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APPLICATION NUMBER:

21-536

PHARMACOLOGY REVIEW(S)

MEMORANDUM

June 16, 2005

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-536

I concur that this application may be approved. Although the primary pharmacology/toxicology reviewer (Dr. Herman Rhee) recommended that the drug product be labeled as _____ I agree that Pregnancy Category C is more appropriate and consistent with available data.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

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/s/

Kenneth Hastings
6/16/05 12:34:15 PM
PHARMACOLOGIST

PHARMACOLOGY AND TOXICOLOGY REVIEW

NDA #: 21-536

**Product Name : Levemir (insulin detemir)
Sponsor: Novo Nordisk Pharmaceuticals, Inc.**

Indication: Diabetes

Division: Metabolic and Endocrine Drug Products

Reviewer: Herman Rhee, Ph.D.

Date: August 4, 2003

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability: Approval.

Preclinical pharmacology and toxicology recommends approval of NDA21-536, based on preclinical findings on insulin detemir as reviewed in this document.

1.2 Recommendation for nonclinical studies: None

1.3 Recommendations on labeling:

Carcinogenicity, Mutagenicity, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed.

Pregnancy: Teratogenic Effects:

In a fertility and embryonic development study, insulin detemir was administered to female rats before mating, during mating, and throughout pregnancy at doses up to 300 nmol/kg/day (3 times the recommended human dose, based on AUC ratio). Doses of 150 and 300 nmol/kg/day produced numbers of litters with visceral anomaly. Doses up to 900 nmol/kg/day (approximately — times the recommended human dose based on AUC ratio) were given to rabbits during organogenesis. Drug-dose related increases in the incidence of fetuses with gall bladder abnormalities such as small, bilobed, bifurcated and missing gall bladders were observed at dose of 900 nmol/kg/day. The rat and rabbit embryofetal development studies indicated that detemir and human insulin had similar effects regarding embryotoxicity and teratogenicity.

Summary of nonclinical findings

1.4 Brief overview of nonclinical findings

Insulin detemir (ϵ -Lys^{β29}-myristoyl, des-^{β30} Human Insulin) binds extensively to albumin with a fatty acid side-chain which may be the basis of its rather long duration of action compared to regular human insulin. The potency of insulin detemir in binding and activating the insulin receptor was low compared to human insulin and other insulin analogues in animal studies. The metabolic potency of insulin detemir in mouse primary adipocytes was reduced 4-5 fold compared to human insulin, in agreement with the reduced receptor binding affinity.

Binding affinity of insulin detemir to structurally related IGF-I receptors was low to a similar extent as its affinity for the insulin receptor. The ratio between insulin and IGF-I receptor affinities is therefore approximately the same for insulin detemir and human

insulin. The mitogenic potency of insulin detemir was reduced about 10-fold as compared to human insulin, which might be due to reduced affinity for the insulin receptors and IGF-I receptors.

Safety studies conducted in mice and rats after s.c. doses of up to 200 nmol/kg/day indicated that insulin detemir had no remarkable effects on neurological and renal systems. A cardiovascular and pulmonary safety study with insulin detemir in dog after s.c. doses of up to 18 nmol/kg/day did not establish remarkable adverse effects. The sponsor performed single dose subcutaneous toxicity studies in mice and rats and repeated-dose toxicity studies in rats and dogs for various duration. In 6-month toxicity study in rats, there were no treatment-related effects on body weight, food consumption, hematological, or ophthalmoscopic parameters after s.c. doses up to 300 nmol/kg/day. NOEL was below 30 nmol/kg/day while NOAEL appeared to be 96 nmol/kg/day. Therapeutic exposure ratio at the NOAEL was estimated to be 0.5 in male rats, while it was 1 in females, based on human AUC data (73 nmol.hr/ml after 11.76 nmol/kg/day in diabetic subjects). Slightly elevated antibody levels with insulin detemir treatment were noted in 50% of the animals, independent of the dose.

In a 6-month sc toxicity study in dogs, there were increases in adrenal weight at 3.6 and 7.2 nmol/kg/day, and at 7.2 nmol/kg/day NPH human insulin. There were no clear associated macroscopic and microscopic changes in the adrenal glands. It appears that there is no gender difference in major PK and PD parameters in dogs and no antibody reaction towards the test article could be measured. Based on the effect of insulin detemir on adrenal gland weight, NOAEL would be below 3.6 nmol/kg/day. Therapeutic exposure ratios at the NOAEL were 0.6 and 0.7 in females and males, respectively (based on human AUC values after 11.76 nmol/kg/day in diabetic subjects). There were no remarkable mitogenic and immunogenic adverse effects of the test article.

An ICH genotoxicity test battery (Ames test, cytogenetic test in human lymphocytes and mouse micronucleus test) was performed by the sponsor, which indicated that genotoxic potential of insulin detemir was negative. The sponsor did not perform standard 2-year carcinogenicity studies because this Division waived animal carcinogenicity studies for insulin detemir. Effects of insulin detemir on fertility, embryo-fetal development, pre- and postnatal development were evaluated in rats and rabbits. Effects observed were comparable to human insulin.

Effects of insulin detemir on local tolerance were evaluated in mini-pigs and immunogenic potential was evaluated in rabbits without the test article related actions. Potential mitogenic action of insulin detemir was evaluated in 1) CHO-K1 and human B10 osteosarcoma cells, 2) Human hepatoma cells (HepG2) in the presence of 0.1% human serum albumin (HSA) and 3) MCF-7 cells, which were largely negative.

2.2 Nonclinical safety issues relevant to clinical use

There were insulin detemir dose-dependent prolonged hypoglycemic episodes in mice, rats, rabbits, mini-pigs, and dogs in toxicology studies.

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-536

Review number: 001

Sequence number/date/type of submission: 000 /Dec.5, 2002/Commercial

Information to sponsor: Yes (x) No ()

Sponsor and/or agent: Novo Nordisk Pharmaceuticals, Inc., Princeton, NJ [(609)987-5852]

Manufacturer for drug substance: Novo Nordisk A/S, Novo Alle, Bagsvaerd, Denmark

Reviewer name: Herman Rhee, Ph.D.

Division name: Metabolic and Endocrine Drug Products

HFD #: 510

Review completion date: June 30, 2003

Drug:

Trade name: Levemir or —

Generic name: Insulin detemir(rDNA origin) injection, SoLongIns

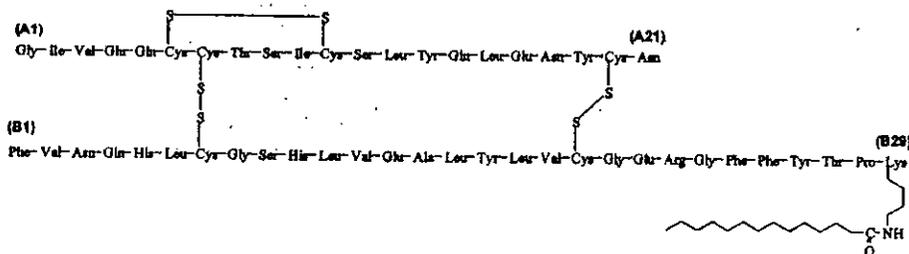
Code name: NN304

Chemical name: Lys^{B29} (N^E-tetradecanoyl)des (β30) human insulin

CAS registry number:

Molecular formula/molecular weight: C₂₆₇H₄₀₂ O₇₆N₆₄S₆/5916.9

Structure:



Relevant INDs/NDAs/DMFs: IND#34,945 (LysProInsulin), #37,159(Ly197535), #51,789(InsulinNN304)/NDA#19-938(Novolin R), #20-986(NovoLog), #21-081(Lantus)

Drug class: Long acting, soluble human insulin analogue

Indication: Diabetes

Clinical formulation: Three formulations of insulin detemir are: 600 nmol/mL, 1200 nmol/mL and 2400 nmol/mL. Each milliliter of insulin detemir has appropriate amount of insulin detemir, 65.4 µg zinc, 2.06 mg meta-cresol, 30 mg mannitol, 1.8 mg phenol, 0.89 mg disodium phosphate dihydrate, 1.17 mg sodium chloride, and water for injection (pH=7.4).

Route of administration: Subcutaneous

Proposed use: —

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

Studies reviewed within this submission: Please see the subsequent reviews below.

Studies previously reviewed under IND #51,789:

Rev#1

Insulin receptor binding studies, Safety studies, Pharmacokinetic studies, Single iv toxicity study in SD rats, Single sc toxicity study in mice, 4-Week sc toxicity study in rats, 4-Week sc toxicity study in dogs, Genotoxicity, Range finding studies for fertility, embryofetal development in the rat and rabbits, Immunogenicity study in rabbits(NN940479)

Rev#2: Acute single iv toxicity study in rats and Irwin Test

Rev#3: NN304 6-Month sc toxicity studies in the rat and dog, Insulin detemir with CytP450 metabolism in SD rats (NN970040), Insulin detemir with CytP450 metabolism in SD rats (NN970040), and Drug disposition study in dogs (NN9700050)

Rev#4: Mitogenic toxicity of Insulin Detemir (NN304)

Rev#5: Binding of NN304 to Insulin and IGF-1 receptors in HepG2 cells

3.2 PHARMACOLOGY

3.2.1 Brief summary

Insulin detemir (ϵ -Lys^{β29}-myristoyl, des-(β30 Human Insulin) exists in hexameric state in the presence of zinc and phenol. Its extensive albumin binding with the fatty acid side-chain is the basis of long acting property, which produced delayed hypoglycemia in animals. The duration of action for 0.3 U/kg insulin detemir was estimated to be 17 hours compared to an estimated 12.7 hours for 0.3 U/kg NPH insulin in human. The

estimation of variance for PD endpoints was approximately 3 to 6 times lower than for NPH insulin and 2.5 to 4 times lower than for insulin glargine within individual subjects.

Since all biological effects of human insulin are mediated via the insulin receptor, characterization of the insulin receptor binding properties of insulin detemir is important in its molecular pharmacological evaluation. The potency of insulin detemir in binding and activating the insulin receptor is reduced as compared to human insulin and other insulin analogues as shown in Table 1. The metabolic potency of insulin detemir in mouse primary adipocytes was reduced 4-5 fold compared to human insulin, in agreement with the reduced receptor binding affinity.

Insulin detemir, human insulin, human IGF-1 and iodinated insulin and IGF-1 were compared in binding to insulin receptors. The affinity of insulin detemir for the insulin receptor was 0.54 ± 0.14 nM (mean \pm S.D.) whereas that of insulin was 0.10 ± 0.03 nM, resulting in a relative affinity of insulin detemir of $18 \pm 3\%$. For the IGF-1 receptor the affinity of IGF-1 was 0.06 ± 0.015 nM whereas insulin and insulin detemir both exhibited low affinity. The affinity of insulin detemir relative to insulin was $16 \pm 2\%$ for the IGF-1 receptor.

The affinity of insulin detemir for the structurally related IGF-I receptor was reduced to a similar extent as its affinity for the insulin receptor. In addition to its primary metabolic effects, human insulin can stimulate mitogenesis via insulin receptors or IGF-I receptors. The mitogenic potency of insulin detemir was reduced about 10- fold as compared to human insulin, in studies with three different cell lines. The reduced mitogenic potency of insulin detemir is thought to be caused by the reduced affinity for the insulin and IGF-I receptors, combined with potential changes in PK parameters such as an increased dissociation rate of insulin detemir from the insulin receptor.

The relationships between insulin structure, insulin receptor binding, IGF-I receptor binding, metabolic potency and mitogenic potency have been investigated using a series of insulin analogues designed for clinical use. These include the rapid-acting analogues insulin lispro (B28Lys, B29Pro human insulin) and insulin aspart (B28Asp human insulin) and the long- acting analogues insulin glargine (A21Gly, B31Arg, B32Arg human insulin) and insulin detemir (Lys^{B28}(N^E-tetradecanoyl) des (B30) human insulin) as discussed subsequently.

Table 1 Receptor binding and metabolic and mitogenic potencies of insulin analogues relative to human insulin

Analogue	Insulin receptor affinity (%)	Insulin receptor off-rate (%)	Metabolic potency (lipogenesis) (%)	IGF-I receptor affinity (%)	Mitogenic potency (%)
Human insulin	100	100	100	100	100
B10Asp	205 \pm 20	14 \pm 1	207 \pm 14	587 \pm 50	975 \pm 173
Insulin aspart	92 \pm 6	81 \pm 8	101 \pm 2	81 \pm 9	58 \pm 22
Insulin lispro	84 \pm 6	100 \pm 11	82 \pm 3	156 \pm 16	66 \pm 10
Insulin glargine	86 \pm 3	152 \pm 13	60 \pm 3	641 \pm 51	783 \pm 132
A21Gly	78 \pm 10	162 \pm 11	88 \pm 3	42 \pm 11	34 \pm 12
B31B32diArg	120 \pm 4	75 \pm 8	75 \pm 5	2049 \pm 202	2180 \pm 390
Insulin detemir	18 \pm 3	204 \pm 9	Approx. 29	16 \pm 2	Approx. 11

Reference Kurtzhals et al., Diabetes 2000; 49:999-1005³

Insulin detemir absorption appears drug-dose dependent as revealed in AUC elevation after subcutaneous administration in mice, rats and dogs. It is distributed slowly to peripheral target tissues compared to NPH insulin since it binds to albumin extensively. In rats and dogs, over 30% of the dose was excreted as CO₂ following a single-dose administration. There were several polar components that were excreted in the urine, which was C₆- moiety of the fatty acid linked to Lys^{B29}. In SD rats, after insulin detemir 50 U/kg/day administration, there was significant decrease in 2 α - and 16 α -hydroxylation of testosterone, indicating minor down regulation on the male specific rat CYP2C11. In female rats, it produced a small but significant increase in total Cytochrome P450 without specific effects on CYP1A1/2, CYP2A1, CYP2B1 or CYP3A1/2 activities or protein level detected by immunoblotting (CYP2C11, CYP2E1, and CYP4A).

A safety study was conducted in NMRI mice and Han Wistar rats evaluating s.c. doses of approximately 2 to 200 nmol/kg/day. The study indicated that insulin detemir had no remarkable effects on the neurological and renal systems. A cardiovascular and pulmonary safety study was carried out in Han Wistar rats. An acute cardiovascular safety study was also conducted in anesthetized Beagle dogs at doses 0.18, 1.8 and 18 nmol/kg/day. The studies did not demonstrate remarkable adverse effects of insulin detemir, although there was a marginal decrease in blood pressure after the high dose in dogs.

Acute toxicology studies were carried out in mice and rats after iv or subcutaneous administration of insulin detemir (2 to 24 μ mol/kg/day) for up to 2 weeks without clear treatment-related toxicities. In a 6-month rat toxicity study, 24 SD rats/sex/group were administered subcutaneously either vehicle or NN304 at doses of 5, 16, and 50 U/kg/day (30, 96, 300 nmol/kg/day). There were no treatment-related effects on body weight, food consumption, hematological, or ophthalmoscopic parameters except dose-dependent decreases in blood glucose. NOEL was below 30 nmol/kg/day because all doses reduced blood glucose levels while NOAEL appeared to be 96 nmol/kg/day. Therapeutic exposure ratio at the NOAEL was estimated to be 0.5X in male rats, while it was 1.1X in females (based on human AUC value, 73 nmol.hr/ml after 11.76 nmol/kg/day in diabetic subjects). Slightly elevated antibody levels with NN304 treatment were noted in 50% of the animals in all groups, independent of the dose.

A 6-month toxicity study was carried out in Beagle dogs after daily subcutaneous injection at a dose of 1.8, 3.6, and 7.2 nmol/kg/day (0.3, 0.6 and 1.2 U/kg/day). There were increases in adrenal weight at 3.6 and 7.2 nmol/kg/day, and at 7.2 nmol/kg/day NPH insulin. There were no clear associated macroscopic and microscopic changes in the adrenal glands. It appears that there is no gender difference in major PK and PD parameters in dogs and no clear antibody reaction towards the test article could be measured in dogs in 6-month period. NOAEL may be near 1.8 nmol/kg/day, based on the insulin detemir effect on adrenal weight. Therapeutic exposure ratio at the NOAEL would be less than 0.2 and 0.05 in males and females, respectively, based on human AUC values.

Effects of NN304 on Adrenal Weight in Beagle Dogs After 6-Month Treatment*

Group	Dose(U/kg/day)	Mean Weight(g)	P value
1	0(Vehicle)	1.05	--
2	0.3	1.20	0.1463
3	0.6	1.25	0.0322
4	1.2	1.34	0.0009
5' —	1.2	1.24	0.0531

* Represent adjusted group mean values for both sexes — is NPH insulin.

3.2.2 Primary pharmacodynamicsMechanism of action:

Detemir insulin has removed the amino acid residue in position B30 and a ¹⁴C fatty acid chain has been attached to position B29. In the presence of zinc and phenol it exists predominantly in the state of hexamer. The fatty acid side-chains contribute to the aggregation of hexamers. In the monomeric state, the 14-C fatty acid chain attached to position B29 binds to the fatty acid binding sites of albumin in the subcutaneous tissue, delaying absorption to the bloodstream. Approximately 98% of insulin detemir is bound to albumin in plasma. The prolonged duration of hypoglycemic action is attributable to increased self-association at the injection site and albumin binding. Thus, the rate of absorption is limited by the low concentration of free insulin detemir available for diffusion through tissues and passage across the capillary wall.

The affinity of insulin detemir for the insulin receptor was found to be 0.54 ± 0.14 nM (EC₅₀ + SD) as compared to 0.10 ± 0.03 nM for human insulin, resulting in a relative affinity of insulin detemir equal to 18 + 3% of human insulin. For the IGF-I receptor, the affinity of insulin detemir was 590 ± 310 nM as compared to 94 ± 43 nM for human insulin and 0.06 ± 0.02 nM for IGF-I. Thus, for the IGF-I receptor, the affinity of insulin detemir relative to human insulin was 16+ 2%. The relative affinity of insulin detemir for the IGF-I receptor is slightly lower than for the insulin receptor, but this difference was not statistically significant.

Drug activity related to proposed indication:

The pharmacological effects of insulin detemir and NPH insulin — were compared in 5 female non-diabetic pigs under 24-hour euglycemic clamp setting. The animals were randomly allocated to receive 216 nmol s.c. of the two preparations during variable-rate of i.v. infusion of glucose. The total amounts of glucose infused (gram), peak effect (mg/kg min) and time to peak effect in hour were summarized below.

Table 2 Effect of insulin detemir versus NPH insulin

Main variables (Mean \pm SD, n=5)	Insulin detemir	NPH insulin	Paired t-test
Total glucose infused, g	336 \pm 167	347 \pm 85	n.s.
Peak effect, mg/kg min	6.6 \pm 2.2	9.9 \pm 0.8	P<0.05
Time to peak effect, h	6.0 \pm 2.2	3.4 \pm 0.2	P<0.05

The hypoglycemic effects of insulin detemir were also compared in type 1 diabetic mini-pigs during chronic s.c. administration. Eight streptozotocin-induced type 1 diabetic male pigs were treated with twice-daily porcine NPH insulin two weeks before and two weeks after chronic dosing with insulin detemir. Insulin detemir was dosed for 4 weeks in between the dosing periods with porcine NPH insulin while the dose for each pig was kept constant. The results were summarized in Table 3. Mean fasting plasma glucose (mmol/l) was reduced during treatment with insulin detemir in type 1 diabetic mini-pigs.

Table 3 Pharmacodynamic parameters during chronic s.c. dosing in type 1 diabetic mini-pigs

	Porcine NPH insulin		Insulin detemir	
	Before insulin detemir treatment period	After insulin detemir Treatment period	2 weeks of treatment	4 weeks of treatment
Mean morning fasting plasma glucose (mmol/l)	13.6 \pm 5.1	13.6 \pm 5.0	9.2 \pm 5.3	11.5 \pm 4.51
Areas under plasma glucose curves (mmol/l \times h)	230 \pm 64 (p=0.075)	268 \pm 97 (p<0.05),	171 \pm 66	171 \pm 66
Fructosamine *(μ mol/l)	328 \pm 72	364 \pm 48	304 \pm 18	297 \pm 25

*Fructosamine values in non-diabetics pigs are below 300 μ mol/l.

3.2.3 Secondary pharmacodynamics

The sponsor performed safety study only under GLP regulations because insulin detemir will not produce other secondary pharmacodynamic actions.

3.2.4 Safety pharmacology

(Please see Pharmacology Review (IND#51,789, Review#2) dated July 28, 1997 for detail information)

Neurological effects:

Neurological, behavioral and autonomic studies were conducted in 6 male NMRI mice/group after s.c. doses of 0, 1.8, 18, and 180 nmol/kg. Pharmacological effects were monitored at 5, 30 and 60 minutes, as well as 2, 4, and 22 hours after dosing. Control and the LD had no effects while the MD and HD produced minor short-lasting effects such as decreased activity and exploration.

A test was designed to investigate whether insulin detemir has any anti-convulsant effect on pentylenetetrazol (PTZ)-induced convulsions and mortality in 12 CD-1 male mice/group. Animals were dosed intraperitoneally with 100 mg/kg PTZ, 45 minutes after subcutaneous treatment with insulin detemir at doses of 1.8, 18 and 180 nmol/kg or vehicle (control animals). Clonazepam was used as a positive reference compound. In insulin detemir-treated animals, the times to onset of convulsions, number of animals convulsing and incidence of mortality per group were similar to those recorded for the control animals.

In a different group of 6 male mice, effects of insulin detemir on hexobarbital sleeping time were studied after s.c. doses of 0, 1.8, 18, and 180 nmol/kg. Chlorpromazine (10 mg/kg) was used as positive reference agent. Insulin detemir had no effect on the time to onset of sleep or duration of sleep induced by the barbiturate. Effects of insulin detemir on alcohol-induced sleeping time were also studied under similar conditions. The HD dose (180 nmol/kg) of insulin detemir increased mortality of mice because 3 out of seven in the HD group died during the alcohol-induced sleeping period.

Cardiovascular and Pulmonary effects:

This safety pharmacology study was designed to investigate whether insulin detemir has any effect on the cardiovascular and respiratory systems of the anaesthetized Han Wistar male rats (four rats/group). Systolic and diastolic blood pressure, heart rate and electrocardiograms were the cardiovascular parameters measured. Respiratory rate, tidal volume and minute volume were the respiratory parameters measured. In addition, the pO₂, pCO₂ and pH of arterial blood samples were determined. The rats were treated subcutaneously with insulin detemir at doses of 1.8, 18 and 180 nmol/kg or vehicle (control animals). Adrenaline was used as a positive reference compound.

Slight increases in systolic and diastolic blood pressures following treatment with all three doses of insulin detemir and the vehicle were observed during the first 10-20 minutes after dosing. Statistically significant elevations in diastolic pressures were noted in different time intervals, although insulin detemir had no effects on the heart rate or electrocardiogram. There were no clear effects of insulin detemir on the blood gas parameters measured, at any dose tested. Respiratory parameters were stable throughout the experiment in all animal groups and were unchanged by treatment with insulin detemir.

The cardiovascular safety study was performed in anesthetized Beagle dogs (2 dogs/sex/group) after treatments with insulin detemir at doses of 0.18, 1.8 and 18 nmol/kg. Epinephrine served as a positive reference compound. The higher dose of 18 nmol/kg induced a marginal decrease in blood pressure. No significant differences in the absolute values of the 18 nmol/kg dose group and control animals were apparent when expressed as percentage change from pre-dosing values. Some isolated significant differences in the diastolic blood pressure (real values) were recorded at 185 ($p<0.05$), 200 ($p<0.05$) and 235 ($p<0.05$) minutes after dosing. Insulin detemir had no effect on the heart rate, electrocardiogram and blood flow of the anaesthetized dog at doses up to 18 nmol/kg. The respiratory parameters varied considerably before dosing and throughout the experiment in all animal groups. Based on this consideration, it was only relevant to compare the percent change values, which revealed no inter-group differences.

Renal effects:

Renal effects of insulin detemir were evaluated in Han Wistar male rats (6 rats/group). Urine was collected for 24 hours (at 2, 4 and 24 hours) after s.c. injection at doses of 1.8, 18 and 180 nmol/kg. Vasopressin and acetazolamide were used as positive reference compounds. The low dose of 1.8 nmol/kg had no effect on the urine volume, electrolyte excretion, specific gravity and osmolality, or creatinine clearance. A dose of 18 nmol/kg insulin detemir induced a slight but statistically significant ($p<0.05$) reduction in the specific gravity and a non-significant increase in the urine volume two hours after dosing.

All parameters were comparable with the control values at four and 24 hours after dosing. The high dose of insulin detemir (180 nmol/kg) similarly produced a non-significant increase in urine volume two hours after administration, accompanied by a slight decrease in osmolality and significant decrease in specific gravity ($p<0.01$). These effects were still apparent and reached statistical significance ($p<0.01$) at four hours after dosing. In addition, non-significant increases were recorded in sodium, potassium and chloride excretion. At 24 hours after administration of insulin detemir, all parameters had returned to control values. These results suggest that insulin detemir has induced hypoglycemia, leading to slight changes in the proximal tubular compliance and reabsorption rate due to an increased functional load (glomerular filtration minus proximal tubular reabsorption). All effects were minimal and fully reversible within 24 hours.

Gastrointestinal effects:

Isolated guinea pig ileum was the model chosen to test insulin detemir at concentrations of 0.1, 1, 10 and 100 nM. Direct effects of the test article on baseline tension, contractility and its effects on dose-response curve for the agonists histamine (0.1 ng/ml to 3mg/ml) and acetylcholine (0.1 ng/ml to 0.1µg/ml). Reference compounds were diphenhydramine (0.1µg/ml) and atropine (10 ng/ml). Insulin detemir had no direct effect on the baseline tension of the isolated guinea pig ileum at bath concentrations up to 100 nM. The maximal contractile responses to histamine and acetylcholine achieved in the

presence of all four concentrations of insulin detemir tested were unchanged from the control.

To test the effects of insulin detemir on GI motility NMRI male mice(6/group) were used to determine the distance traversed by the charcoal after subcutaneous treatment at doses of 1.8, 18 and 180 nmol/kg. The test article had no effect on the distance traversed by the charcoal meal. Scores of the number and size of mucosal lesions were given in order to assess gastric irritation, which were not significantly different from the vehicle control.

Abuse liability:

No relevant study has been performed.

Other:

To test analgesic effects of insulin detemir on acetic acid-induced writhing, eight male CD-1 mice/group were used. The animals were dosed intraperitoneally with 0.6% acetic acid 45 minutes after subcutaneous treatment with insulin detemir at doses of 1.8, 18 and 180 nmol/kg. Morphine sulfate was used as a positive reference compound. The incidence of writhing and latency to the first writhe per group were similar to those recorded for the control animals. The middle dose, 18 nmol/kg, significantly increased the total number of writhes/mouse and caused writhing in all mice. Since this effect was not replicated at either the higher or the lower dose, it was not considered pharmacologically significant.

In conscious 6 Han Wistar male rats/group, the effect of insulin detemir on body temperature was evaluated. The animals were treated with insulin detemir at doses of 1.8, 18 and 180 nmol/kg and body temperature was measured at 2, 4, 6, 8, and 24 hours after subcutaneous treatment of either the test article or vehicle. Propylene glycol was used as a positive reference compound.

The treatment with insulin detemir had very little effect on the body temperature of rats. The diurnal fall in temperature over the first eight hours was slightly less in treated than in control animals. This was evident in all dose groups, but was not clearly dose-dependent. Six hours after treatment with the high dose of 180 nmol/kg, the body temperature was significantly higher ($p < 0.05$) than for the control animals, but when calculated in percent change from pre-dosing values, there was no statistical significance.

3.2.5 Pharmacodynamic drug interactions

It is anticipated that blood glucose lowering agents in combination with insulin detemir will potentiate its hypoglycemic action. Alcohol interaction with insulin detemir was studied as discussed under Safety Pharmacology. Limited drug interaction studies with other drug products were also carried out as discussed subsequently.

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief summary

Utilizing radiolabeled insulin detemir (I^{125} or C^{14}), the following PK studies were performed in mouse, rat and dog including human subjects as summarized in Table 5 below.

Table 5 Inter species comparison of pharmacokinetic parameters for insulin detemir based on studies using non-labelled material (Means of males and females)

Species	Mouse		Rat		Dog		Human	
	i.v.	s.c.	i.v.	s.c.	i.v.	s.c.	i.v.	s.c.
AUC/Dose (h/l)	3.2	1.8	2.4	1.2	9.9	4.6	6.8	4.1
t_{max} (h)	-	0.75	-	0.75	-	3.1	-	7.4
CL (l/(h/kg))	0.32	-	0.41	-	0.11	-	0.15	-
V (l/kg)	0.25	-	0.14	-	0.14	-	0.14	-
$t_{1/2}$ (h)	0.54	1.49	0.22	0.89	0.85	2.15	0.59	5.90
f (%)	-	52-60	-	47-50	-	38-58	-	59
Protein bound (%)	97.3		98.1		98.3		98.8	
K_a^* (M^{-1})	5×10^4		7×10^4		8×10^4		10×10^4	
Study No.	Table 2.6.5.3.1/ NN 960024, Table 2.6.5.6.2/ NN 970176		Table 2.6.5.3.4/ NN 950474, Table 2.6.5.6.2/ NN 970176		Table 2.6.5.3.7/ NN 960271 (Medium dose) Table 2.6.5.6.2/ NN 970176		(Module 5 Vol. 4- 5/NN304 -1451 Table 2.6.5.6.1/ NN 970174	

*Tables 2.6.5.6.1/NN 970174 and 2.6.5.6.2/NN 970176

3.3.3 Absorption

The rate of absorption is limited by the low concentration of insulin detemir available for diffusion through the tissue and passage across the capillary wall. More than 98% of insulin detemir in the bloodstream is albumin bound. However, there were drug dose dependent increases in AUC values after insulin detemir administrations (0.1 to 1.6 U/kg). Steady state with insulin detemir was reached quite rapidly by the end of 24 hours after two doses administered in 12-hour intervals and trough concentrations at this point were almost identical to those after 7 days of twice-daily administration. The absorption of insulin detemir is lower after a subcutaneous injection in the thigh than after a subcutaneous injection in the deltoid or the abdomen.

3.3.4 Distribution

Insulin detemir is distributed more slowly to peripheral target tissues compared to NPH insulin. It has a small volume (0.1 L/kg) of distribution as a result of large quantity of plasma binding. In both muscle and adipose tissue, the concentration ratio between

serum and interstitial fluid at steady state after continuous i.v. infusion was substantially higher for insulin detemir than for human insulin, according to the clinical PK data.

Tissue distribution of insulin detemir in rats following single-dose administration of ¹²⁵I-insulin detemir, single- and multiple-dose administration of ¹⁴C-insulin detemir was investigated, which is summarized the findings in Table 6 below.

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Table 6 Overview of tissue distribution studies

Technique	Reference	Isotope	Dose (nmol/kg)	Male rat (Albino)	Male rat (Pigmented)	Female rat (Albino)	Female rat (Pregnant albino)
QTD ^a	NN 960497	¹²⁵ I	6	×	×	×	×
QTD	NN 960497	¹²⁵ I	6000	×		×	×
WBA ^b	NN 960497	¹²⁵ I	6	×		×	×
QTD	NN 960497	¹⁴ C	6000	×		×	×
WBA	NN 960497	¹⁴ C	6000	×		×	×
QTD	NN 980249	¹⁴ C	600 × 7	×			
WBA	NN 980249	¹⁴ C	600 × 7	×			×

^a: QTD, Quantitative tissue distribution; ^b: WBA, Whole-body autoradiography

3.3.5 Metabolism

In vitro metabolism of insulin detemir in human plasma or whole-blood was stable because insulin detemir was remained unchanged (66% in plasma and 80% in blood) after 7-hour incubation period. The in vitro metabolism study was also conducted in primary hepatocytes from the mouse, rat, rabbit, dog, mini-pig, monkey and human as well as in kidney homogenate and liver cytosol from the rat, dog, pig and human. Insulin detemir is metabolized by the cleavage of the disulfide bridges between the A and the B chain, leading to a free A-chain and an acylated B-chain, which are both inactive products of insulin detemir.

Following s.c. administration of ¹⁴C-insulin detemir to rats and dogs, it was extensively metabolized and mainly excreted as metabolites. In the rat, 24% and 53% of the dose was excreted as CO₂ following single and multiple dose administration, respectively. In the dog, 32% of the dose was excreted as CO₂ following single-dose administration. There were several polar components that were excreted in the urine from rats and dogs, which accounted for 13-26% of the total dose. The major component in rat urine was identified as a C₆-moiety of the fatty acid linked to Lys^{B29}, a product of ω-oxidation followed by β-oxidation of the fatty acid and peptide cleavage of the protein moiety.

Metabolic effects of insulin detemir were also investigated in SD rats (5/sex/group) after 7-day treatment with pentobarbital as positive control. Cytochrome P450 concentration, CYP1A1/2, CYP2A1, CYP2B1, CYP2C11, CYP2E1, CYP3A, and CYP4A were determined in hepatic microsomal fractions. Insulin detemir had no effect on metabolic parameters, although there was significant decrease in 2α- and 16α-hydroxylation of testosterone (P<0.05; P<0.01, respectively), indicating minor down regulation on the male specific rat CYP2C11. In female rats, NN304 produced a small but significant (1.2-fold; P<0.01) increase in the liver weight at the low dose (1 U/kg/day).

3.3.6 Excretion

Excretion of radioactive insulin detemir in rat and dog following single and multiple dose administration is summarized in Table 8 below.

Table 8 Excretion of radioactivity following single and multiple dose administration of ¹⁴C-insulin detemir

Species	Dose (nmol/kg)	Excretion (% of dose)				
		Urine	Faeces	Expired air	Carcass	Total ^a
Rat	6000	25.7	10.5	23.7	17.9	77.8 ^b
Rat	600 × 7	13.3	11.2	52.9	20.3	97.8
Dog	9	19.1	7.0	32.4	36.7	95.9

^aIncludes cage wash and carcass. Collection completed 168 hours post last dosing.

^bLow recovery was consistent following dosing of additional animals. The low recovery may be due to incomplete collection of ¹⁴CO₂.

3.3.7 Pharmacokinetic drug interactions

Insulin detemir competes with long-chain fatty acids for common binding sites in the presence of varying concentrations of lauric acid. But the binding of up to one molar equivalent of lauric acid did not affect the albumin binding of insulin detemir. Selected albumin-bound drugs such as warfarin, furosemide, tolbutamide, diazepam, glibenclamide, nicardipine, repaglinide, acetylsalicylic acid and valproic acid had no remarkable effect on the binding of insulin detemir in competition binding study. There were no clinically relevant influences of renal insufficiency, hepatic impairment, sex or ethnic group on the pharmacokinetics of insulin detemir. In elderly (>70 years) subjects, the AUC_(0-∞) was higher compared with younger subjects after administration of insulin detemir.

3.3.10 Tables and figures to include comparative TK summary

Please see Table 18 and figures 4-6 below for PK summary data of insulin detemir for repeated toxicology study in rats and dogs including reproductive studies.

Table 18 The main toxicokinetic parameters at the high dose levels

Exposure	AUC (pM·h)		C _{max} (pM)	
	M	F	M	F
Animal				
Repeat-dose studies				
Rats (300 nmol/kg - 0-6h)*	163765	321351	51956	100033
Exposure ratio animal/human***	2.3	4.4	10.3	19.8
Dogs (7.2 nmol/kg - 0-24h)**	40901	37449	5543	5294
Exposure ratio animal/human***	0.6	0.5	1.1	1.0
Reproductive toxicology				
Rats (300 nmol/kg) (NN 960122)	184400	221200	68300	42230
Exposure ratio animal/human***	2.5	3.0	13.5	8.3
Rabbits (900 nmol/kg) (NN 960301)	-	9720667	-	979833
Exposure ratio animal/human***	-	134	-	193
Human				
11.76 nmol/kg Trial NN304-1448 (mean exposure in diabetic patients)	72659 (calculated)		5064 (calculated)	

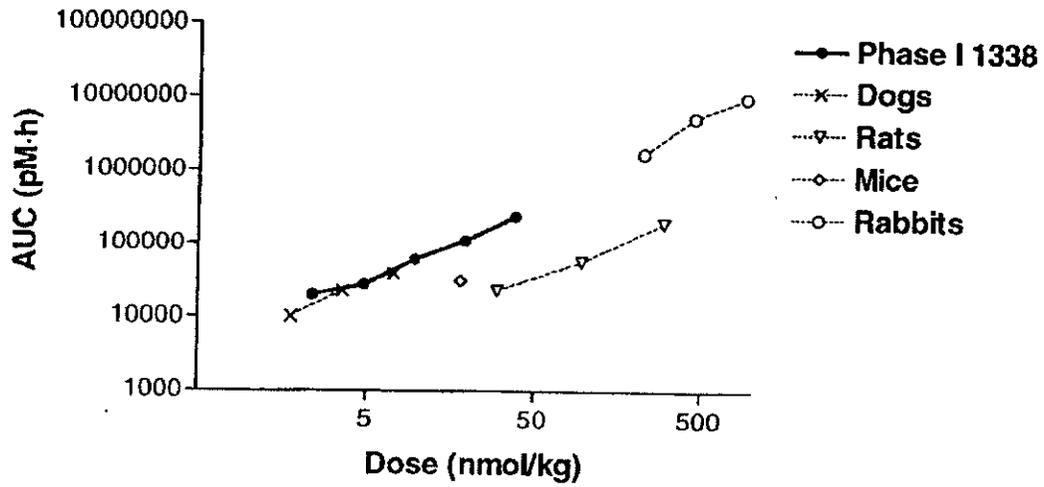
* Mean values from 3 and 6 months studies (NN 970194, NN 980225)

** Mean values from 3 and 6 and 12 months studies (NN 970193, NN 980224, NN 990052)

*** $Value_{Animal} / Value_{Human(Trial\ NN\ 304-1448)}$

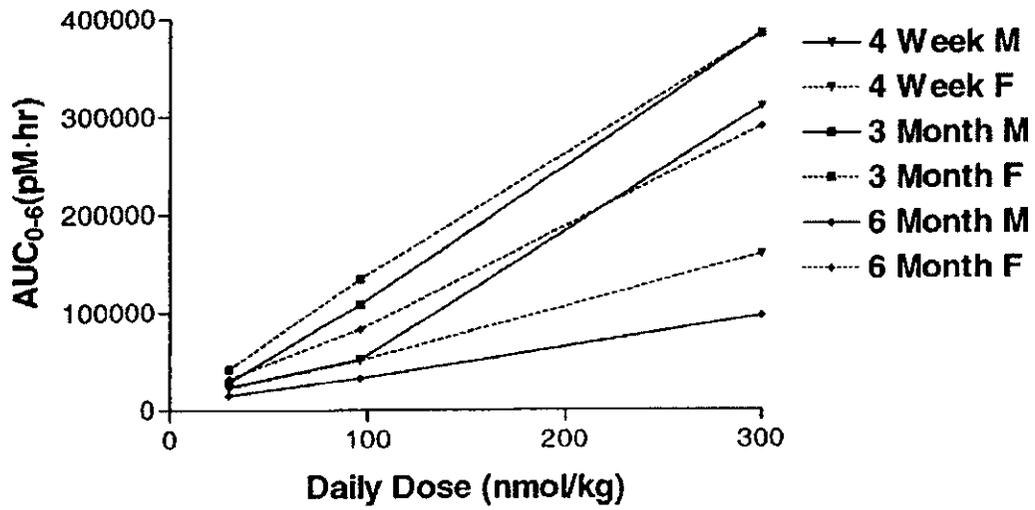
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Figure 3 Overview of toxicokinetics from long-term studies for the species investigated.



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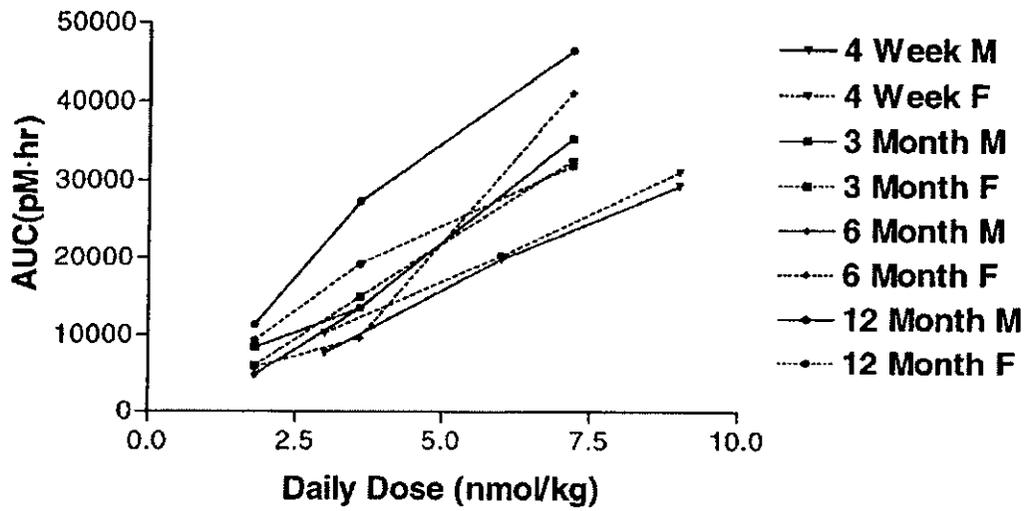
Figure 4 Overview of toxicokinetics performed in rats.



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Figure 5 Overview of toxicokinetics performed in dogs.



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3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

The sponsor performed the following in vitro or in vivo toxicological studies in mouse, rat, dog, or rabbit utilizing insulin detemir in final formulation. The studies were performed under current GLP regulations, which are summarized in a table below.

Study type and duration	Route of administration	Species
Single-dose toxicity	s.c. & i.v.	Mouse & Rat
Repeat-dose toxicity		
- 1 month	s.c.	Rat & Dog
- 3 month	s.c.	Rat & Dog
- 6 month	s.c.	Rat & Dog
- 12 months	s.c.	Dog
Genotoxicity		
- Ames test	n.a.	n.a.
- Cytogenetic test (human lymphocytes.)	n.a.	n.a.
- Mouse micronucleus test	s.c.	Mouse
Reproductive and Developmental toxicity studies		
- Fertility and embryo-foetal development	s.c.	Rat
- Pre- and postnatal	s.c.	Rat
- Embryo-foetal development	s.c.	Rabbit
Local Tolerance	s.c.	Pig
Other Toxicity studies		
- 4 week rat study on aged product	s.c.	Rat
- Immunogenicity in rabbit	s.c.	Rabbit
- Mitogenicity	n.a.	n.a.

n.a. Not applicable

General toxicology:

Single- and multiple-dose toxicology studies were carried out in mice, rats and dogs after various doses of insulin detemir. In general, there were drug dose dependent decreases in blood glucose concentrations. There were also minor clinical signs after a large dose of insulin detemir, which appeared to be secondary effects due to the drug's primary effect on hypoglycemia. However, in long-term study in dog (6-month), enlarged adrenals were observed at 3.6 and 7.2 nmol/kg, which was also produced by regular NPH insulin (7.2 nmol/kg). There were no histopathological changes in the study so that the sponsor concluded that the enlarged adrenal gland was determined to be an unspecific reaction.

Genetic toxicology:

Insulin detemir was tested for genotoxicity in the Ames assay, in vitro chromosomal aberrations assay in human lymphocytes, and in vivo mouse micronucleus assay. No evidence of genotoxic potential was observed.

Carcinogenicity:

The sponsor did not perform standard 2-year carcinogenicity studies because this Division waived animal carcinogenicity studies for insulin detemir. However, the sponsor evaluated the potential mitogenic action of insulin detemir in 1) CHO-K1 and human B10 osteosarcoma cells, 2) Human hepatoma cells (HepG2) in the presence of 0.1% human serum albumin (HSA) and MCF-7 cells including drug binding study to IGF-1 and insulin receptors as described subsequently.

Reproductive toxicology:

Potential effects of insulin detemir on fertility, early and late embryo-fetal development, and pre- and postnatal development were studied in rats and rabbits. Using NPH insulin as a reference substance the following investigations were performed as summarized in a table below. The main significant findings with brief methodology are summarized under table below.

Study No	NN 950460	NN 960122
Species/Strain	Rat /SD,	Rat/SD,
Drug	Insulin detemir, NPH insulin	Insulin detemir, NPH insulin
Dose route	s.c.	s.c.
Animals Sex/Group	Range-finding study: 5 groups: 12 males, 12 females/group. Satellite study: 5 groups: 6 males, 6 females/group	5 groups: 24 males, 24 females/group
Dose Groups nmol/kg/day	Insulin detemir: 0, 30, 150, 300 NPH: 300/150	Insulin detemir: 0, 30, 150, 300 NPH: 75
Dosing period	Males: 4 weeks precoitus up to sacrifice of females Females: 2 weeks precoitus up to and including day 17 of gestation	Males: 4 weeks precoitus up to sacrifice of females Females: 2 weeks precoitus up to and including day 17 of gestation
Results	Insulin detemir had no direct effect on fertility or embryo-foetal development. NPH was more severe and for the main study, this dosage was reduced to 75 nmol/kg.	Treatment with insulin detemir up to 300 nmol/kg had no direct effect on fertility or embryo-foetal development; all effects were consistent with treatment induced hypoglycaemia. The NOAEL was above 300 nmol/kg.

1. A range-finding study for insulin detemir fertility and teratology studies in rat (NN95046).

Twelve SD — rats/sex/group were administered subcutaneously at doses of 0(control vehicle), 30, 150 or 300 nmol insulin detemir/kg/day. Males received the treatment 4 weeks precoitus up to sacrifice while females had the treatment 2 weeks precoitus up to and including day 17 of gestation. The dose of a reference substance, NPH insulin (protaphane) was reduced from 300 to 150 nmol/kg/day on the day prior to pairing due to the occurrence of sporadic deaths in this group of animals.

Clinical signs, body weight, food and water consumption data were collected. On Day 20 of pregnancy, all surviving females were killed and subjected to macroscopic postmortem examination. Litter values were determined and fetuses examined macroscopically. At termination, males were subjected to macroscopic postmortem examination and specified organs of the reproductive system were weighed and examined histopathologically. Seminology assessment was performed to detect any changes in sperm motility and count. Blood samples were taken from additional satellite animals prior to mating (females) and on the last day of treatment (males and females) for determination of plasma glucose concentration and to determine absorption of the test substance.

There was insulin detemir dose-related reduction of plasma glucose. In the HD group (300 nmol/kg/day), one rat died and there were slight increases in body weight gain (male only) and food consumption (both sexes). Slight reductions in sperm motility and count were observed in this group, although there was no impairment of mating performance. In the LD and MD groups, there were no remarkable effects of insulin detemir.

Treatment with reference compound, NPH insulin (300 nmol/kg), resulted in the death of five male rats and one female rat. Five deaths occurred within the first two weeks of treatment and the dose level was therefore reduced from 300 to 150 nmol/kg the day prior to pairing. NPH insulin treatment produced a severe and persistent reduction of plasma glucose concentration, which was considered to be the cause of these deaths. This response was similarly associated with significantly increased body weight gain, food and water intake during treatment at 300 nmol/kg. For all parameters, the effect was more marked among males than females. The large pharmacological response to NPH insulin may also account for the reduced sperm motility and count, lower organ weights and associated microscopic testicular and epididymal changes which were noted following termination. These effects on the male reproductive system were considered to be largely responsible for the poorer mating performance noted in this group.

No clear adverse effects of insulin detemir were observed on embryo-fetal survival, growth or gross morphological development. The reference substance (NPH insulin) was, however, produced early in utero deaths and reduction in mean fetal weight, which might be as a secondary effect of induced maternal hypoglycemia. The AUC ratio of animal to human exposure of insulin detemir based on the HD was approximately 2 to 3 in male and female rats, based on $AUC_{0-6 \text{ hr}}$. In conclusion, insulin detemir produced dose-dependent hypoglycemic effect and no direct effects on fertility or embryo-fetal development were produced at a dose that was approximately 2 X of clinical exposure ratio in rats. NOAEL must be less than 300 nmol/kg/day, based on deaths as a result of drug-induced hypoglycemia.

2) A study of fertility and embryo-fetal development by subcutaneous administration of insulin detemir in the rat(NN960122).

Twenty four SD rats/sex/group were dosed subcutaneously with 0(control vehicle), 30, 150 or 300 nmol insulin detemir/kg/day. Based on findings from the range-finding study (NN950460) as discussed above, the dose of the reference drug (NPH insulin) was reduced to 75 nmol/kg/day in this study. Study design, experimental procedures, and methodology were similar to those described previously under study number NN960122.

Treatment with insulin detemir resulted in a dosage-related reduction of plasma glucose concentration at 150 and 300 nmol/kg/day (both sexes). This pharmacological response was associated with increased body weight gain for females receiving 300 nmol/kg/day during the two week pre-mating treatment period. Treatment at 30 nmol/kg/day had no obvious effects even on the blood glucose level in adult animals of either sex, which suggested that 30 nmol/kg/day might be NOEL.

Treatment with NPH insulin produced a similar but more pronounced plasma glucose response in both sexes than insulin detemir. Associated effects included two sporadic deaths among females in the early stages of the study, increased body weight gain and food consumption (both sexes). In addition, terminal studies showed low sperm counts in the vas deferens and an increased incidence of focal seminiferous epithelial atrophy with vacuolation of Sertoli cells in males treated with NPH insulin. For adult animals receiving either insulin detemir or NPH insulin, it appeared that there were no direct adverse effects on fertility or reproductive performance.

Treatment with insulin detemir was associated with a low incidence of fetal microphthalmia/small eye at the 150 and 300 nmol/kg/day. There was no such finding in the animal group that was treated with 30 nmol/kg/day, although NPH insulin treatment was also associated with a low incidence of microphthalmia. It appeared that 30 nmol/kg/day insulin detemir was NOEL because no clear changes in blood glucose were associated with the dose. NOAEL may be near the MD (150 nmol/kg/day) because there was a low incidence of fetal microphthalmia in the MD group. At this dose, therapeutic exposure ratio was approximately 1, based on AUC values obtained at the dose.

3) A dose range-finding study in the pregnant rabbit after subcutaneous administration(NN960121)

Six New Zealand white female rabbits/group were administered subcutaneously at doses of 0(control vehicle), 300, 600 or 900 nmol insulin detemir/kg/day. Animals were treated from days 6 to 18 of pregnancy, inclusive. The dose of a reference substance, NPH insulin (protaphane) was 30 nmol/kg/day. Blood samples were taken from all animals on day 6 of pregnancy (first day of treatment) for determination of plasma glucose concentration and to study the toxicokinetics of the test substance.

For the main study (NN960301), 24 pre-mated female rabbits were dosed subcutaneously with 0, 225, 450 or 900 nmol/kg/day insulin detemir. The dose of NPH insulin was the same (30 nmol/kg/day) to the dose-finding study and experimental procedures were essentially identical to the dose-finding study.

Clinical signs, mortalities, body weight, food and water consumption, toxicokinetic data were collected. For terminal observations (Day 29 of pregnancy), all surviving females were killed and subjected to macroscopic postmortem examination. Litter values were determined and fetuses examined macroscopically. The ovaries and uteri were examined for number of corpora lutea, number of implantation sites, number and distribution of live young, number and distribution of embryo-fetal deaths, individual fetus weights, fetal gross abnormalities, and placental abnormalities were determined.

At termination, litters/offspring were examined externally then sacrificed, weighted, sexed and dissected to examine for visceral abnormalities. Young were skinned, eviscerated and fixed in 74 OP industrial methylated spirit; after fixation the heads were

sliced through the line of the frontoparietal suture and the brain examined for visible abnormalities before clearing and staining of the carcasses for skeletal examination.

Treatment with insulin detemir resulted in a reduction of plasma glucose concentrations at all dose levels, reaching a minimum at four hours post-dose for all dosages and persisting up to eight hours post-dose at 900 nmol/kg only. At the HD, five rabbits died due to presumed hypoglycemia. All decedents were found dead at the pre-dose check after at least five days of treatment; post-mortem findings included abnormal gastrointestinal tract observations for three out of five animals. One decedent experienced a hypoglycemic episode at three hours after dosing on the day prior to death. Other associated effects were evident at all three dosages, including a dosage-related increase in maternal body weight gain and food consumption during the treatment period, with reversal of the effect following cessation of treatment.

Treatment with NPH insulin produced a plasma glucose profile similar to that seen with insulin detemir, but of smaller magnitude. Again, this response was associated with a sporadic death, the decedent being found dead pre-dose after six days of treatment and having stomach findings at post-mortem. Apparent hypoglycemic episodes were observed for two surviving animals in this group, between two and four hours after dosing on one occasion each. The effect of NPH insulin on maternal body weight gain and food intake was qualitatively similar to that seen with insulin detemir, with the degree of effect approximating the low dosage response.

Treatment with insulin detemir was associated with a dose-related increase in early embryonic death at all dosages, with resultant lower litter size and weight. Mean fetal weight in these groups was lower than expected from the litter size. In addition, two animals at 900 nmol/kg/day, one intermediate-dose female and one low-dose female, aborted in the later stage of pregnancy. NPH insulin treatment produced a similar effect on the litter, including one abortion, with the degree of effect approximating that seen at the low or intermediate insulin detemir dosage.

Maternal treatment with insulin detemir at all dosages was associated with a generally dosage-related increase in the incidence of gall bladder abnormalities, extra ribs and an additional thoracolumbar vertebrae (not dose-dependent). Maternal NPH insulin treatment was associated with the same visceral and skeletal changes seen with insulin detemir, the degree of the effects being similar or less than that noted at the lowest dosage of insulin detemir. The incidence of fused jugal to maxilla was higher in the NPH insulin group than in any other. Microphthalmia/anophthalmia also was observed in this group alone. The dose dependent increases in gall bladder abnormality should be recorded.

NOEL cannot be determined because blood glucose levels were reduced at all doses. NOAEL may be lower than 225 nmol/kg/day in rabbit because of the fact that there was generally dose-related in the incidence of abnormalities in gall bladder and extra ribs, although the abnormalities were observed with NPH treatment. The sponsor considers the abnormalities might be related to the pharmacological effects of hypoglycemia-

induced by the insulin detemir because the HD did not have direct effects on pregnancy or embryo-fetal development in the rabbit. The sponsor summarized the study results in a table below. The reviewer does not agree with the sponsor's view that NOAEL was 900 nmol/kg/day because there was insulin detemir dose-related increase in gall bladder abnormalities in rabbits.

Table 15 Embryo-foetal development in rabbits

Study ID	NN 960121	NN 960301
Species/Strain	Rabbit/New Zealand White,	Rabbits/New Zealand White,
Drug	Insulin detemir, NPH insulin	Insulin detemir, NPH insulin
Dose route	s.c.	s.c.
Animals Sex/Group	Range-finding study: 5 groups: 6 pregnant female rabbits/group	5 groups: 24 females/group
Dose Groups nmol/kg /day	Insulin detemir: 0, 300, 600, 900 NPH: 30	Insulin detemir: 0, 225, 450, 900 NPH: 30
Dosing period	13 days; day 6-18 of pregnancy	13 days; day 6-18 of pregnancy
Results	All of the dosages were tolerated, no toxic effects were noted and dosages of 0, 225, 450 and 900 nmol insulin detemir/kg/day and 30 nmol NPH/kg/day were selected for the subsequent main study of embryo-foetal development in rabbits.	Treatment with insulin detemir up to 900 nmol/kg had no direct effect on pregnancy and embryofoecal development; all effects observed being a consequence of treatment induced hypoglycaemia. The no-observed adverse effect level for both dams and foetuses was 900 nmol/kg.

3) Prenatal and postnatal development studies in SD rat(990122 and 990123).

Virgin female SD rats were mated with stock male rats of the same strain and source. Five groups of 24 female rats were dosed subcutaneously from day 6 after mating to day 14 of lactation. The 5 groups were 0(control vehicle), 30, 150, 300 nmol/kg/day and NPH positive control (75 nmol/kg/day). For the dose-finding study 8 female rats/group were used at doses of 0, 150, 300 nmol/kg/day insulin detemir and 75 nmol/kg/day NPH insulin for positive control. Fo female rats were allowed to give birth and development of the F₁ animals was assessed.

Clinical condition, body weights and food consumption were assessed for the Fo females through gestation and lactation. Fo females were allowed to litter. F₁ offspring were

assessed for survival, locomotor activity, learning ability, neuromuscular function and other development. Females were bled for toxicokinetic assessment on days 6 and 17 of their pregnancy and on days 4 and 14 of lactation; plasma glucose levels were also measured in these samples. Female rats and their offspring were killed on Day 14 of lactation for gross macroscopic examination. The development of F₂ offspring was assessed until day 7 of age.

Twelve Fo females died in the late gestation or early lactation periods at 150 nmol insulin detemir/kg (2) and 300 nmol insulin detemir/kg (5) and 75 NPH insulin/kg (5). Slow/shallow/gasping respiration was seen before death in several animals. Body weights in gestation were unaffected. Body weights in lactation at 300 nmol/kg or NPH insulin were significantly lower as compared to controls. Food consumption in late gestation (all treated groups) and early lactation (300 nmol insulin detemir/kg and 75 nmol NPH insulin/kg) was high compared to controls. Littering, the numbers, survival and development of the F^o offspring were unaffected. It appeared that F₁ locomotor activity, learning ability, neuromuscular function, sexual development, mating performance and fertility were unaffected. F₁ littering and survival and development of F₂ progeny were similar in all groups.

The mortalities that occurred in the Fo generation females were considered to be the result of the pharmacological actions of the insulins, i.e., hypoglycaemia. Neither insulin detemir nor NPH insulin, at the dosages tested, was considered to have any unexpected or toxic effects on the Fo females nor any subsequent detrimental effects on their progeny, nor in turn the F₂ offspring. The no-observed-adverse-effect level (NOAEL) for effects on pre- and post-natal development was considered to be 300 nmol/kg for insulin detemir and 75 nmol/kg for NPH insulin as summarized below.

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Table 16 Prenatal and postnatal development including maternal function

Study No	NN 990122	NN 990123
Species/Strain	Rat/SD	Rat/SD
Drug	Insulin detemir, NPH insulin	Insulin detemir, NPH insulin
Dose route	s.c.	s.c.
Animals Sex/Group	Range-finding study; 4 groups: 8 females/group.	5 groups: 24 females/group
Dose Groups nmol/kg/day	Insulin detemir: 0, 150, 300 NPH: 75	Insulin detemir: 0, 30, 150, 300 NPH: 75
Dosing period	Dosing from day 6 after mating to day 14 of lactation.	Dosing from day 6 after mating to day 20 of lactation. Group 5: Dosing discontinued from day 20 of gestation to day 2 of lactation
Results	Insulin detemir had no adverse toxic effects on the F ₀ animals or on their progeny. 300 nmol/kg was considered suitable as a high dose in a the main study.	Insulin detemir had no adverse toxic effects on the F ₀ animals and no subsequent detrimental effects on their progeny or in turn the F ₂ offspring

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Special toxicology:

The sponsor performed a mitogenicity study because insulin detemir binds insulin receptors as well as IGF-1 receptor as presented earlier (Table 1).

Mitogenic Potency of Insulin Detemir in CHO-K1 Cells.

In order to evaluate the potential mitogenic action of insulin detemir, insulin and IGF-1 receptor binding studies were performed in human hepatoma cells (HepG2) in the presence of 0.1% human serum albumin (HSA). The relative affinity of insulin detemir to either insulin or IGF-1 receptor was much lower than that of insulin (insulin being 100%) as summarized under IND#51,789(Review#4) on 4/11/2002.

Effects of insulin detemir on stimulation of radiolabeled thymidine incorporation into newly synthesized DNA were compared to that of human insulin using CHO-K1 cells. The cell line was chosen because it responds well to growth factor stimulation, as a cell expresses approximately 50,000 IGF-1 receptors compared to 3000 insulin receptors. ED₅₀ values were estimated based on log dose-response profiles for stimulation of thymidine incorporation into DNA by insulin detemir. Mean ED₅₀ value for insulin detemir was determined to 30 nM while that of human insulin was 2 nM.

3.4.2 Single-dose toxicity

The following single dose toxicity studies were performed by the sponsor as summarized in a table below. Each individual study will not be reviewed again since they were reviewed previously (Please see IND#51,789, Review#1).

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Study ID	NN 950426	NN 950427	NN 950424	NN 950425
Species/ Strain	Mouse / — /	Mouse / — /	Rat/SD, — /	Rat/SD. — /
Drug	Insulin detemir	Insulin detemir	Insulin detemir	Insulin detemir
Dose route	s.c.	i.v.	s.c.	i.v.
Animals Sex/Group	5 groups: 5 males, 5 females/group	7 groups: 5 males, 5 females/group	5 groups: 5 males, 5 females/group	6 groups: 5 males, 5 females/group
Dose Groups nmol/kg/day	0, 375, 1500, 6000, 24000	0, 375, 750, 1500, 3000, 6000, 12000	0, 375, 1500, 6000, 24000	0, 375, 1500, 6000, 12000, 24000
Duration	1 day, 2 week ob- servation	1 day, 2 week ob- servation	1 day, 2 week observation	1 day, 2 week observation
Results/ Conclusion	The highest non- lethal dose was 1500 nmol/kg in both males and fe- males. The no toxic effect level was 1500 nmol/kg in males and greater than 24000 nmol/kg in females.	The highest non- lethal dose was 1500 nmol/kg and the no toxic effect level was 1500 nmol/kg in both males and females.	The highest non- lethal dose was 24000 nmol/kg and the no toxic effect level was 1500 nmol/kg in both males and females.	The highest non- lethal dose was 6000 nmol/kg and the no toxic effect level was 6000 nmol/kg in both males and fe- males.

3.4.3 Repeat-dose toxicity

The sponsor performed repeated-dose toxicity studies in rats and dogs. A partial list of the repeated studies in rat is attached below. Most of the studies were reviewed in previous reviews under IND 51,789 (1/14/1997, 7/29/1997 and 10/29/19998), which is omitted in this review except the 6-month toxicology studies in rat and dog.

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Study ID	NN 950176	NN 950175	NN 960006	NN 960243
Species/ Strain	Rat/SD	Rat/SD	Rat/SD	Rat/SD
Drug	Insulin detemir	Insulin detemir	Insulin detemir, NPH insulin	Insulin detemir, NPH insulin
Dose route	s.c.	s.c.	s.c.	s.c.
Animals Sex/Group	Range-finding study 5 groups: 5 males, 5 females/ group	Main study: 4 groups: 12 males, 12 females/group Satellite study: 4 groups: 6 males, 6 females/group	Main study: 5 groups: 20 males, 20 females/group. Recovery study: 3 groups: 10 males, 10 females/group Satellite study: 4 groups: 6 males, 6 females/ group	Main study: 5 groups: 20 males, 20 females/group. Satellite study: 4 groups: 6 males, 6 females/ group
Dose Groups nmol/kg/day	0, 150, 300, 600, 1200	0, 30, 96, 300	Main study: insulin detemir: 0, 30, 96, 300 NPH: 144/72 Recovery study: insulin detemir: 0 (vehicle), 96, 300 Satellite study: insulin detemir: 0 (vehicle), 30, 96, 300	Main study: insulin detemir: 0, 30, 96, 300 NPH: 72 Satellite study: insulin detemir: 0 (vehicle), 30, 96, 300
Duration	2 weeks	4 weeks	Main and satellite study: 13 weeks. Recovery study: 4 weeks	Main and satellite study: 26 weeks.
Results/ Conclusion	Highest dose level should be no higher than 300 nmol/kg	NOEL was <30 nmol/kg and NOAEL was >300 nmol/kg	Apart from the lo- cal effects, the NOAEL was above 300 nmol/kg.	Apart from the local effects the NOAEL was above 300 nmol/kg.

Study title: Six-month Subcutaneous Toxicity Study in the Rat

Key study findings: (The final report is essentially identical to the original submission (AmendmentS#008, which was reviewed on Oct. 29, 1998). New relevant information for GRP format is documented below.

Twenty five Sprague-Dawley rats/sex/group were administered subcutaneously either vehicle or NN304 at doses of 5, 16, and 50 U/kg/day (30, 96, 300 nmol/kg/day) for 6 months. There was no treatment-related mortality, although there were three incidental deaths. There were no treatment-related effects on body weight, food consumption, hematological, or ophthalmoscopic variables at any doses. NN304 caused a dose-dependent decrease in blood glucose, of which peak effects were seen 1, 3 and 6 hours after dosing of 5, 16 and 50 units/kg/day, respectively.

Slight elevated antibody levels with NN304 treatment were noted in 50% of the animals in all groups, independent of the dose. Liver weights were decreased in males by 10% and 8% at 16 and 50 units/kg/day, respectively. In conclusion, subcutaneous administration of NN304 to SD rats for 6 months at 5, 16 and 50 units/kg/day caused a dose-dependent reduction in blood glucose. Hepatic weight was also reduced by the treatment in the mid- and high-dose groups by 10%. There were no other remarkable adverse effects.

It appears that NOEL must be near 30 nmol/kg/day, while NOAEL may be 96 nmol/kg/day because the HD produced multiple adverse effects, although they appear to be secondary to the primary hypoglycemic action. Therapeutic exposure ratio at the NOAEL was estimated to be 0.5X in male rats, while it was 1X in females, based on human AUC value, 7.3 nmol.hr/ml after 11.76 nmol/kg/day in diabetic patients.

Study no.: NN960243

Volume #1.14-16, and page # Vol.14(1-339); Vol.15(1-360); Vol.16(1-307): Module 4

Conducting laboratory and location: Novo Nordisk A/S, DK-2760 Maaloev, Denmark

Date of study initiation: Sept. 5, 1997

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Batch# 317708. Tks#142

Methods

Doses: 30, 96, 300 nmol/kg/day insulin detemir and 72 nmol/kg/day NPH

Species/strain: Rat/SD,

Number/sex/group or time point (main study): 20 rats/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous administration, The final formulation had vehicle medium containing phenol(— , m-cresol(— , sodium chloride(—) and —

with pH= —

Satellite groups used for toxicokinetics or recovery: 6 rats/sex/group

Age: 4-5 weeks old

Weight (nonrodents only): 70-90 g

Unique study design or methodology (if any):

Observation times and results

Mortality: Twice daily.

Male rat (#10) in control vehicle group was found dead on Day 90 without showing clinical signs. Male (#64) in NN304 30 nmol/kg/day had a short episode of convulsion before dosing on Day 134 and found dead. Female (#185) in NN304 300 nmol/kg/day was subdued, dehydrated with a markedly distended abdomen, piloerection and had blood in the urine on Day 161 when she was killed on that day.

Clinical signs: Twice daily

Male (#220) in NPH 72 nmol/kg/day group was subdued and had convulsions after dosing on Day 177, which was recovered after glucose treatment. On Day 103 the rat had a short episode with slight convulsions. Male (#108) in NN304 96 nmol/kg/day group had short episodes of convulsions on Day 142, 146, 162, 163 and 173 while male rat (#122) in 96 nmol/kg/day group had convulsion on Day 82.

Body weights: All rats were weighed on arrival, on Day 1 and weekly thereafter. There was no treatment related effect on body weight.

Food consumption: Weekly

There was no treatment related effect on food consumption, although a few intergroup differences were significant sporadically.

Ophthalmoscopy: On Day -10 and in week 13 (day 88) of dosing and before termination of dosing (Day 177 or 180). There was slight central lenticular opacities occurred at the end of the dosing period in a majority of females in several groups including the control vehicle group. Thus, the sponsor concluded that insulin detemir had no direct effect on ophthalmologic action in the rat.

EKG: Not determined in this rat study.

Hematology: In week 14 of dosing (Day 94-96) and before termination of treatment (male Day 183-187; females Day 183-190) blood samples were taken from all main animals. There were no consistent and remarkable changes on these parameters after insulin detemir treatment.

Clinical chemistry:

There were some sporadic changes in the parameters without clear relationship with the test-article treatment. For instance, in Week 14 of dosing both sexes in the HD group, the NPH group and both sexes in MD groups had high concentrations of alkaline phosphatase ($p < 0.05$). Serum total bilirubin concentrations were also high in the HD group in both sexes ($p < 0.01$). Serum triglyceride concentrations were significantly decreased in both sexes in the HD and MD groups ($p < 0.05$). Serum carbamide (urea) was low in both sexes in the HD group.

Before termination of dosing the concentration of ornithine carbamyl transferase was low in the male of MD group and in the females in HD group. Serum AST was low in both sexes in the HD group while alkaline phosphatase level was high in both sexes in the HD group.

Urinalysis: In week 13 of dosing (male Day 86-88; females Day 86-87) and before termination of treatment (male Day 178-180; females Day 178-179) urine samples were taken from all main animals. Results: Not remarkable.

Gross pathology: Tissue specimens were collected and fixed in phosphate buffered neutral 4% formaldehyde. The testes were fixed in Bound's fixative and the eyes in Division's fluid while the bone marrow smear was fixed in methanol.

Organ weights (specify organs weighed if not in histopath table): See histopath table.

Histopathology: Adequate Battery: yes (), no ()—explain

Peer review: yes (), no ()

Results: No remarkable effects on organ weights, gross or histopathology.

Toxicokinetics:

Toxicokinetic findings are summarized in a table below by the sponsor.

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Health Care, Discovery and Preclinical
Development
Pharmacokinetics
Project: NN304
Study No 980225

Date: 30 December 1998
Version No.: 1
Status: Final
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Novo Nordisk

RESULTS

NN304 was detected in serum from all dosed animals and exposure increased proportionally with the dose. No gender differences were observed. The pharmacokinetic parameters are tabulated below.

Dose (nmol/kg)	Sex	Day	AUC(0-6)	C _{max}	λ _z	t _{1/2}	T _{max}
30	F	79	33775	13533	0.7296	1.0	1
30	F	170	32231	12047	0.6529	1.1	1
30	M	79	20010	6167	0.4881	1.4	1
30	M	170	14920	3857	0.4188	1.7	3
192	F	79	80429	20133	0.5436	1.3	1
192	F	170	82596	27433	0.5088	1.4	1
192	M	79	41326	14500	0.2681	2.6	1
192	M	170	32661	12093	0.4504	1.5	1
300	F	79	290951	90433	0.3221	2.2	1
300	F	170	289002	79033	0.6222	1.1	3
300	M	79	146655	44467	0.2582	2.7	1
300	M	170	96239	33633	0.2322	3.0	1

CONCLUSIONS

- Exposure of the rats to NN304 was confirmed in all groups treated with NN304.
- Exposure increased proportionally with dose.
- No gender dependent pharmacokinetics were observed.

**APPEARS THIS WAY
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Study title: Six-Month Subcutaneous Toxicity Study in the Dog

Key study findings: (The final report is essentially identical to the original submission (Amendment S#008, which was reviewed on Oct. 29, 1998). New relevant information for GRP format is documented below.

Study no.: NN960241

Volume #1.19-20, and page # Vol#19(162-321); Vol#20(1-325: Module 4

Conducting laboratory and location: Novo Nordisk A/S, DK-2760 Maaloev, Denmark

Date of study initiation: Nov. 1997

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Batch# 317708.

Methods

Doses: 1.8, 3.6, 7.2 nmol/kg/day insulin detemir and 7.2 nmol/kg/day NPH

Species/strain: Beagle dog

Number/sex/group or time point (main study): 4 dogs/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous administration. The final formulation had vehicle medium containing phenol(—), m-cresol(—), sodium chloride(1.17 mg/ml) and — with pH= —

Satellite groups used for toxicokinetics or recovery: 2 dogs/sex/group

Age: 4-5 months old

Weight (nonrodents only): 5.3-9.4 kg

Unique study design or methodology (if any): None

Observation times and results

Mortality: Twice daily.

There were no deaths in this study.

Clinical signs: Twice daily

There were no drug treatment related clinical signs. Male (#28) in the HD group had conjunctivitis twice, which was treated. Female (#32) in the HD group had a wound of the top of the tail, which was treated with local antibiotic cream during days 64-71. One or a few cases of loose stools, local swellings, small nodules in the skin or vomiting occurred on one or two days, which appeared to be incidental.

Body weights: All dogs were weighed on arrival, on Day -11 and -7 including the first day of dosing (Day 1) and weekly thereafter.

There were sporadic changes in body weight in females, which was not drug dose dependent. Thus, it appears that there was no treatment related effect on body weight.

Food consumption: Daily

There were no treatment related changes on food consumption after insulin detemir treatment.

Ophthalmoscopy: On Day -4, in week 6 (day 36) and 13 (Day 86) of dosing and before termination of dosing (Day 177). There were no ophthalmologic changes were seen except for transient conjunctivitis in female No. 29 of the HD group on Day 36.

EKG: Before start of dosing (Day -6), in week 6 (Day 37) and 13 (Day 87) of dosing and before termination of dosing (Day 182). All animals were subjected to electrocardiographic examination. There were some isolated statistically significant group mean differences in P-wave or PR-interval duration, but none of them showed any relationship to treatment.

Hematology: Before start of dosing (Day -6), in week 6 (Day 36) and 13 (Day 91) of dosing and before termination of dosing (Day 178), blood samples were collected for hematology and clinical chemistry investigations. The mean cell volume (MCV) in NPH treated dogs was increased in both sexes on Day 178. Neither the hematocrit nor the red blood cell count differed statistically, so the finding was considered incidental. The percentage of eosinophils, but not the absolute number of eosinophils, was higher for both sexes in the HD group ($p < 0.01$), but the data were within the range of historical control data. It is concluded that insulin detemir had no consistent and remarkable effects on the hematological parameters.

Clinical chemistry:

On Day 38, TG and Creatinine were high for both sexes in NPH treated group. Insulin detemir had no remarkable effects on clinical chemistry parameters. Before termination of dosing (Day 178), the serum magnesium concentration was high in both sexes in NPH treated group, which was still within the range of historical control data.

Urinalysis: Before start of dosing (day -5), in week 6 (Day 39) and 13 (Day 91) of dosing and before termination of dosing (Day 178), urine samples were collected overnight.

Insulin detemir had no remarkable effects on parameters of urinalysis.

Gross pathology: On the day of necropsy, animals were weighed, examined externally, anesthetized with an i.v. injection of a barbiturate. A macroscopic examination was performed after opening the cranial, thoracic, and abdominal cavities and by observing the appearance of the tissues in situ. Hemorrhages at the injection sites were seen in all dogs including the control vehicle group. The remaining macroscopic findings appeared to be incidental in Beagle dogs.

Organ weights: Please see histopathology table. The absolute weight of the adrenals and the adjusted weight of the adrenals were high in HD group ($p < 0.01$) when data for males and females were analyzed simultaneously. In the MD group, the trend of adrenal weight was still higher than the control vehicle group, but p value was greater than 0.05 in combined assays of males and females, which was true in NPH treated group as shown below. The implication of increased adrenal weight in the HD group is not known,

although treatment with high doses of insulin may be stressful to the animals. The adjusted weight of the spleen in the MD males was higher than in the control group. Variations in the weight of the spleen are often observed, which was considered to be due to differences in the exsanguination, according to the sponsor. There were no additional organ weight changes that were associated with insulin detemir treatment.

Effects of Insulin Detemir on Adrenal Weight in Beagle Dogs After 6-Month

Treatment*

<u>Group</u>	<u>Dose(nmol/kg/day)</u>	<u>Mean Weight(g)</u>	<u>P value</u>
1(control vehicle)	0	1.05	--
2	1.8	1.20	0.14
3	3.6	1.25	>0.05
4	7.2	1.34	<0.01
5(NPH insulin)	7.2	1.24	0.0531

* Represent adjusted group mean values for both sexes.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (x), no ()

At the injection site subcutaneous hemorrhage, inflammation, fibrosis and/or necrosis were observed with no difference between the groups. There were no histopathological changes that correlated with the increased adrenal weights in the MD and HD detemir insulin group and NPH treated group. All other microscopic findings were not related to the treatment.

Toxicokinetics:

On the first day of dosing, in week 13 (Day 85) and just prior to termination of dosing (Day 176), blood samples for TK and measurement of blood glucose were collected from all animals that were treated with insulin detemir or NPH at the following time points. Before dosing (time 0) and 1, 2, 4, 6 and 24 hours after dosing. For groups of control vehicle and NPH treatment, only blood samples for blood glucose analysis was taken on the above mentioned time points. Attached are the summary TK findings for the 6-month dog toxicology study.

Toxicokinetic findings are summarized in a table below by the sponsor. In the three doses of insulin detemir tested, there were increases in AUC values as the study progressed from Day 1, 85, and 176, which indicate the test article may be accumulated in both sexes. Based on the effect of insulin detemir on adrenal gland weight at the HD, NOAEL would be 3.6 nmol/kg/day. At the NOAEL, the exposure ratios were 0.6 and 0.7 in females and males, respectively, based on clinical dose of 11.76 nmol/kg.

Health Care, Discovery and Preclinical Development Pharmacokinetics Project: NN304 Study No: 980224	Date: 30 December 1998 Version No.: 1 Status: Final Page: 9 of 36	Novo Nordisk
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RESULTS					
NN304 was detected in serum from all dosed animals and exposure increased proportionally with the dose. No gender differences were observed. The pharmacokinetic parameters are tabulated below.					
Group No	Daily Dose (nmol/kg)	Sex	Day	C _{max} (pM)	AUC (pM·hr)
2	1.8	M	1	1001 (192)	4065 (681)
		M	85	3890 (2093)	11745 (2943)
		M	176	6745 (1274)	38786 (29172)
2	1.8	F	1	1092 (399)	5302 (633)
		F	85	2950 (917)	12096 (1983)
		F	176	5440 (1298)	28895 (5465)
3	3.6	M	1	1337 (301)	5527 (1542)
		M	85	2401 (652)	13892 (4558)
		M	176	7670 (2714)	50448 (39282)
3	3.6	F	1	1568 (370)	6832 (840)
		F	85	2903 (540)	13062 (2967)
		F	176	6023 (1619)	40353 (9493)
4	7.2	M	1	1159 (453)	4747 (911)
		M	85	2103 (247)	13439 (4347)
		M	176	6258 (2624)	35344 (15339)
4	7.2	F	1	1021 (381)	5700 (3256)
		F	85	1938 (625)	9624 (3142)
		F	176	5390 (524)	40140 (3933)
CONCLUSIONS					
<ul style="list-style-type: none"> • Exposure of the dogs to NN304 was confirmed • Exposure increased proportionally with dose. • No gender dependent pharmacokinetics were observed. 					

Other:

Data were processed to give group mean values and SD. Each continuous variable was tested for homogeneity of variance with Bartlett's test. If the variance was homogeneous, ANOVA was carried out for the variable. If the variance were heterogeneous, intergroup differences were assessed with Dunnett's test.

Histopathology inventory (optional)

Study	NN960243	NN960241
Species	Rat	Dog
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	
Bone (femur)		X
Brain	X*	X*
Cecum	X	X
Cervix		
Colon	X	X
Duodenum	X	X
Epididymis	X*	X*
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions		X
Harderian gland		
Heart	X*	X*
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		
Larynx		
Liver	X*	X*
Lungs	X*	X*
Lymph nodes, cervical		
Lymph nodes mandibular		
Lymph nodes, mesenteric		
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	
Ovaries	X*	X*
Pancreas	X	X
Parathyroid		
Peripheral nerve		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland		X
Sciatic nerve	X	X
Seminal vesicles	X*	
Skeletal muscle	X	X

Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes	X	X*
Thymus	X*	X*
Thyroid	X*	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed
 *, organ weight obtained

3.4.4. Genetic toxicology (Please also see genetic toxicity studies that were reviewed (Original IND Review) on January 14, 1997). The genotoxicity studies that were performed by the sponsor are summarized below.

Study ID	NN 960002	NN 960004	NN 960003
Drug	Insulin detemir	Insulin detemir	Insulin detemir
Type of Study	Insulin detemir potential to induce reverse mutations in bacteria (<i>S. typhimurium</i> and <i>E. coli</i>)	Insulin detemir chromosome damaging potential in human peripheral blood lymphocytes, <i>in vitro</i> cytogenetics assay	Insulin detemir potential to induce Micronuclei in the Polychromatic Erythrocytes in CD-1 Mice
Results	Insulin detemir did not induce mutation in 4 strains of <i>Salmonella typhimurium</i> and 2 strains of <i>E. coli</i> at a concentration up to 5000 µg/ml (equals 850 000 nM) with or without metabolic activation	Insulin detemir did not induce chromosome aberrations in cultured human peripheral blood lymphocytes	Insulin detemir did not induce micronuclei in polychromatic erythrocytes of bone marrow at dosages up to 7500 nmol/kg body weight

3.4.5. Carcinogenicity

The sponsor did not perform standard 2-year carcinogenicity studies because this Division waived animal carcinogenicity studies for insulin detemir. However, the sponsor evaluated the potential mitogenic action of insulin detemir in 1) CHO-K1 and human B10 osteosarcoma cells, 2) Human hepatoma cells (HepG2) in the presence of 0.1% human serum albumin (HSA) and MCF-7 cells including drug binding study to IGF-1 and insulin receptors as described subsequently.

3.4.6. Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: A study of effects on fertility and embryofetal development in the rat

Key studies findings: The present study demonstrated that insulin detemir did not impair the fertility and early embryonic development in rats at the doses of 5, 25 or 50 U/kg/day. However, in females, significant increases in body weight gain were noted in the MD, HD and NPH treated group. Fetal examination indicated that numbers of litters with fetuses with visceral anomalies appeared to be increased in MD and HD insulin detemir groups. The exposure ratios of insulin detemir at the MD were 1.8 and 2.1 in males and females, respectively, based on human AUC values after 11.76 nmol/kg/day. An increased incidence and degree of focal seminiferous epithelial atrophy with vacuolation of Sertoli cells was seen in NPH insulin treated males, although this finding was not confirmed in the groups treated with insulin detemir.

Study no.: 960122

Volume #32 and page #: 247-310

Conducting laboratory and location: —

Date of study initiation: June 17, 1996

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #3176004, and — purity:

Methods

Doses: 0, 5, 25, and 50 U/kg/day (0, 30, 150, 300 nmol/kg/day) insulin detemir and 75 nmol/kg/day NPH insulin

Species/strain: Pathogen free male rats — CDBRVAF/Plus

Number/sex/group: 24/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous/ 100 U/ml

Satellite groups used for toxicokinetics: Not specified.

Study design: Standard fertility and embryofetal study. In brief, males were treated 4 weeks before mating and up to termination of the females. Females were treated for 2

weeks prior to mating with dosing continued up to Day 17 of pregnancy (during organogenesis).

Parameters and endpoints evaluated: Please see the results below.

Results

Mortality: There were no treatment-related deaths among animals receiving insulin detemir.

Clinical signs: There were no clear treatment-related clinical signs including the hypoglycemic episodes.

Body weight: Body weight gain for males was not changed throughout the study in insulin detemir groups. There were significant increase in the parameters in 12.5 U/kg/day male group. In females, significant increases in body weight gain were noted in the MD, HD and NPH treated group (Table 2)

Food consumption: Not remarkable.

Toxicokinetics: (Please see a summary table below)

Toxicokinetic Data in Fertility and Embryofetal Development Study in Rats						
Drug Dose (nmol/kg/day)	C _{max} (pM)			AUC _{0-6h} (pM.h)		
	F before	F After	M after	F before	F After	M after
30	10240	10150	9373	32910	41540	25690
150	47900	31830	40830	152700	133100	157500
300	61170	42230	68300	185600	221200	184400

F and M stand for females and males while before and after indicate before and after mating.

Necropsy: Organ weights were not affected by the treatment of insulin detemir or NPH insulin. Macroscopic pathological findings were fur loss and hemorrhage at injection sites which were seen all groups including the control. Histopathological examination of testes and epididymides from males in the treated groups was not remarkably different from the control group. An increased incidence and degree of focal seminiferous epithelial atrophy with vacuolation of Sertoli cells was seen in NPH insulin treated males (Table 16).

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): Mating performance (incidence of successful matings), distribution of pre-coital time and vaginal smear were not considered to be affected by treatment with insulin detemir. Mean litter values and the incidence and distribution of embryofetal deaths for all groups treated with insulin detemir were similar to controls as shown below. The high pre-implantation loss at the MD group compared with control was considered not to be significant since the incidence was not dose-dependent.

Fetal examination indicated that numbers of litters with fetuses with visceral anomalies were increased in MD and HD insulin detemir groups as shown in Table 9 below. It appeared that the increases were drug dose dependent and NPH insulin did not produce the effect, which should be included in labeling. However, the sponsor attributed the difference was due to hemorrhage affecting the brain and surrounding tissues. Sperm count and motility in vas deferens or epididymal tissue were not affected significantly after the treatment since there was no difference in the parameters from the control group.

Table 2 Bodyweight - group mean values (g)

Week	Group and dosage (IU/kg/day)									
	1♂ Control	2♂ NN304	3♂ NN304	4♂ NN304	5♂ — 12.5	1♀ Control	2♀ NN304	3♀ NN304	4♀ NN304	5♀ — 12.5
-1	325	324	325	324	324					
OT♂	370	373	369	371	373					
1	403	406	400	403	413	233	233	233	233	233
2T♀	433	438	427	438	452	250	249	247	251	250
3	461	467	455	463	478	260	258	257	263	265
4P	482	489	476	487	504	267	269	269	276	277
5	504	514	500	510	527	301	305	305	310	312
6	530	540	527	535	557	340	341	342	346	347
7	540	551	539	547	574	409	405	412	415	407
8	545	554	543	552	581					
9	562	574	559	570	603					
Gain (g/rat):	♂ Weeks 0 - 4					♀ Weeks 2 - 4				
% control	112	116	108	116	132	16	20	22	25	26
	-	104	96	104	118	-	125	138	156	163
	♂ Weeks 0 - 9									
% control	193	202	190	199	230					
	-	105	98	103	119					

^a $p \leq 0.05$, ^b $p \leq 0.01$ for Control versus Groups 2 to 4

^c $p \leq 0.01$, ^d $p \leq 0.001$ for Control versus Group 5

T Treatment commenced

P Animals paired (1♂ : 1♀)

Table 16 Microscopic pathology incidence summary

	Group 1	Group 2	Group 3	Group 4	Group 5
Sex: Males					
Males on study	24	24	24	24	24
Animals completed	24	0	0	24	24
Right Epididymis					
Examined	24	0	0	24	24
No abnormalities detected	24	0	0	24	24
Right Testis					
Examined	24	0	0	24	24
No abnormalities detected	23	0	0	22	18
Focal seminiferous epithelial atrophy with vacuolation of Sertoli cells (Total)	F 1	0	0	2	6*
Trace	1	0	0	2	2
Minimal	0	0	0	0	4

No statistical significance ($p > 0.05$) for control versus Group 4

* $p \leq 0.05$ for control versus Group 5

F Fisher's exact test applied

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Table 9 Foetal abnormalities - prevalence and distribution in litters

Category	No. of affected fetuses/litter (n)	1	2	3	4	5
		Control	NN304	NN304	NN304	—
			5	25	50	12.5
		No. of litters with 'n' fetuses affected				
Number of litters examined		24	22	22	23	19
Malformation	0	22	21	20	20	18
	1	1	1	2	3	1
	2	1	-	-	-	-
Visceral anomaly	0	16	12	8 ^a	6 ^b	10
	1	4	7	9 ^a	11 ^b	4
	2	4	1	4 ^a	3 ^b	2
	3	-	2	-	2 ^b	3
	4	-	-	-	1 ^b	-
	5	-	-	1 ^a	-	-
Skeletal anomaly	0	12	12	14	18	12
	1	9	7	5	4	6
	2	3	2	2	-	1
	3	-	-	1	1	-
	6	-	1	-	-	-
	Mean % fetuses affected per litter					
Malformations		0.8	0.3	0.6	0.8	0.4
Visceral anomalies		7.0	8.8	14.4	15.2	13.5
Skeletal anomalies		8.7	9.4	8.2	4.3	6.4

^a $p \leq 0.05$, ^b $p \leq 0.01$ for control versus Groups 2 to 4

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Embryofetal development

Study title: Insulin detemir effects on embryofetal development in the rabbit

Key study findings: Maternal body weight gain showed a dose-related increase during the treatment period, compared with the controls, as food consumption increased. In utero deaths of litters were increased 5 to 10-fold compared to the control group, although the NPH human insulin also increased the parameter. Post-implantation loss of litters was also increased in the treated group as much as 10-fold of the control group. Thus, NOAEL appeared to be 37.5 U/kg/day (225 nmol/kg/day) based the effects of insulin detemir on in utero deaths and post-implantation loss of litters. The exposure ratio at the NOAEL is 23-fold of human exposure, based on human AUC values (72659 pM.hr) after 11.76 nmol/kg. It appears that the high litters loss might be related to pharmacological effects of insulin detemir on blood glucose.

Study no.: 960301

Volume #24, and page #: 253-297

Conducting laboratory and location: —

Date of study initiation: July 22, 1996

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #h96012/Lot#3176004, and % purity: —

Methods

Doses: 0(vehicle), 37.5, 75, and 150 U/kg/day (0, 225, 450, and 900 nmol/kg/day) insulin detemir including 5 U/kg/day (30 nmol/kg/day) NPH insulin

Species/strain: Rabbit/New Zealand White rabbits

Number/sex/group: 24 females/group

Route, formulation, volume, and infusion rate: Subcutaneous, 100-400 U/ml

Satellite groups used for toxicokinetics: Not specified.

Study design: The three doses were given to groups of 24 time-mated female rabbits by subcutaneous injection. A reference product —, was included at 5 U/kg/day (30 nmol/kg/day) and animals were treated from Day 6 to 18 post coitum inclusive.

Parameters and endpoints evaluated: The following items listed under results were measured as endpoints for the evaluation of insulin detemir on pregnancy and embryofetal development in the rabbit.

Results

Mortality (dams): There were no deaths in the LD and MD groups. Five females in HD group were found dead in the morning, approximately 24 hours after the previous dosing. One rabbit (#413) experienced an apparent hypoglycemic episode 3 hours after dosing. Autopsy findings in 3/5 decedents had abnormal hemorrhagic depressions in the stomach, changes in content of cecum and colon.

Clinical signs (dams): One female in HD group of insulin detemir and two females receiving 5 U/kg/day NPH had hypoglycemic episodes as confirmed by plasma glucose levels. The animals were rescued by glucose administration, although one died eventually (#413) as indicated above. There were no other treatment-related clinical signs.

Body weight (dams): Maternal body weight gain showed a dose-related increase during the treatment period, compared with the controls as shown below.

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Table 3 **Bodyweight and bodyweight change of dams with live young - group mean values (g)**

Group/ dosage (U/kg/day)	No. of animals	Bodyweight (g) at Day of pregnancy								
		0	2	6	8	10	14	19	23	29
1 Control	22	3280	3375	3444	3456	3505	3634	3740	3822	3985
2 NN304 37.5	19	3353	3407	3471	3502	3574	3704	3831	3877	3994
3 NN304 75	21	3294	3383	3456	3509	3560	3743	3896	3908	4032
4 NN304 150	16	3228	3305	3369	3439	3497	3688	3860	3778	3889
5 <hr/> 5	21	3306	3413	3506	3555	3599	3759	3863	3896	4026

Group/ dosage (U/kg/day)	No. of animals	Bodyweight change (g) from start of treatment at Day of pregnancy								
		0	2	6	8	10	14	19	23	29
1 Control	22	-164	-70	0	12	61	190	295	378	541
2 NN304 37.5	19	-117	-63	0	31	104	233	361	407	524
3 NN304 75	21	-162	-73	0	53	104	287	440	452	576
4 NN304 150	16	-142	-64	0	69	128	319	490	409	520
5 <hr/> 5	21	-200	-93	0	49	93	252	357	390	520

Statistical analysis of bodyweight change:

^a $p \leq 0.05$, ^b $p \leq 0.01$ for Control versus Groups 2 to 4

^c $p \leq 0.05$ for Control versus Group 5

Treatment period: Days 6 to 18 of pregnancy inclusive

Food consumption (dams): There were dose dependent increases in food consumption similar to the dose-related increases in body weight.

Litter data:

Two dams in the HD group and one at 37.5 U/kg/day aborted their litters before termination on Day 29 of pregnancy (7 and 9 fetuses each), which appeared to be related to treatment because one dam at the MD resorbed her single implant. There were no drug-related effects on the number of corpora lutea, number of implantations or pre-implantation loss. Insulin treatment (detemir or NPH) resulted in increased in utero deaths. Insulin treatment (detemir or NPH) at all doses produced marked increases in post-implantation loss as shown below.

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Table 6 Litter data and sex ratio - group values

Group		1	2	3	4	5
Dosage (U/kg/day)		Control	NN304	NN304	NN304	—
			37.5	75	150	5
Dams with live young						
No. of litters		22	19	21	16	21
Group mean values						
No. of corpora lutea		10.5	9.9	10.0	10.8	10.1
No. of implantations		9.0	9.2	9.3	9.2	9.3
Pre-implantation loss (%)		12.9	7.5	7.5	13.4	8.7
No. of <i>in utero</i> deaths:						
- early		0.3	1.6 ^a	1.9 ^b	3.3 ^b	1.6
- late		0.1	0.7	0.4	0.6 ^a	0.4
- total (early and late)		0.5	2.3 ^b	2.3 ^b	3.8 ^b	2.0 ^c
Post implantation loss (%)		4.4	24.1 ^b	24.9 ^b	41.6 ^b	20.4 ^c
No. of live young		8.5	6.9	7.0	5.4 ^b	7.3
Sex ratio (% ♂)		43.4	44.2	50.8	47.2	51.0
Litter weight (g)		394.0	324.1	333.3	253.4 ^b	334.9
Foetal weight (g)		47.0	47.9	48.7	49.1	47.1
Litter incidence	'n'	No. of litters with 'n' losses/ <i>in utero</i> deaths				
Pre-implantation losses	0	13	12	10	6	10
	1	-	4	6	4	5
	2	6	1	5	3	5
	3	-	1	-	1	1
	4	1	-	-	-	-
	5	-	1	-	1	-
	8	2	-	-	1	-
<i>in utero</i> death: total (early and late)	0	14	5 ^d	3 ^d	2 ^d	6 ^d
	1	6	3	6	-	6
	2	2	4	2	1	5
	3	-	1	6	5	-
	4	-	3	3	2	-
	5	-	1	-	3	1
	6	-	2	-	1	2
	7	-	-	-	1	-
	8	-	-	-	1	-
	9	-	-	1	-	1

Analysis of group mean values:

^a $p \leq 0.05$, ^b $p \leq 0.01$ for Control versus Groups 2 to 4^c $p \leq 0.01$ for Control versus Group 5

Analysis of litter incidence:

^d $p \leq 0.001$

Toxicokinetics:

At the three doses of insulin detemir relevant TK data were calculated as presented below. The exposure ratios were increased as the dose was increased as shown except the HD, which was actually reduced from the MD value 72 to 35. The reason for the discrepancy is not clear because the Cmax values were increased drug dose-dependently, although there were large variabilities in raw data.

Toxicokinetic Data in Embryofetal Development Study in Rabbits*					
Drug Dose		Cmax(pM)	t _{1/2} (h)	AUC ₀₋₂₄ (pM.h)	AUC ratio@
37.5 (U/k/d)	225 (nmol/k/d)	224167	2.8	1706000	23
75	450	530667	2.6	5213667	72
150	900	979833	3.3	2559117	35

*Data were obtained from 6 rabbits on Day 6 of pregnancy. @Based on human AUC values (72659 pM.hr) after 11.76 nmol/kg in diabetic subjects.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Insulin detemir treatment was associated with a dosage-related increase in the incidence of fetus with gall bladder abnormalities (small, bilobed, bifurcated and absent). In addition, all of animal groups have dose-related increases in the percentage of fetus per litter with extra lumbar ribs and of additional thoracolumbar vertebra, compared with control. A low incidence of fetuses with microphthalmia and anophthalmia was noted for some animals.

Offspring (malformations, variations, etc.):

There were drug-dose related increases in the incidence of fetuses with gall bladder abnormalities such as small, bilobed, bifurcated and missing gall bladder as shown below. In the animals, dose-related increases in the percent of fetus per litter with extra lumbar or thoracolumbar vertebra were observed (See Table 9 below). There was no other apparent effect of insulin detemir treatment on the incidence or type of fetal malformations.

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Table 8 (Foetal abnormalities - continued)

Group	Foetuses					Litters					
	1	2	3	4	5	1	2	3	4	5	
Compound	Control	NN304	NN304	NN304	—	Control	NN304	NN304	NN304	—	
Dosage (U/kg/day)		37.5	75	150	5		37.5	75	150	5	
No. examined	188	132	146	86	153	22	19	21	16	21	
Description of visceral anomalies											
HEAD											
Eyes	corneal/lenticular opacity	1	-	1	-	-	1	-	1	-	-
THORAX											
Anomalous cervicothoracic arteries		5	6	7	3	4	4	6	5	3	4
Anomalous systemic/pulmonary arteries		-	-	-	-	1	-	-	-	-	1
Lungs	atelectatic	-	-	-	1	-	-	-	-	1	-
absent intermediate lobe		3	1	-	3	1	3	1	-	2	1
ABDOMEN											
Distended with fluid		-	-	-	1	-	-	-	-	1	-
Liver	cyst	-	-	-	1	-	-	-	-	1	-
misshapen		-	-	-	-	1	-	-	-	-	1
pale and swollen		-	-	-	1	-	-	-	-	1	-
abnormal lobation		-	-	-	1	1	-	-	-	1	1
Gall bladder	bilobed/bifurcated/absent	1	8	7	10	5	1	5	7	6	4
small		-	-	-	4	-	-	-	-	4	-
Gonads	cystic/haemorrhagic	1	2	-	1	1	1	2	-	1	1
ovary											

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Table 9 Skeletal variants - group values

Group	1	2	3	4	5
Compound	Control	NN304	NN304	NN304	—
Dosage (U/kg/day)		37.5	75	150	5
Number of litters examined	22	19	21	16	21
Number of fetuses examined	188	132	146	86	153
Ribs					
Number of fetuses with 12 ribs	112	48	51	24	53
Number of fetuses with 12/13 or 13/13 ribs	76	84	95	62	100
Mean % fetuses per litter with 12/13, 13/13	39.7	61.2 ^a	69.5 ^b	76.0 ^b	63.9 ^c
20 Thoracolumbar vertebrae					
Number of fetuses affected	25	63	46	54	61
Mean % fetuses per litter	12.7	48.2 ^b	36.7 ^b	67.6 ^b	41.3 ^d
Sternebrae					
No. of fetuses with i.o 5th sternebrae	68	47	49	20	51
No. of fetuses with i.o other sternebrae	11	15	13	5	12
Total number of affected fetuses	74	52	55	22	57
Mean % fetuses per litter with i.o sternebrae	39.1	36.7	33.7	30.5	35.4

i.o. Incomplete ossification

Analysis of % fetuses affected:

^a $p \leq 0.05$, ^b $p \leq 0.01$ for control versus Groups 2 to 4^c $p \leq 0.05$, ^d $p \leq 0.01$ for control versus Group 5

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Conclusion: NOAEL appeared to be 37.5 U/kg/day based on abnormalities of gall bladder and skeletal variations. The following observations after insulin detemir treatment in rabbits should be included in label: significant increases in gall bladder abnormalities and in skeletal variations were observed in the fetuses of dams treated with insulin detemir and NPH insulin. Incidences were comparable in all insulin treated groups and were observed with doses of detemir \geq 37.5 U/kg/day (23 times human exposures based on AUC).

Prenatal and postnatal development

Study title: Insulin detemir effects on pre-and post-natal development in CD rats

Key study findings: The HD (50 U/kg/day = 300 nmol/kg/day) was high enough to kill 5 rats, although comparator human insulin also kill 5 rats at a dose of 12.5 U/kg/day. The deaths appeared to be related to the hypoglycemia induced by the agents. There were no remarkable findings in Fo. In F1 generation, insulin detemir had no remarkable effects on physical development and reproduction parameters such as fertility, mating performance, and litter size, although survival, viability, and lactation index were slightly reduced at the HD. There were no remarkable effects of insulin detemir and human insulin on F2 offspring.

Study no.: 990123

Volume #26, and page #: 1-267

Conducting laboratory and location: —

Date of study initiation: August 19, 1999

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, Batches#317809, and % purity: Not specified

Methods

Doses: 0, 5, 25, and 50 U/kg/day (0, 30, 150 and 300 nmol/kg/day) Insulin detemir, and 12.5 U/kg/day —

Species/strain: Rat/CD

Number/sex/group: 24 female rats/group

Route, formulation, volume, and infusion rate: Subcutaneous, 100 Units/ml

Satellite groups used for toxicokinetics: 4 rats/group were bled on Day 6 and 17 after mating, Day 4 and 14 of lactation at before, 1, 3, and 6 hours after drug administration.

Study design: All Fo generation females were dosed subcutaneously from Day 6 after mating to Day 20 of lactation inclusive, with the exception of females receiving Insulatard. Dosing was suspended after Day 20 of gestation and recommenced on Day 2 of lactation. The F1 and F2 offspring were not treated directly.

Parameters and endpoints evaluated: Maternal observational parameters were carried out as other fertility study while Litter (Fo, F1, and F2) observations are described briefly under each individual result section below.

Results

F₀ in-life and mortality:

There were no deaths in control and LD groups. In MD group, two rats died: one each on Day 21 and 23 of gestation while no rats died during lactation in this group. In the HD group, five rats died: one each on gestation day 22 and lactation day 2 and three on lactation Day 3. Five rats died in the — group. The deaths were appeared to be related to the pharmacological effects of the test articles such as hypoglycemia.

Clinical Signs: There was slow and/or shallow gasping respiration before deaths. Many animals had hypoactive and prostrate posture, which might be related to the treatment.
Body weight and Food consumption: There were no clear drug-related changes in the parameters in the control and treated groups during gestation, although there were sporadic changes between groups during lactation.

F₀ necropsy: Not remarkable.

F₁ physical development:

There were no apparent effects of treatment on litter size or survival before or after culling of the offspring at Day 4 after birth. The ratio of male to female offspring in the litters was unaffected by treatment. Offspring bodyweights were largely unaffected by treating their mothers with either insulin detemir or —

F₁ behavioral evaluation: Behavioral effects of insulin detemir were not observed.

F₁ reproduction:

The mean number of implantation sites for the F₀ females was unaffected by treatment and litter size to Day 28 of age was comparable in all groups. There was a slight reduction in the post-implantation survival index, viability index and lactation index for females receiving 25 or 50 U/kg/day insulin detemir or 12.5 U/kg/day — as shown below. It appeared that the no statistically significant reductions were due to females in those groups dying before parturition. There were no macroscopic findings that were considered indicative of an effect of treatment on the F₀ generation females.

TABLE 9

Offspring survival indices - group values (F₁)

Group	:	1	2	3	4	5
Compound	:	Control		----- NN304 -----		
Dosage (U/kg/day)	:	0	5.0	25.0	50.0	12.5

F₀ Females treated

Group		Post Implantation Survival Index (%)	Live birth Index (%)	Viability Index (%)	Lactation index					
					7	11	14	18	21	28
1	Mean	94.1	98.4	95.8	99.6	99.2	99.2	99.2	99.2	99.2
	n	24	24	24	24	24	24	24	24	24
2	Mean	92.0	100.0	99.4	99.6	99.6	99.6	99.6	99.6	99.6
	n	24	24	24	24	24	24	24	24	24
3	Mean	67.7	92.6	97.1	99.1	99.1	99.1	99.1	99.1	99.1
	n	24	23	22	22	22	22	22	22	22
4	Mean	87.8	97.9	78.3	96.7	96.7	96.7	96.7	96.7	96.7
	n	24	23	23	18	18	18	18	18	18
5	Mean	89.6	98.1	91.0	100.0	95.0	95.0	95.0	95.0	95.0
	n	24	23	23	20	20	20	20	20	20

n Number of females/litters.

Sex ratios in each litter were not affected by treatment and F1 body weight from birth to day 28 of age was considered to be not affected the treatment of the F₀ females. Vaginal opening and preputial separation was also unaffected by treatment of the F₀ females with insulin detemir or — The auditory and visual responses to the F1 offspring were similar in all groups. It appeared that locomotor activity and learning ability were unaffected by the treatment. There were slight inter-group variations in neuromuscular function of treated groups, although they appeared not to relate to the treatment.

Pre-coital interval and mating performance and fertility of F1 were not affected by the treatment.

F₂ findings:

General conditions of F2 offspring were similar in all groups including group mean bodyweights in F2. The mean number of implantation sites, total litter size at Day 1 of age and survival to Day 7 was comparable in all groups. There were no apparent differences in sex ratios and bodyweight up to Day 7 of lactation in all groups. Necropsy of F2 offspring indicated that there was no clear inter-group difference in gross pathological findings.

Toxicokinetic Data:

TK parameters were estimated after mating and/or lactation in MD and HD groups on different dates as summarized below. In the HD group there were significant decreases in Cmax values after lactation with shortened half-life, which were reflected in reductions in AUC and AUC ratio. The variability in the MD group was somewhat less than that in the HD group. AUC ratios were approximately 1.5 after mating or lactation in the 25 U/kg/day group while the ratios in the HD group were variable after mating and after lactation, depending on the day of lactation. In general, drug exposure to test animals was low, compared with that of human.

Toxicokinetic Data in Pre- and Post-natal Development Study in Rats						
Dose (U/kg/day)	Day after mating or lactation	Cmax(pM)	Tmax (hr)	t _{1/2} (hr)	AUC _{0-6hr} (pM.hr)	AUC Ratio*
25	6(mating)	28350	0	1.4	85388	1.2
	17(mating)	29630	1	3.6	126339	1.7
	4(lactation)	27850	1	5.1	113320	1.6
	14(lactation)	29900	1	1.0	106707	1.5
50	17(mating)	60000	1	4.5	240086	3.3
	4(lactation)	31400	3	2.8	11959	0.2
	14(lactation)	28900	1	2.2	75415	1.0

*Based on human AUC values (72659 pM.hr) after 11.76 nmol/kg/day human dose.

3.4.7 Local tolerance

Study title: Local toxicity after subcutaneous injection in pigs(NN980106)

This study is summarized in Table 17 below.

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Table 17 Overview of local tolerance studies in pigs

Study No	NN 980106	NN 990162
Drug (nmol/ml)	Insulin detemir (IDet): 1200 and 2400 Vehicle for insulin detemir NPH insulin (—):600 0.9% NaCl	Insulin detemir (IDet): 1200 and 2400 Vehicle for insulin detemir NPH insulin (—): 600 0.9% NaCl
Dose route	s.c	s.c.
No. of Animals	8	6
Dosing	Injections given 5 days and 2 days before sacrifice: NPH: 200 µl IDet: 2400 nmol/ml & vehicle: 200 µl IDet: 1200 nmol/ml & vehicle: 400 µl NaCl: 400 µl	Injections given 5 days and 2 days before sacrifice: IDet: 1200 nmol/ml: 200 µl or 400 µl IDet: 2400 nmol/ml: 200 µl 0.9% NaCl: 200 µl or 400 µl, NPH: 200 µl
Results	IDet 1200 and 2400 nmol/ml caused mild inflammations at the injection site. Two days after injection the changes were similar to those of vehicle and 0.9% NaCl but different from NPH. 5 days after injection the reaction was similar for all products.	The local reaction was mild and comparable with saline for both IDet formulations 2 and 5 days after the injection when given in the volume of 200 µl. The reaction was slightly enhanced when the IDet 1200 nmol/ml was given in a volume of 400 µl. The reaction for both IDet formulations was milder than that for NPH.

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3.4.8 Special toxicology studies

The sponsor performed several mitogenicity and antigenicity studies, which are briefly reviewed below.

A. Mitogenicity Study:

1. Mitogenic potency of insulin detemir in CHO-K1(Disc/Idet/07)

Effects of insulin detemir on stimulation of ^3H -thymidine incorporation into newly-synthesized DNA were compared to that of human insulin in Chinese Hamster Ovary cells (CHO-K1 cells). This cell line was chosen because it responds strongly to growth factor stimulation. These cells express approximately 50000 IGF-I receptors and approximately 3000 insulin receptors. Dose-response profiles were generated for human insulin and insulin detemir and EC_{50} values were calculated.

The mitogenic potency of insulin detemir was determined to be $7.01 \pm 1.68\%$ relative to human insulin. Correction for the amount bound to albumin can be estimated if the binding constant is known. An albumin concentration of 0.02% will bind approximately 23% of the insulin detemir molecules, thus leaving 77% available for the proliferative system. The potency of insulin detemir corrected for albumin binding is 9% relative to human insulin (correction factor $1 \times 10^5 \text{ M}^{-1}$).

2. Mitogenic potency of insulin detemir in human osteosarcoma cell line B10 (Disc/Idet/08)

The mitogenic potential of insulin detemir was compared with insulin X10 in human osteosarcoma-derived cell line B10. The mitogenic effect of the test substances was determined by measuring dose-dependent stimulation of ^3H -thymidine incorporation into newly-synthesized DNA. Human insulin and the insulin analogues stimulated DNA synthesis with ED_{50} values in the nanomolar range. The mitogenic potency (mean \pm SE) of insulin X10 was determined to be $1094.75 \pm 166.40\%$ relative to human insulin ($=100\%$; $\text{ED}_{50} = 6.11 \pm 0.51 \text{ nM}$). In contrast, the relative mitogenic potency of insulin detemir was determined to be $0.36 \pm 0.06\%$ relative to human insulin. However, the mitogenic potency of insulin detemir in absence of albumin was estimated to be approximately 11% relative to human insulin (Correction factor $4 \times 10^5 \text{ M}^{-1}$).

3. Insulin detemir mitogenicity in MCF-7 cells (NN970014)

MCF-7 cells (human, mammary cancer fibroblasts) were incubated with insulin detemir, human insulin or — . The percentage of cells synthesizing new DNA (the S-phase) was determined using flow cytometry and cell cycle analysis. An increase in S-phase percentage reflects a mitogenic stimulation of cells which leads to an increased fraction of cells capable of producing new DNA.

Dose-response profiles were generated and the relative mitogenic potency of the insulins was calculated. The mitogenic potency of insulin detemir relative to that of human insulin was determined to be 5.0% (95% CI: 2.2%-11.7%). The buffer contained 0.03% bovine serum albumin (BSA). Correcting for albumin binding, the mitogenic potency of insulin detemir relative to human insulin was determined to be 14.6% (Correction factor $4 \times 10^5 \text{ M}^{-1}$).

B. Immunogenicity Study:**Immunogenicity of insulin detemir in rabbits(NN940479)**

Thirty New Zealand white rabbits were divided randomly into three equal groups with an equal number of male and female rabbits. The animals had free access to a standard feed and water containing glucose. Insulin detemir was compared to porcine and bovine insulins. Dosing was twice a week s.c. 200 µl (0.72 mg corresponding to 120 nmol) for 98 days, injected between the shoulders. Antibody formation was measured in serum using RIA.

Antibody response in insulin detemir-treated animals was very low, only in three out of ten animals a low response was seen during the treatment period. Whereas, in the groups of porcine or bovine insulin treated animals, four to seven animals out of ten developed antibodies. Statistical analysis showed that there was no significant difference between the groups being treated with either insulin detemir or porcine insulin, whereas a significant difference was found between the antibody response in the insulin detemir- and bovine insulin-treated rabbits.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Compared to human insulin, insulin detemir has been shown to have lower receptor binding affinity, metabolic and mitogenic potency. Insulin detemir has the anticipated protracted actions as compared to human insulin. The safety study has demonstrated that except for the kinetic differences between insulin detemir and human insulin, the ADME profile is similar to what is known for regular insulins such as NPH insulin or Insulartard. The safety profile of insulin detemir is comparable to that of human insulin. Toxicology studies indicate that insulin detemir adverse effects appeared to be secondary to its primary hypoglycemic action, although there were some unclear toxicology issues as indicated under "Pharmacology Recommendation". In conclusion, the non-clinical testing program has indicated that insulin detemir is an insulin with a protracted duration of action and a safety profile comparable to human insulin.

Unresolved toxicology issues (if any): None.

Pharmacology Recommendations:

1. The following preclinical findings should be included in labeling instructions.

a. Carcinogenicity, Mutagenicity, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed. Insulin detemir

b. Pregnancy: Teratogenic Effects: —

2. In future submissions, please use uniform unit (international unit, mg, or nmol) throughout the submission.

3.7. SUGGESTED LABELING: PLEASE SEE APPENDIX/ATTACHMENTS

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

1 Page(s) Withheld

 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

 ✓ § 552(b)(5) Draft Labeling

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/s/

Herman Rhee
9/4/03 05:36:14 PM
PHARMACOLOGIST

Jeri El Hage
9/5/03 08:16:32 AM
PHARMACOLOGIST

**45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY**

NDA No. 21-536/Novo Nordisk Pharmaceuticals Inc./Insulin Detemir/Jan.27, 03

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?	X		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		
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.)?	X		
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)	X		<p>Have electronic files of the carcinogenicity studies been submitted for statistical review? N/A</p> <p><u>Studies completed:</u></p> <p>1) 2-, 4-Week s.c. toxicity in rats 2) 3-, 6-Month s.c. toxicity in rats 3) 4-W, 3-, 6-M s.c. toxicity in dogs 4) 12-M s.c. toxicity, TK in dogs 5) Genotoxicity (Ames, MNA, CAT) 6) Embryofetal development in rabbits and Pre- & Post natal studies in rats 7) Local toxicity study 8) Immunogenicity study in rabbits 9) Mitogenicity studies in CHO-K1, osteosarcoma, and MCF-7 cells.</p>

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	X		
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 (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		
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?	X		

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/m ² or comparative serum/plasma AUC levels?	X		
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ITEM	YES	NO	COMMENT
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file:			

Herman Rhee, Ph.D. _____
 Reviewing Pharmacologist

Jeri Elhage, Ph.D. _____
 Supervisory Pharmacologist

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

/s/

Herman Rhee
1/31/03 09:01:00 AM
PHARMACOLOGIST

Jeri El Hage
1/31/03 09:05:54 AM
PHARMACOLOGIST