites (no difference to vehicle dosed animals). All these changes were reversible after 4-week recovery period.

Furthermore, systemic exposure was confirmed in all animals dosed with IDeg. Antibodies towards IDeg were seen in a few rats but not in dogs. All in all, changes seen were considered related to pharmacological effect of insulin and there were no unexpected adverse findings. Based on these studies, the NOAEL for IDeg was determined as 60 nmol/kg/day in rats and 8 nmol/kg/day in dogs.

- **Genotoxicity**

In accordance with the ICH S6 guidance, genotoxicity studies were not conducted.

- **Carcinogenicity**

In accordance with the ICH S6 guideline, 2-yr carcinogenicity study was not conducted. However, the carcinogenic potential of IDeg was assessed by evaluating hyperplastic and neoplastic lesions in all pivotal repeated dose toxicity studies in rats and dogs and by evaluating female mammary cell proliferation particularly in the 52-week toxicity study in rats. In this 52 week toxicity study in rats, IDeg showed no carcinogenic potential.

- **Reproductive and developmental toxicity** (Seg I/II/III in rats and Seg II in rabbits).

In accordance to ICH S5A guidance, the reproductive and development toxicity of IDeg were investigated in Seg I/II/III studies in rats and/or rabbits. The dose levels of 20, 80, and 125 nmol/kg/day were used in rats (Seg I/II/III) where the dose levels of 5, 10, and 20 nmol/kg/day were used in rabbits (Seg II).

In rats, treatment with IDeg up to 125 nmol/kg/day prior to mating, and in females during gestation had no major adverse effects on mating performance and fertility, or on embryo-fetal survival and growth. The incidences of the fetal skeletal abnormalities seen in rats at 20, 80, and 125 nmol/kg/day IDeg were above those in concurrent control animals but were below the incidences in the NPH insulin group and/or within the historical range (attributed to gestational hypoglycaemia). In addition, maternal mortality was observed with 80 and 125 nmol/kg/day during gestation and lactation periods (also seen in NPH insulin group). The mortality was associated with insulin-induced hypoglycaemia. Moreover, due to decreased maternal food consumption and body weight, lowered live birth index, viability index, lower offspring body weigh and viability, and delayed preputial separation were all considered secondary changes to

expected pharmacological effect on maternal blood glucose levels. This was also seen with NPH insulin. Based on the historical control data provided by the applicant, the incidences of these findings seemed to be within the historical control range.

In rabbits, treatment with IDeg up to 20 nmol/kg/day in females during gestation had some effect on embryo-fetal survival, growth, and development (i.e. skeletal malformation/variations at 5 nmol/kg/day and visceral variations at 20 nmol/kg/day). Based on the historical control data provided by the applicant, the incidences of these findings were within the historical control range (attributed to gestational hypoglycaemia).

- **Local tolerance**

Local tissue reaction was mild and similar to that of vehicle or NPH insulin. This is common when insulin is administered by sc route and there was no difference when compared the reactions after injecting with 600 nmol/ml (100 U/ml) and 1200 nmol/ml (200 U/ml) formulations.

- **Impurity Profile of Insulin Degludec**

Drug product Impurities were identified and qualified as (b) (4) The HMWP (high molecular weight proteins) was also listed.

- Based on the NOAEL (150 nmol/kg – high dose group) of 1 month rat toxicity study (aged vs. non-aged IDeg, Study No 210227), three impurities were tested at the higher concentrations (1-2.3 fold) than proposed limits. The animal exposure was 33-78 fold the human exposure for the three groups of impurities.

- Based on the NOAEL (20 nmol/kg – low dose group) of 6 month rat toxicity study (Study No 206315), the HMWP was tested at lower concentration (0.45 fold) than proposed limit. The animal exposure was 2 fold the human exposure for the HMWP. Note: In the 6 month rat study, the sponsor set the NOAEL at the high dose group (125 nmol/kg); therefore, the exposure was 13 fold the human exposure.

Leachables (i.e. (b) (4), and etc) were identified and determined for (b) (4) months shelf life and 8 weeks in use period of 100 U IDeg.

Based on CMC review, there was no issue with regards to impurities and leachables. CMC commented on the long term and accelerated stability data where a shelf-life of 30 months at (b) (4) and in-use period of ≤ 56 days at ≤ 30°C was recommended for the drug product. This was in agreement with the sponsor's proposed expiry.

Overall Review of IDeg and Safety Margin

The nonclinical program showed no unexpected safety concerns for chronic sc administration of IDeg in human. Toxicity findings observed in animals were mostly associated with hypoglycemia which was an exaggerated pharmacology of insulin. The PK/PD findings also showed that IDeg did not change its metabolic efficacy or its safety profile compared to human insulin. Based on AUCs obtained from the repeat-dose toxicity studies, there were exposure multiples of 5.2 in rats and 1.3 in dogs to that of in humans.

Table 9 Safety Margins.

Toxicity	Species	NOAEL (nmol/kg/day) M+F	Animal AUC0-24 (hxnmol/L)	Safety Margin Based on Animal AUC*
Repeat dose toxicity	Rat (52 wk)	60	883	**5.2x**
	Dog (26 wk)	8	227.5	**1.3x**
Reproductive and developmental toxicity**	Rat (Seg I/II)	<20	146	**0.86x**
	Rabbits (Seg II)	< 5	430	**2.5x**
	Rat (Seg III)	20	146	**0.86x**

*AUC in human: 170 hxnmol/L at clinical dose of 4.5 nmol/kg (0.75 U/kg) based on two clinical studies: NN1250-1993 (exposure in steady state) and NN1250-3582 (clinical exposure)
** AUC in repro studies were adopted from NN206075 (rat) and NN206073 (rabbit).

12 Appendix/Attachments

n/a

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/s/

MIYUN M TSAI-TURTON
06/01/2012

KAREN L DAVIS BRUNO
06/04/2012

Pharmacology/Toxicology Mid-Cycle Deliverables

Drug Information: This NDA 203313 is for Ryzodeg™, insulin degludec/insulin aspart (IDegAsp), by Novo Nordisk to treat diabetes. Two NDAs were referenced in this submission: NDA 203314 (insulin degludec) and NDA 20986 (insulin aspart – Novolog). This drug product (100 U/ml) contains 420 nmol/ml insulin degludec (70%) and 180 nmol/ml insulin aspart (30%). This drug product is intended for once daily or twice-daily sc administration in diabetic patients.

The PD/PK/TOX profile of IDegAsp is mainly based on NDA 203314 (IDeg). The sponsor submitted 11 additional studies to further support the use of insulin 454 and insulin aspart combination.

3 PD studies
Study 6ulr051108-100-mar-2006: clamp study in pigs
Study ars-23-aug-2005: additive effect of the combo
Study UIR060904-0100 feb 2007: clamp study in pigs

4 PKstudies
Study 204383: PK I in pigs
Study 205053: PK II in pigs
Study 205220: PK III in pigs
Study 205419: PK IV in pigs

4 TOX studies
Study 208289: 4 week toxicity study in rats
Study 208337: 13 week toxicity (pivotal) study in rats
Study 208333: Seg II DRF rat
Study 208334: Seg II (pivotal) rat

Mid-Cycle Deliverable	Goal Date	Status
Filing Review Meeting • **Deliverable: Regulatory history, summary of pharmacology and toxicology findings from preliminary review of existing data, adequacy of NDA submission**	**45- day of NDA submission**	Completed
Genetox Study Review (impurities and drug substance) • **Deliverable: Draft review, label recommendation/interact with genetox committee**	**2-3 months of NDA submission**	N/A
Carcinogenicity Study Review • **Deliverable: Identify statistical reviewer**	**1-month of NDA submission**	N/A
• **Deliverable: Schedule ECAC meeting**	**1-month of NDA submission**	N/A
• **Deliverable: Draft review of Carcinogenicity study with statistical input, *Interact with statistician***	**6-months of NDA submission**	N/A
• **Deliverable: ECAC review of carcinogenicity study, incorporation of eCAC comments in the review, Identification of issues from Carcinogenicity study & related post marketing commitments, labeling recommendation**	**7- month of NDA submission**	N/A
ReproTox Study Review	**5- month of NDA**	Two Seg II rat studies have

• **Deliverable: Draft review, Identify issue/s (special studies for post marketing commitments), labeling recommendation, *Interact with Reprotox committee***	**submission**	been reviewed.
Impurity/Extractable Qualification • **Deliverable: Begin review; *interact with CMC***	**3-month of NDA submission-will depend on CMC also**	CMC reviewer: Muthukumar Ramaswamy. His review is in DARRTS dated Feb 8th 2012. No additional deficiency is addressed bedside the ones already listed in NDA 203314.
• **Deliverable: Identify whether the impurities are qualified, Identify approvability/post marketing commitment issues**	**5-6 month of NDA submission-if sponsor has final formulation**	N/A
Toxicokinetic Study Review • **Deliverable: Draft review; *Interact with Clinical Pharmacology* for dose and ADME, *Interact with Medical Reviewer* for adverse event comparison and dose multiples**	**6- months of NDA submission**	In progress - four PK studies in pigs.
Chronic Toxicity Study Review • **Deliverable: Draft review**	**6- months of NDA submission**	Two repeat-dose (4 wk and 13 wk) rat toxicity studies have been reviewed.
Pharmacology Study Review • **Deliverable: Draft review, identify PD issues**	**6- months of NDA submission**	In progress - two clamp studies in pigs and one additive effect study.
Tox findings compared in animal chronic studies and human phase 3 studies • **Deliverable: Input in ODS database**	**8- months of NDA submission**	The final recommendation is pending completion of comprehensive review.

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/s/

MIYUN M TSAI-TURTON
02/27/2012
p/t MCR

KAREN L DAVIS BRUNO
02/27/2012

Pharmacology/Toxicology Mid-Cycle Deliverables

Drug Information: This NDA 203314 is for Tresiba™, insulin degludec (IDeg), by Novo Nordisk to treat diabetes. This long acting insulin analog is in two strengths: U-100 (600 nmol/ml) and U-200 (1200 nmol/ml). This drug product is intended for once daily sc administration at any time of the day, impendent of meals, in diabetic patients.

Mid-Cycle Deliverable	Goal Date	Status
Filing Review Meeting • **Deliverable: Regulatory history, summary of pharmacology and toxicology findings from preliminary review of existing data, adequacy of NDA submission**	**45- day of NDA submission**	Completed
Genetox Study Review (impurities and drug substance) • **Deliverable: Draft review, label recommendation/interact with genetox committee**	**2-3 months of NDA submission**	N/A
Carcinogenicity Study Review • **Deliverable: Identify statistical reviewer**	**1-month of NDA submission**	N/A
• **Deliverable: Schedule ECAC meeting**	**1-month of NDA submission**	N/A
• **Deliverable: Draft review of Carcinogenicity study with statistical input, *Interact with statistician***	**6-months of NDA submission**	N/A
• **Deliverable: ECAC review of carcinogenicity study, incorporation of eCAC comments in the review, Identification of issues from Carcinogenicity study & related post marketing commitments, labeling recommendation**	**7- month of NDA submission**	N/A
ReproTox Study Review • **Deliverable: Draft review, Identify issue/s (special studies for post marketing commitments), labeling recommendation, *Interact with Reprotox committee***	**5- month of NDA submission**	Studies have been reviewed: Seg I (rat), Seg II (rat/rabbit), and Seg III (rat) studies. Sponsor is seeking Category (b) (4) labeling.
Impurity/Extractable Qualification • **Deliverable: Begin review; *interact with CMC***	**3-month of NDA submission-will depend on CMC also**	CMC reviewer: Joseph Leginus. His review is in DARRTS dated Feb 8th 2012. The CMC has listed few deficiencies with regards to impurities in their drug product (See page 141-142 in his review). *The sponsor identified product related impurities were identified (i.e.* (b) (4)

		(b) (4) *impurities, HMWP, which all impurities were related to insulin degludec) as well as other impurities (i.e. leachables).*
• **Deliverable: Identify whether the impurities are qualified, Identify approvability/post marketing commitment issues**	**5-6 month of NDA submission-if sponsor has final formulation**	The sponsor submitted 1-month rat toxicity study to investigate an aged vs. non-aged insulin 454. The review of this study is in process.
Toxicokinetic Study Review • **Deliverable: Draft review; *Interact with Clinical Pharmacology* for dose and ADME, *Interact with Medical Reviewer* for adverse event comparison and dose multiples**	**6- months of NDA submission**	Studies are currently under reviewed.
Chronic Toxicity Study Review • **Deliverable: Draft review**	**6- months of NDA submission**	Studies have been reviewed. 12-month rat toxicity study (pivotal) was conducted with mammary tissue examination.
Pharmacology Study Review • **Deliverable: Draft review, identify PD issues**	**6- months of NDA submission**	Studies have been reviewed. Primary PD studies (clamp studies, mitogenicity, binding affinities of IR and IGF-1R, glycogen synthesis, lipolysis), secondary PD study, and safety pharm studies (CV, behavioral, respiratory, QT, ECG)
Tox findings compared in animal chronic studies and human phase 3 studies • **Deliverable: Input in ODS database**	**8- months of NDA submission**	The final recommendation is pending completion of comprehensive review.

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/s/

MIYUN M TSAI-TURTON
02/27/2012
p/t MCR

KAREN L DAVIS BRUNO
02/27/2012

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

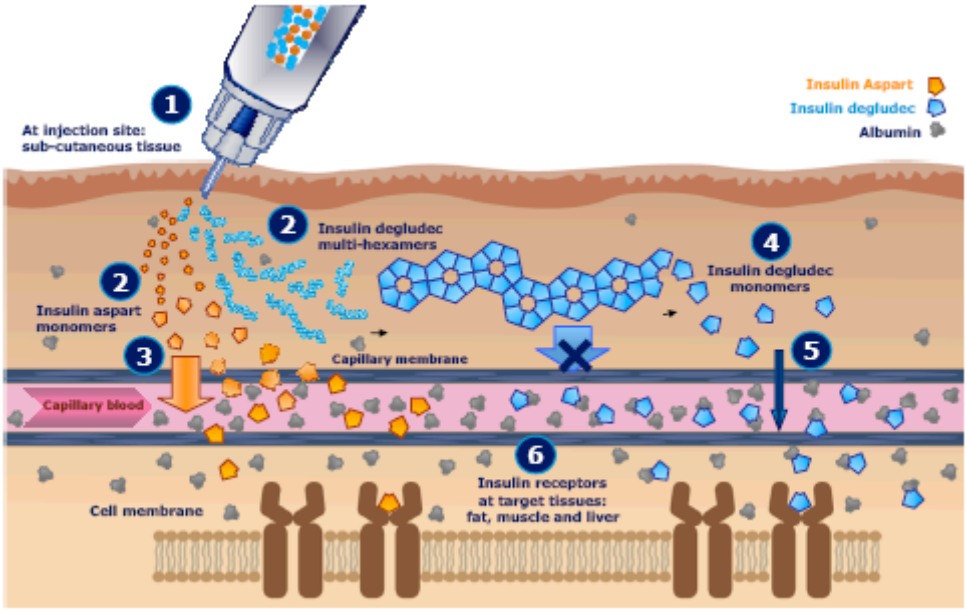
NDA 203313: This NDA is a 505(b)(1) application.
Submission date: Sept 29th 2011
Sponsor: Novo Nordisk Inc.
Drug: Ryzodeg (insulin degludec/insulin aspart, sc injection) for diabetes

Drug Information:

This submission is a new NDA application for insulin degludec/insulin aspart (IDegAsp), an insulin analog. It was first submitted in March 2008 under IND 73198. The sponsor references two of their own NDAs: NDA 203314 for insulin degludec and NDA 20986 for insulin aspart (Novolog; as the listed drug). This drug product is a 100 U/ml co-formulation of 420 nmol/ml insulin degludec (70%) and 180 nmol/ml insulin aspart (30%). This is intended for once-daily or twice-daily sc administration in diabetic patients. During development of this drug product, several names have been used:

- Insulin degludec: insulin 454, SIBA, and NN1250
- Insulin degludec + insulin aspart: insulin 454/insulin aspart, SIAC, and NN5401

A step-wise illustrations of the rapid absorption of insulin aspart and slow absorption of insulin degludec in the IDegAsp co-formulation



Nonclinical Development of IDegAsp:
The nonclinical development of IDegAsp focused on safety and efficacy evaluation of IDeg for which a complete nonclinical development program was conducted (under NDA 203314). Based on ICH M3 guideline on combination medical products, the efficacy of IDegAsp had been assessed in primary PD studies as well as tolerance and toxicity studies following sc administration.

- PK profile

(Only IDegAsp-related studies were described here)

In vitro experiments showed that the effect of IDeg and Asp were additive and there were no synergistic or inhibitory interactions between the two. In addition, pig studies demonstrated that a 2:1 ratio of IDeg/Asp resulted in improved PD/PK profiles compared to those observed for

biphasic insulin aspart 30 (NovoMix/NovoLog Mix). A sharper peak and longer duration of action was observed.

Overview of Pharmacology studies

Study type	Test compound	Administration	Species
Efficacy Pharmacology			
Insulin receptor binding	IDeg	*in vitro*	rat, pig, dog, human
IGF-1 receptor binding	IDeg	*in vitro*	rat, dog, human
Insulin receptor signalling and kinetics	IDeg	*in vitro*	human
Additive effect	IDegAsp	*in vitro*	rat
Metabolic effect (adipocyte, hepatocyte, muscle cell)	IDeg	*in vitro*	rat, human
Mitogenic effect (COLO205, HMEC, L6-hIR, MCF-7 cells)	IDeg	*in vitro*	human
Receptor selectivity	IDeg	*in vitro*	various
Pharmacodynamic (normal and diabetic animals)	IDeg	i.v.	rat
PK/PD	IDeg, IDegAsp	s.c.	pig
Safety Pharmacology			
Effect on action potential	IDeg	*in vitro*	rabbit
Effect on CNS and respiration	IDeg	s.c.	rat
Effect on cardiovascular system	IDeg	s.c. and i.v.	dog

- PD profile

(Only IDegAsp-related studies were described here)

The PK of IDegAsp was studied in rat with to-be-marketed formulation and in pigs with various formulations (to support early clinical studies). These studies demonstrated that IDeg and Asp can be co-formulated without changing the individual PK profiles of the two insulins.

Overview of Pharmacokinetic studies

Toxicokinetic	Test compound	Administration	Species
DRF/MTD, 4-, 13-, 26- and 52-weeks	IDeg	s.c.	mouse[b], rat, dog[a]
4- and 13-week	IDegAsp	s.c.	rat
Fertility[c] and embryo-foetal development	IDeg	s.c.	rat, rabbit
Embryo-foetal development	IDegAsp	s.c.	rat
Pharmacokinetic			
Single dose	IDeg, IDegAsp[d]	s.c. and i.v.	rat, rabbit, dog, pig
Multiple dose (7-days)	IDeg	s.c. and i.v.	rat, dog
Single dose (radioactivity)	IDeg	s.c. and i.v.	rat, dog
Distribution			
QWBA	IDeg	s.c. and i.v.	rat
Placenta transfer	IDeg	s.c.	rat
Protein binding	IDeg	*in vitro*	rat, dog, rabbit, pig, human
Metabolism			
Hepatocytes, Cathepsin D	IDeg	*in vitro*	rat, dog, human
Plasma, urine[c], bile[c], faeces[c], milk[c]	IDeg	s.c.	rat, dog
Excretion			
Urine, bile, faeces	IDeg	s.c.	rat
Milk	IDeg	s.c.	rat

a – DRF/MTD, 4- and 26-week
b – 4- and 13-week
c – rat only
d – pig only

- Tox profile

(Only IDegAsp-related studies were described here)

The general toxicity of IDegAsp was investigated in a 13-week repeat-dose toxicity study in rats. NPH insulin was included as comparator. The lowering effects on blood glucose levels with IDegAsp was similar in nature and magnitude to those induced by NPH insulin and IDeg administrated alone. Changes observed in this study were considered related to exaggerated pharmacological effects of insulin. In addition, the pivotal embryo-fetal study in rats with IDegAsp was conducted. The effects observed were also considered related to pharmacological effects of insulin. Local tolerance studies at injection site with early development and to-be-marketed drug products were conducted in pigs. The local tissue reaction was mild and comparable to that of vehicle or NPH insulin. Note: Since the sponsor uses their own approved insulin aspart (NDA 20-986), the-13 week repeat-dose rat study is sufficient to characterize the IDeg/Asp combination and allow bridging to each components' nonclinical developments.

Overview of pivotal toxicity studies

Study type and duration	Test compound	Administration	Species
Single-dose toxicity	IDeg	s.c.	rat and dog[b]
Repeat-dose toxicity			
4 week	IDeg, IDegAsp[a]	s.c.	rat and dog
13 week	IDegAsp	s.c.	rat
26 week	IDeg	s.c	rat and dog
52 week including carcinogenicity assessment	IDeg	s.c.	rat
Reproductive and developmental toxicity studies			
Fertility	IDeg	s.c	rat
Embryo-foetal development	IDeg, IDegAsp[a]	s.c.	rat and rabbit
Pre- and post-natal development	IDeg	s.c.	rat
Local tolerance			
Early development drug product and "to be marketed" drug product	IDeg, IDegAsp	s.c.	Pig/minipig
"To be marketed" drug product	IDeg, IDegAsp	i.m., i.v., i.a.	rabbit

a – rat only

b – integrated part of maximum tolerable dose study (204317); this study was conducted as a non-GLP activity

Moreover, 6 groups of product related impurities and HMWP were identified and tested in nonclinical studies and they were related to either IDeg or Asp. There were no process-related impurities detected in IDegAsp drug product. Leachables are being determined for the (b) (4) months shelf-life and 4 weeks in-use periods for 100 U/ml IDegAsp. Excipients of IDegAsp (i.e. phenol and m-cresol (b) (4), glycerol and sodium chloride (b) (4), and zinc acetate (b) (4) were well-known chemicals present in similar or higher levels in marketed insulin products.

- Safety margin

The animal/human exposure ratios were calculated based on NOAELs in the animal studies at steady state and human exposure at the highest mean clinical dose (1.08 U/kg) in the confirmatory clinical study with T2MD on a twice-daily dose regime.

Animal/human exposure ratios

	Rat		Dog		Rabbit	
Study type	$AUC_{(0-24)}$	C_{max}	$AUC_{(0-24)}$	C_{max}	$AUC_{(0-24)}$	C_{max}
Insulin degludec: Repeated dose toxicity[a]	5.1	13	1.3	1.5	-	-
IDegAsp: Repeated dose toxicity[b]	7.0/12[f]	26/41[f]	-	-	-	-
Insulin degludec: Carcinogenicity[c]	5.1	13	-	-	-	-
Insulin degludec: Reproduction toxicity[d]	5.0	15	-	-	9.7	13
IDegAsp: Reproduction toxicity[e]	7.6/24[f]	19/101[f]	-	-	-	-

References

a – Study no.:206539 (rat), 206315 (dog);

b – Study no.:208337

c – Study no.:206539

d – Study no.:206075, 206076, 208335, 208336 (rat), 206073, 206074 (rabbit);

e – Study no.:208334

f – Exposure ratio for "insulin degludec"/"insulin aspart"

Insulin degludec: Human exposure from Trial NN1250-1993 (PK); NN5401-3592 (dose)

IDegAsp: Human exposure from Trial NN1250-1993 (PK – IDeg); NN5401-3539 (PK – IAsp), NN5401-3592 (dose)

FILING CHECKLIST

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	Yes		This electronic submission contains 4 modules – regional (forms), common technical document summary, quality (CMC), nonclinical study reports (pharm/tox), and clinical study reports (clinical trials).
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	Yes		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	Yes		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA. (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	Yes		Timeline: March 2008 – IND submission Feb 2009 – EOP2 meeting June 2011 – pre-NDA meeting Sept 2011 – NDA submission Genotoxicity and carcinogenicity were not assessed with IDegAsp.
5) Were the studies adequately designed (i.e. appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	Yes		
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (i.e. adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?			Three groups of IDeg and three groups of Asp impurities and related substances were identified in addition to HMWP. Leachables from the container closure system have been identified and qualified. Excipients used in IDegAsp were seen in other marketed products at similar or higher concentrations.

ITEM	YES	NO	COMMENT
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	Yes		SC injection
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	Yes		Proposed labeling related to nonclinical: 8.1 Pregnancy Category C 13 Nonclinical Toxicology Human exposure related to animal exposure levels were calculated based on AUC and Cmax levels.
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	Yes		
10) Reasons for refusal to file: None			

Reviewing Pharmacologist: Miyun Tsai-Turton

Supervisory Pharmacologist: Karen Davis-Bruno

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/s/

MIYUN M TSAI-TURTON
11/15/2011

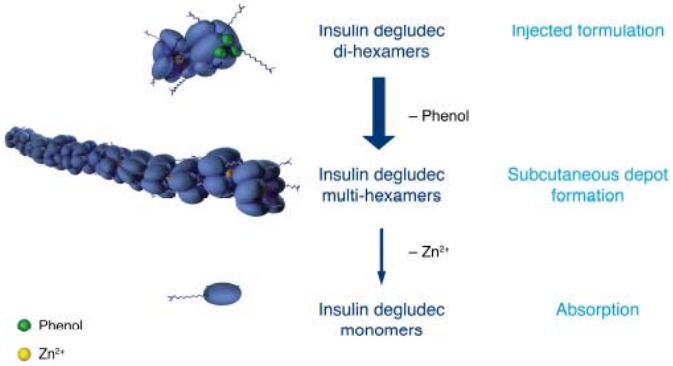
KAREN L DAVIS BRUNO
11/15/2011

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA 203314: This NDA is a 505(b)(1) application.
Submission date: Sept 29th 2011
Sponsor: Novo Nordisk Inc.
Drug: Tresiba™ (insulin degludec) for diabetes

Drug Information:

This submission is a new NDA application for insulin degludec (IDeg), a long acting soluble insulin analogue. It was first submitted in Sept 2007 under IND 76496. The sponsor is filing for approval of this drug product in two strengths: U-100 and U-200, containing 600 nmol/ml and 1200 nmol/ml drug substance respectively. This is intended for once-daily sc administration at any time of the day, independent of meals, in diabetic patients. During development of this drug product, several names have been used: insulin degludec: insulin 454, SIBA, and NN1250. The diagram below provided by the sponsor is the proposed mechanism of protraction for IDeg from injection into tissue for absorption into circulation.



Nonclinical Development of IDeg:

Since IDeg is designated to alter absorption rate, the nonclinical development strategy focused on evaluating the ultra-long PK/PD effects of IDeg without affecting efficacy and safety compared to human insulin.

- PK/PD profile

A series of biological in vitro studies were done to demonstrate that IDeg is a specific agonist to the human insulin receptor and the MOA is identical to that of human insulin and other insulin analogues. In vivo studies showed the blood glucose lowering effect of IDeg in rats and pigs and the slow absorption resulting in the ultra-long/stable PK/PD profiles in pigs. In addition, a series of safety pharmacology studies were conducted to assess its potential effects on CNS, CV, and respiratory in rats and/or dogs.

Overview of Pharmacology studies

Study type	Administration	Species
Efficacy Pharmacology		
Insulin receptor binding	*in vitro*	rat, pig, dog, human
IGF-1 receptor binding	*in vitro*	rat, dog, human
Insulin receptor signalling and kinetics	*in vitro*	human
Metabolic effect (adipocyte, hepatocyte, muscle cell)	*in vitro*	rat, human
Mitogenic effect (COLO205, HMEC, L6-hIR, MCF-7 cells)	*in vitro*	human
Receptor selectivity	*in vitro*	various
Pharmacodynamic (normal and diabetic animals)	i.v.	rat
PK/PD	s.c.	pig
Safety Pharmacology		
Effect on action potential	*in vitro*	rabbit
Effect on CNS and respiration	s.c.	rat
Effect on cardiovascular system	s.c. and i.v.	dog

Overview of pharmacokinetic studies

Toxicokinetic		
DRF/MTD, 4-, 13-, 26- and 52-weeks	s.c.	mouse[b], rat, dog[a]
Fertility[d] and embryo-foetal development	s.c.	rat, rabbit
Pharmacokinetic		
Single dose (insulin degludec)	s.c. and i.v.	rat, rabbit, dog, pig
Multiple dose (7-days)	s.c. and i.v.	rat, dog
Single dose (radioactivity)	s.c. and i.v.	rat, dog
Distribution		
QWBA	s.c. and i.v.	rat
Placenta transfer	s.c.	rat
Protein binding	*in vitro*	rat, dog, rabbit, pig, human
Metabolism		
Hepatocytes, Cathepsin D	*in vitro*	rat, dog, human
Plasma, urine[c], bile[c], faeces[c], milk[c]	s.c.	rat, dog
Excretion		
Urine, bile, faeces	s.c.	rat
Milk	s.c.	rat

a – DRF/MTD, 4- and 26-week **b** – 4- and 13-week **c** – rat only

- Tox profile

The toxicity of IDeg was evaluated after sc single dose administration in rats and dogs and after sc repeat-dose administration in rats (up to 52 weeks) and dogs (up to 26 weeks). Hypoglycaemia and hypoglycaemia-related adverse effects were results of the exaggerated pharmacological effect of insulin. In addition, genotoxicity and standard carcinogenicity studies were not performed with IDeg. Reproductive and development toxicity of IDeg were assessed in rats and rabbits. The adverse effects were considered related to pharmacological effects of insulin. Local tolerance studies showed that local tissue reaction (at injection site) was mild and comparable to that of vehicle or NPH insulin. Note: The 52-week repeat-dose tox study in rats included NHP insulin as comparator and mammary tumor assessment. This study was reviewed in details by Lee Elmore and his review is in DARRTS (dated August 2008).

Overview of pivotal toxicity studies

Study type and duration	Route of administration	Species
Single-dose toxicity	s.c.	Rat and dog[a]
Repeat-dose toxicity		
4 week	s.c.	Rat and dog
26 week	s.c	Rat and dog
52 week including carcinogenicity assessment	s.c.	Rat
Reproductive and developmental toxicity studies		
Fertility	s.c	Rat
Embryo-foetal development	s.c.	Rat and rabbit
Pre- and post-natal development	s.c.	Rat
Local tolerance		
Early development drug product and "to be marketed" drug product	s.c.	Pig/Minipig
"To be marketed" drug product	i.m., i.v., i.a.	Rabbit

a – integrated part of maximum tolerable dose study (204317); this study was conducted as a non-GLP activity

Moreover, product related impurities (i.e. (b) (4) were identified and qualified. All impurities were related to IDeg. Other impurities (i.e. leachables including (b) (4)) from the container-closure system) are currently been determined for (b) (4) months shelf-life and 8 weeks in-use periods of the 100 U/ml IDeg. Excipients of IDeg (i.e. phenol and m-cresol (b) (4) and glycero (b) (4)) were well-used excipients which were present in similar of higher concentrations in currently marketed insulin products for sc administration such as insulin aspart.

- Safety Margin

The animal/human exposure ratios were calculated based on NOAELs in the animal studies of longest duration (as AUC and Cmax at steady state) and human exposure at the mean clinical dose (0.75 U/kg) in the confirmatory clinical trial using the highest insulin dose.

Animal/human exposure ratios

	Rat		Dog		Rabbit	
Study type	$AUC_{(0\text{-}24)}$	C_{max}	$AUC_{(0\text{-}24)}$	C_{max}	$AUC_{(0\text{-}24)}$	C_{max}
Repeated dose toxicity[a]	5.2	13	1.3	1.6	-	-
Carcinogenicity[b]	5.2	13	-	-	-	-
Reproduction toxicity[c]	5.1	15	-	-	9.7	13

a – Study no: 206539 (rat), 206315 (dog);
b – Study no: 206539
c – Study no: 206075, 206076, 208335, 208336 (rat), 206073, 206074 (rabbit);
Human exposure calculated from NN1250-1993 (PK) and NN1250-3582 (clinical dose)

FILING CHECKLIST

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	Yes		This electronic submission contains 4 modules – regional (forms), common technical document summary, quality (CMC), nonclinical study reports (pharm/tox), and clinical study reports (clinical trials).
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	Yes		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	Yes		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA. (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	Yes		Timeline: Sept 2007 – IND submission Feb 2009 – EOP2 meeting June 2011 – pre-NDA meeting Sept 2011 – NDA submission Genotox/Carci studies were not done but the sponsor justified their rationales with published literature/reference for insulin. ▪ The 52-week repeat-dose tox study in rats used NHP insulin as comparator to assess mammary tumor potential.
5) Were the studies adequately designed (i.e. appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	Yes		
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (i.e. adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	Yes		IDeg-related Impurities in the drug product have been tested in nonclinical studies. No process-related impurities have been detected. Leachables from the container closure system have also been identified and qualified. Excipients are well-known chemicals present at similar or higher levels in marketed insulin products.

ITEM	YES	NO	COMMENT
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	Yes		SC injection
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	Yes		Proposed labeling related to nonclinical: 8.1 Pregnancy Category C 13 Nonclinical Toxicolog Human exposure related to animal exposure levels were calculated (based on AUC and Cmax).
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	Yes		
10) Reasons for refusal to file: None			

Reviewing Pharmacologist: Miyun Tsai-Turton

Supervisory Pharmacologist: Karen Davis-Bruno

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MIYUN M TSAI-TURTON
11/15/2011

KAREN L DAVIS BRUNO
11/15/2011