

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**21-148**

**PHARMACOLOGY REVIEW**

**NDA 21-148**

**March 17, 2000**

**DRUG: Norditropin® SimpleXx™**

**INDICATION: Long term treatment of children who have growth failure due to inadequate secretion of endogenous growth hormone**

**TEAM LEADER MEMO TO FILE REGARDING  
PRECLINICAL PHARMACOLOGY/TOXICOLOGY ISSUES  
FOR NDA 21-148 (Norditropin® SimpleXx™, somatropin (rDNA origin))**

**SUMMARY OF PRINCIPLE PHARMACOLOGY/TOXICOLOGY ISSUES WITH NDA 21-148**

The following discussion is based upon the primary NDA pharmacology review provided by David Hertig, pharmacologist.

**INTRODUCTION:**

The product covered by this NDA represents a new formulation of recombinant human Growth Hormone (GH). The currently approved product is available as a lyophilized powder, which has to be reconstituted prior to injection. The product under review is a liquid, ready-to-use formulation. It is also to be available in a modified NovoPen to improve the convenience of dosing. Indication and dosing will remain the same as that recommended for the currently approved lyophilized product.

The key issues related to the pharmacology/toxicology evaluation are as follows:

1. Stability, immunogenicity and activity of the stored liquid product
2. Safety of the excipient Poloxamer 188 which is currently used as a \_\_\_\_\_ and is present in many IV products, but for which there is little information regarding subcutaneous exposure.

1. Stability, immunogenicity and activity of the stored liquid product.

To address these issues, the sponsor performed a variety of toxicology studies to determine the activity and toxicity of the degraded product. The degraded Liquid Norditropin® product was considered to be similar to a Liquid Norditropin® product that would have been stored for \_\_\_\_\_ . These included studies to characterize the biological activity of degraded products, receptor binding of degraded products, safety pharmacology studies including Irwin evaluation, evaluation of acute cardiovascular effects, renal function and acute toxicology in mice. A standard multi-dose 3-month subcutaneous toxicology study was performed in rats and a tissue irritation study was performed in rabbits. In addition, an immunotoxicity assessment was made in transgenic mice which carry the human somatropin gene.

To summarize briefly, the degraded product performed essentially identically in all studies when compared to the intact product, indicating that the liquid formulation storage and the excipient components do not adversely alter the safety profile of the Norditropin®. The effects of growth hormone administered subcutaneously have been previously well characterized and there has been extensive human use. With regards to growth hormone content, it would appear that the available knowledge we have about growth hormone applies to the product currently under consideration. The sponsor has adequately addressed concerns regarding stability, toxicity, immunogenicity and activity of the stored liquid product.

## **2. Safety of the excipient Poloxamer 188.**

In order to provide a stable liquid product, a suitable vehicle had to be developed. The formulation developed for Norditropin®SimpleXx™ contains the following (amounts are specified in the primary review):

Somatropin  
Histidine  
Poloxamer 188  
Phenol  
Mannitol  
HCL/NaOH  
Water for injection

Of this listing, there was insufficient safety data available to support the subcutaneous administration of Poloxamer 188 as would be used for the proposed human indication. The sponsor performed an extensive series of preclinical studies to establish the safety of Poloxamer 188 to support the use of the proposed formulation.

The studies to support safety of the Poloxamer 188 included multi-dose toxicology studies in rats and dogs of up to 13 weeks, Developmental and Reproductive Toxicology (DART) studies in rats and rabbits, genetic toxicology studies including Ames, *in vitro* assessments of chromosomal aberrations and mutation (human peripheral lymphocyte assay and Mouse lymphoma assay) and an *in vivo* assessment of clastogenicity (mouse micronucleus test).

To briefly summarize the findings with Poloxamer 188:

1. Poloxamer 188 was not considered mutagenic under the conditions used in the complete battery of genotoxicity studies provided.
2. Results of the 3-month toxicology studies indicated a No Observed Effect Level (NOEL) of 10 mg/kg in rats and 0.5 mg/kg in dogs. Comparing to human exposure on a surface area basis, these represent multiples of approximately 40 and 7 fold the human daily exposure. Although the apparent safety margin for dogs appears to be relatively smaller, it is noted that at the next higher dose in dogs (3 mg/kg or approximately 40 times the human exposure based on surface area comparisons) the primary findings were increased in kidney and liver weights (<20%) in females only with no histopathology or clinical chemistry correlates. Thus, the 3 mg dose could be considered a No Observable Adverse Effect Level (NOAEL) and would provide an approximate 40 fold safety margin compared to proposed human exposure.
3. Results of the DART battery indicated the following safety margins based on surface area comparisons:
  - a. NOAEL for Rat fertility and early embryonic development: 10 mg/kg/day ~ 40 times expected human exposure.
  - b. Effects on litter parameters in rats: NOAEL in rats 500 mg/kg/day ~2000 times expected human exposure.
  - c. NOAEL for Rat teratology study: 10 mg/kg for dams and fetuses ~40 times expected human exposure
  - d. NOAEL for Rabbit teratology study: 200 mg/kg/day ~1600 times expected human exposure.
  - e. NOAEL for Rat Pre-and Postnatal Developmental study: 10 mg/kg/day ~40 times expected human exposure

Thus, there is sufficient safety established for the potential for reproductive effects of Poloxamer under the proposed human use.

## CONCLUSION

All preclinical issues for the proposed use of this product have been addressed. The pharmacology team leader recommends that this application should be approved (AP) from a pharmacology standpoint pending appropriate modifications to the label as recommended by the primary reviewer.

LS  
Ronald W. Steigerwalt, Ph.D.  
Pharmacology Team Leader, DMEDP

cc: NDA Arch:  
HFD510  
HFD510/Steigerwalt/Hertig /CKing  
Review Code: AP (pending labeling revisions)  
Filename: 21148.Tlmemo.doc

## REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

**KEY WORDS:** Norditropin SimpleXx; Norditropin; somatropin; Poloxamer 188

**Reviewer Name:** David Hertig

**Division Name:** Division of Metabolism and Endocrine Drug Products

**HFD#:** 510

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**Serial number/date/type of submission:** N-000 30 Jun 99, 1 Jul 99 Original  
29,30 Sep 99 Info/Labeling; 25,28 Feb 00 Labeling

**Information to sponsor:** Yes (X) No ( )

**Sponsor (or agent):** Novo Nordisk Pharmaceuticals, Inc., Suite 200, 100 Overlook Center, Princeton, NJ 08540-7810

**Manufacturer of drug substance:** Novo Nordisk A/S, 2880 Bagsvaerd, Denmark

**Drug:**

**Generic Name:** somatropin (rDNA origin)

**Trade Name:** Norditropin SimpleXx™ Cartridges [Somatropin (rDNA origin) for subcutaneous injection]

**CAS Registry Number:** Chemical Abstracts Registry Number: 12629-01-5

**Molecular Formula/ Molecular Weight:** 191 amino acid residues with a sequence identical to the naturally occurring pituitary human growth hormone. ca 22,000 daltons

**Relevant INDs/NDAs/DMFs:** NDA 19-721 Norditropin® [somatropin (rDNA origin) for subcutaneous injection]; DMF         

**Drug Class:** Human Growth Hormone (hGH)

**Indication:** Norditropin®SimpleXx™ is indicated for the long-term treatment of children who have growth failure due to inadequate secretion of endogenous growth hormone.

**Clinical formulation:** A liquid formulation of somatropin in 5 mg, 10 mg, and 15 mg cartridges. The cartridges are ready to administer when inserted into the NordiPen™ injection devices.

Norditropin® SimpleXx™ is supplied as a solution in ready-to-administer cartridges with a volume of 1.5 ml.

[Sponsor's Table Vol. 1.1 p. 32]

Each Norditropin®SimpleXx™ Cartridge contains the following:

Component	5 mg Cartridge	10 mg Cartridge	15 mg Cartridge
Somatropin	5 mg	10 mg	15 mg
Histidine	1 mg	1 mg	1.7 mg
Poloxamer 188	4.5 mg	4.5 mg	4.5 mg
Phenol	4.5 mg	4.5 mg	4.5 mg
Mannitol	60 mg	60 mg	58 mg
HCl/NaOH	q.s.	q.s.	q.s.
Water for Injection	ad 1.5 mL	Ad 1.5 mL	Ad 1.5 mL

Norditropin®SimpleXx™ is supplied in 5 mg (orange), 10 mg (blue), or 15 mg (green) cartridges that must be administered using the corresponding color-coded NordiPen injection pen.

**Route of administration:** Subcutaneous injection

**Proposed clinical protocol or Use:**

The : Norditropin®SimpleXx™ dosage and schedule for administration must be individualized for each patient. Generally, subcutaneous administration in the evening, 6-7 times a week, is recommended. It is further recommended to give the injections in the thighs and to vary the injection site on the thigh on a rotating basis. Dosage can be calculated according to body weight.

Generally recommended dosage: Subcutaneous injection: \_\_\_\_\_ mg/kg body weight, 6-7 times a week.

**Previous clinical experience (IND only):** No "covered clinical studies" were conducted under the US IND in support of this application.

**Disclaimer – use of sponsor's material:** Note some material may be taken directly from sponsor's submission.

**Introduction and drug history:** Norditropin® SimpleXx™ is a new dosage form of Novo Nordisk's Somatropin (rDNA origin) for subcutaneous injection. Human growth hormone (hGH) itself has been used for a number of years to treat children with short stature due to growth hormone deficiency or growth hormone insufficiency and followed in time by other forms of short stature.

Until recently hGH treatment required a mixing/reconstitution process adding to the burden of drug administration. Novo Nordisk has developed Norditropin SimpleXx as a ready to use formulation of biosynthetic human somatropin supplied in 5, 10, and 15 mg cartridges designed to fit into an injection pen [a modified NovoPen 1.5 used for the administration of insulin] for s.c. administration. This avoids the current mixing procedure needed prior to the s.c. injection of somatropin thus improving convenience for the patient. The product will be used in the already existing indications for Norditropin [somatropin (rDNA origin) for subcutaneous injection] which requires reconstitution prior to use.

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## Studies reviewed within this submission:

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**Bioactivity :** Individual study summaries only. Vol. 1.9/21

**Characterization and Biological Activity of Degraded Liquid Norditropin 10 mg, batch 31796022/Des 15% for preclinical use – Doc ID: 20541**

Liquid Norditropin was stored for \_\_\_\_\_ with the purpose of obtaining a degraded product containing at least the \_\_\_\_\_ content estimated to be reached at the end of shelf life for the Liquid Norditropin product i.e. \_\_\_\_\_ at 5°C. The degradation profile verified that the product contained all the degradation products found in the Liquid Norditropin formulation, and in general in higher amounts. \_\_\_\_\_ unknown degradation products also increased above the detection limits in the degraded Liquid Norditropin batch, reflecting the increased degradation of the product.

The degraded Liquid Norditropin batch 31796022/\_\_\_\_\_ used for preclinical studies corresponded to Liquid Norditropin 10 mg stored for \_\_\_\_\_ at 5°C, and thus surpasses the desired end of shelf life for the product set at \_\_\_\_\_

The potency of the degraded product as determined by Weight Gain Assay was identical to that of pure Somatropin. The receptor binding affinity of the degraded Liquid Norditropin batch was identical to that of pure Somatropin.

**Isolation, Characterization and Biological Activity of \_\_\_\_\_ of Somatropin – Doc ID: 20542**

The deamidated forms of Somatropin (\_\_\_\_\_ - present in concentrations above \_\_\_\_\_ in Liquid Norditropin at the end of shelf-life) were shown to be identical to pure Somatropin with respect to biological potency (weight gain assay in rats), receptor affinity (IM-9 cells) and pharmacokinetics in rats. These degradation products did not show immunological responses in transgenic mice.

**Isolation, Characterization and Biological Activity of the Somatropin Degradation Products: \_\_\_\_\_ Doc ID: 20544**

The \_\_\_\_\_ degradation products \_\_\_\_\_ batch 13, \_\_\_\_\_ and \_\_\_\_\_ - batch 26, \_\_\_\_\_ Somatropin present in concentrations greater than \_\_\_\_\_ in Liquid Norditropin at the end of shelf-life were shown to be identical to pure Somatropin with respect to biological potency (weight gain assay in rats), receptor affinity (IM9 cells) and pharmacokinetics in rats. The degradation products did not show immunological responses in transgenic mice.

**Isolation, Characterization and Biological Activity of Somatropin Degradation Product: \_\_\_\_\_ Part II – Doc ID: 26824**

Analyzed by \_\_\_\_\_ the \_\_\_\_\_ Somatropin sample was found to include \_\_\_\_\_ of \_\_\_\_\_ The contaminants were \_\_\_\_\_ and consisted of \_\_\_\_\_ components in a ratio of \_\_\_\_\_ The \_\_\_\_\_ were between \_\_\_\_\_ a \_\_\_\_\_ respectively. \_\_\_\_\_ are not found in the Liquid Norditropin products. \_\_\_\_\_ of the \_\_\_\_\_ are also \_\_\_\_\_ in \_\_\_\_\_ was indistinguishable from Somatropin in immunogenicity and receptor binding tests. The weight gain assay showed \_\_\_\_\_ bioactivity which was reported to be comparable to the activity of the Somatropin \_\_\_\_\_ peak (isolated and handled as \_\_\_\_\_ No increased response was seen in in-vitro testing in \_\_\_\_\_ measuring the receptor binding, \_\_\_\_\_ and stimulation of the transcription of the Somatropin-regulated reporter gene. It is reported that the enhanced activity is not based on an increased activity on the cells, but can be due to an altered in-vivo stability of the proteins tested.

**Isolation, Characterization and Biological Activity of \_\_\_\_\_ of Somatropin**  
**- Doc ID: 26970**

Reduced biological activity was determined for \_\_\_\_\_ forms of Somatropin being \_\_\_\_\_ respectively, whereas the biological activity of the \_\_\_\_\_ form was \_\_\_\_\_ confidence interval 80-125%) – full biological activity by weight gain assay. The \_\_\_\_\_ forms were characterized by a number of analytical methods.

**Isolation, Characterization and Biological Activity of the Somatropin Degradation Products:**  
**- Doc ID: 26181**

\_\_\_\_\_ is Somatropin where \_\_\_\_\_  
 The biological activity of \_\_\_\_\_ was comparable to that of reference Somatropin in the weight gain assay.  
 Comparable activities of \_\_\_\_\_ and reference Somatropin were seen for binding affinity assay (IM9 cells) and immunogenicity assay in transgenic mice.

**Isolation, Characterization and Biological Activity of the Degradation Product:**  
**- Doc ID: 26968**

Except for a minor content of \_\_\_\_\_ a pure preparation of the Somatropin degradation product \_\_\_\_\_ has been obtained with a low content of \_\_\_\_\_ and \_\_\_\_\_ Biological activity was \_\_\_\_\_ in comparison with a standard Somatropin preparation.

**Receptor Binding Vol. 1.9/25**

Membrane receptors on cultured human lymphocytes bind human somatropin in a specific manner. The measurement of peptide hormones by their ability to bind to their receptors is an important indication of their probable biological activity. The human Somatropin receptor assay uses the human lymphocyte cell line IM-9. The receptor assay is based on the competition between a known labeled Somatropin standard and an unknown (unlabeled) somatropin sample for the somatogenic receptor on the IM-9 cells. The binding affinity of the unknown sample is determined from a standard curve.

Somatropin binds to two receptors before being internalized (Cunningham and Wells, Science, 244, 1081-1085, 1989; Cunningham et al., Science, 254, 821-825, 1991; De Vos et al., Science, 255, 306-312, 1992). In this receptor system only the affinity of the binding to the first binding site can be explored, since only displacement of the binding of an iodinated Somatropin was measured. Thus, from these results it can only be concluded that binding site 1 acts similar to that of Somatropin.

The IM9 receptor assay is an in-vitro assay. It is based on competition between a known labeled Somatropin standard and an unknown and unlabeled Somatropin sample for the somatogenic receptor on IM9 cells. The binding affinity is determined by two parameters:

The following parameters were estimated from the binding data:

1. The efficacy (maximal binding,  $E_{max}$ ) expressed in percentage maximal binding of  $^{125}I$ -Somatropin.
2. The mean, inhibitory potency ( $IC_{50}$ ) expressing the sample concentration causing half maximal inhibition of the bound  $^{125}I$ -Somatropin.

Measurement of Binding Affinity:

Somatropin Product	Study #	$E_{max}$ %	$IC_{50}$ ** ng/ml	Reference Somatropin $E_{max}$ %	Reference Somatropin $IC_{50}$ ng/ml
—	27022 Vol. 1.9/71	—	—	101	17.2
—	27018 Vol. 1.9/25/86	—	—	101	17.2
—	27025 Vol. 1.9/102	—	—	97	9.6
—	27134 Vol. 1.9/118	—	—	94	11.8
—	27003 Vol. 1.9/134	—	—	98	13.2
—	27009 Vol. 1.9/150	—	—	105	6.7
—	27012 Vol. 1.9/166	—	—	98	13.0
—	27153 Vol. 1.9/182	—	—	98	13
—	27151 Vol. 1.9/198	—	—	99	13.9
—	27148 Vol. 1.9/214	—	—	97	11.2
—	27011 Vol. 1.9/230	—	—	106	6.8
—	26819 Vol. 1.9/230	—	—	98	12.9

\* The efficacy (maximal binding,  $E_{max}$ ) expressed in percentage maximal binding of  $^{125}I$ -Somatropin.  
 \*\* The mean, inhibitory potency ( $IC_{50}$ ) expressing the sample concentration causing half maximal inhibition of the bound  $^{125}I$ -Somatropin.  
 \*\*\* Significantly different when comparing to reference Somatropin ( $p < 0.05$ ).

Degraded Liquid Norditropin – Batch 31796022

— Significantly different when comparing to reference Somatropin ( $p < 0.05$ ). The binding affinity of — is significantly reduced compared to that of the reference Somatropin (the — peak purified together with the —. According to the sponsor, a reduction in the  $IC_{50}$  value of — could be due to — of the — when the — molecules bind together, thereby reducing the amount of — available for the

receptor. They further state that it could be explained by a reduced affinity of \_\_\_\_\_ of all the molecules, due to interaction with \_\_\_\_\_ when the \_\_\_\_\_ form a \_\_\_\_\_

\_\_\_\_\_: The binding affinity of \_\_\_\_\_ is significantly different from that of reference Somatropin (the Somatropin \_\_\_\_\_ peak purified together with \_\_\_\_\_). According to the sponsor, the reduction in IC<sub>50</sub> value of \_\_\_\_\_ could be due to one of the \_\_\_\_\_, when the \_\_\_\_\_ bind together and thereby reduce the amount of binding sites available for the receptor. They also indicate that it could be explained by a reduced affinity of \_\_\_\_\_ of all the molecules, due to interaction with \_\_\_\_\_ when the \_\_\_\_\_ molecules form a \_\_\_\_\_

**Isolation, Characterization and Receptor Binding of the Somatropin Degradation Product:** \_\_\_\_\_

Doc ID: 26819. 1.9/246

\_\_\_\_\_ peak in degraded Liquid Norditropin observed by \_\_\_\_\_ analysis - content estimated to be \_\_\_\_\_ at the end of the shelf life of Liquid Norditropin. \_\_\_\_\_ contains a \_\_\_\_\_ in the Somatropin molecule. No other major modifications were found.

Enrichment of a degraded Liquid Norditropin with \_\_\_\_\_ showed \_\_\_\_\_ by \_\_\_\_\_ analysis. According to the sponsor, thus, \_\_\_\_\_ are the \_\_\_\_\_ molecule. In addition the \_\_\_\_\_ showed a similar binding affinity to the IM9 cell receptor as Somatropin.

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**Safety Pharmacology Studies:** \_\_\_\_\_

(Study 960480), and

(Studies 960481 and 960482).

**Liquid Norditropin 10 mg: Assessment of Effects in the Irwin Observation Test Following Single Subcutaneous Administration to CD-1 Mice.** Study 960480 - Irwin Observation Test. 24 Mar 97 to 11 Nov 97 QA - Present. 1.9/298

This test was carried out to assess possible behavioral, autonomic and neurological effects of subcutaneous administration of 8 mg/kg Liquid Norditropin (10 mg batch 31796022), degraded Liquid Norditropin (10 mg batch 31796022), \_\_\_\_\_, and Norditropin (batch 67538) in CD-1 male mice (s.c. to 6 per group). The Vehicle Control for Liquid Norditropin 10 mg was batch 433-970227-60 containing Poloxamer 188, dose volume 10 ml/kg s.c.

The only consistent effect was a temporary slight reduction in spontaneous activity during the first 30 min. after dosing with Liquid Norditropin 10 mg at 8 mg/kg. Two vehicle control mice showed slightly reduced spontaneous activity during the 0 to 30 minute interval after dosing. Degraded Liquid Norditropin 10 mg and Norditropin, 8 mg/kg administered as a single s.c. injection, produced no observable effects.

**Liquid Norditropin 10 mg: Assessment of Effects on Blood Pressure, Heart Rate, ECG and Respiration Following Single subcutaneous Administration to Anesthetized CD Rats.** Study 960481. 15 Apr 97 to 5 Feb 98. GLP compliant. 1.9/378

Effects of s.c. administered Liquid Norditropin (10 mg batch 31796022), degraded Liquid Norditropin (10 mg, batch 31796022), \_\_\_\_\_ and Norditropin (batch 67538R) were assessed on a number of cardiovascular and respiratory parameters in the anesthetized rat. Each of 5 rats (208-310 grams) per group received one of the 3 test compounds, the Vehicle for Liquid Norditropin or 0.9% w/v

sterile saline (batch 433-970227-60) containing Poloxamer 188. The groups received vehicle (1.2 ml/kg), Liquid Norditropin 10 mg at 8 mg/kg, Liquid Norditropin degraded at 8 mg/kg, Norditropin at 8 mg/kg and 0.9% w/v sterile saline at 1.2 ml/kg subcutaneously. Effects were noted for 40 minutes following the dose. [Positive test control - Adrenaline, Lot 117F0607 1 µg/kg (1.2 ml/kg) i.v.]

Results: Liquid Norditropin, degraded Liquid Norditropin and Norditropin given s.c. at a dose of 8 mg/kg produced no apparent biologically significant deviations in BP, HR, ECG(lead II), respiration rate or depth. 1 µg/kg adrenaline produced marked increases in BP.

**Liquid Norditropin 10 mg: Assessment of Effects on Renal Function Following Single Subcutaneous Administration to Rats. Study 960482 15 Apr 97 to 5 Feb 98. GLP compliant. 1:9/451**

The effects of s.c. administered Liquid Norditropin (10 mg, batch 31796022), degraded Liquid Norditropin (10 mg, batch 31796022) and Norditropin (67688R) on renal function were assessed in rats (8 males/group; 243-340 g). Vehicle Control for Liquid Norditropin 10 mg, batch 433-970227-60 contained Poloxamer 188, dose vol. 1.2 ml/kg s.c. Dosages of 0.08, 0.8 and 8 mg/kg were selected by the Sponsor on the basis of the 0.08 mg/kg being clinically relevant, with higher dosages as 10 and 100 multiples thereof. Propantheline bromide was used as a positive control.

Results: Somatropin is known to cause a retention of sodium and chloride ions. Liquid Norditropin and degraded Liquid Norditropin administered s.c. caused a dose-related sodium ion and chloride ion retention with a resultant decrease in urine volume. 8 mg/kg (the highest dose tested) Liquid Norditropin and degraded Liquid Norditropin caused effects which were very similar to that seen with Norditropin. A similar, although lesser, effect was seen at the lower dose levels of 0.8 and 0.08 mg/kg, where the decrease in sodium failed to reach a level of statistical significance when compared with the Vehicle Control group. Similar to Norditropin at a dose of 8 mg/kg and Liquid Norditropin 10 mg at 0.8 mg/kg, all three doses of Liquid Norditropin 10 mg degraded caused a statistically significant decrease in urinary pH when compared to the vehicle-control group.

at a dose of 5 or 10 mg/kg produced a decrease in sodium, potassium and chloride ion excretion similar in degree to that produced by Norditropin, Liquid Norditropin 10 mg or Liquid Norditropin 10 mg degraded each at 8 mg/kg.

**Characterization of Degraded Liquid Norditropin 10 mg Batch 31796022/Des15% for Preclinical Use. Report #: (?) Vol.1.9/46**

Degradation products formed in Liquid Norditropin preformulations have been studied, and have led to identification of degradation products formed after storage at corresponding to approximately

In general degradation products in degraded Liquid Norditropin were found in higher content in the degraded Liquid Norditropin batch than would be expected in the Liquid Norditropin formulation after at storage of 5°C. The content of degradation products in Liquid Norditropin, 10 mg, after at 5°C further verified this.

**Degradation products**

The degradation products

batch.

**1.0% > Degradation products:**

unknown degradation products exceeded in the degraded Liquid Norditropin batch.

**Degradation products**

and unknown degradation products, were detected at levels about in the degraded Liquid Norditropin batch.

and unknown degradation products, were detected at about in the Liquid Norditropin batch.

Fresh and degraded Liquid Norditropin were found to be bioequivalent.

The binding affinity (using IM-9 cells) of degraded Liquid Norditropin was found to be equal to that of the Somatropin standard.

No immune response was observed in the transgenic mouse following immunization with the degraded Liquid Norditropin.

Liquid Norditropin was stored for \_\_\_\_\_ with the purpose of getting a degraded product containing at least the \_\_\_\_\_ content estimated to be reached at the end of shelf life for the Liquid Norditropin product i.e. \_\_\_\_\_, at 5°C.

The degraded Liquid Norditropin batch 31796022 \_\_\_\_\_ used for preclinical studies corresponds to Liquid Norditropin 10 mg stored for \_\_\_\_\_ at 5°C which surpasses the desired end of shelf life for the product set at \_\_\_\_\_

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Sponsor's Tables Vol. 1.9/58

**Table 1** Degradation products in the degraded Liquid Norditropin batch present at \_\_\_\_\_ after storage for \_\_\_\_\_. For comparison the content of the products in Liquid Norditropin, 10 mg, after \_\_\_\_\_ are given.

Degradation product	Analysis	Degraded Liquid Norditropin, 10 mg	Liquid Norditropin 10 mg
	(SOP)	(%)	(%)

a Data from stability studies of Liquid Norditropin 10 mg stored for \_\_\_\_\_ Average Liquid Norditropin, 10 mg (#31696024, #31696026, #31696028)

b Sum of \_\_\_\_\_ that are not separated by this

c Calculated as the content of \_\_\_\_\_ determined by

**Table 2** Degradation products in the degraded Liquid Norditropin batch present at \_\_\_\_\_ of \_\_\_\_\_ after storage for \_\_\_\_\_. For comparison the content of degradation products in Liquid Norditropin, 10 mg, after \_\_\_\_\_ are give

Degradation product	Analysis	Degraded Liquid Norditropin 10 mg	Liquid Norditropin 10 mg
	(SOP)	(%)	(%)

a Data from stability studies of Liquid Norditropin 10 mg stored for \_\_\_\_\_ j. Average Liquid Norditropin, 10 mg (#31696024, #31696026, #31696028).

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Sponsor's Table Vol. 1.9/59

Table 3 Degradation products in the degraded Liquid Norditropin batch present at concentrations \_\_\_\_\_, after storage for \_\_\_\_\_ For comparison the content of these degradation products in Liquid Norditropin, 10 mg, after \_\_\_\_\_ are given.

Degradation product	Analysis	Degraded Liquid Norditropin, 10 mg	Liquid Norditropin 10 mg
	(SOP)	_____ (%)	_____ (%)

a Data from stability studies of Liquid Norditropin 10 mg stored for \_\_\_\_\_ Average of \_\_\_\_\_ batches of Liquid Norditropin, 10 mg (#31696024, #31696026, #31696028). \*: Preliminary identification results.

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**Non-clinical Toxicology:**

The overall toxicology profile for Norditropin (somatropin) has been documented in the approved NDA 19-721. In order to support the current filing for a liquid formulation, the sponsor has conducted additional toxicology studies to demonstrate that the difference in degradation profile would not introduce new toxicity.

In addition, the \_\_\_\_\_ degradation products, \_\_\_\_\_ degradation products and degraded Liquid Norditropin were biologically characterized by immunogenicity testing in mice transgenic to the human somatropin gene and in the control group of non-transgenic mice from the same offspring.

**Acute Toxicology:****Liquid Norditropin 10 mg, Forcedly Degraded. Subcutaneous Single dose Toxicity Study in Mice.**

Study 22197. 10 Sep 97. Novo Nordisk No. 970045.  
Batch 31796022/des 15% - Degradation was induced by keeping at \_\_\_\_\_ Vehicle  
Batch 433970114-60. Q.A - Present. Vol. 1.10/17

Three groups of 5M;5F \_\_\_\_\_ mice (17-19 g) received doses of either 0 (vehicle), 67 or 133 mg/kg. The mice were killed after the two weeks observation period.

Results: No clinical signs were seen and there were no deaths. Macroscopic examination at termination did not show any apparent treatment related pathological alterations.

**Multidose Toxicity Studies:****TOXICOLOGY**

**Study Title:** Liquid Norditropin 10 mg, Degraded - Three Month Subcutaneous Toxicity Study in the Rat.

**Study No:** \_\_\_\_\_ Study 22643; Novo Nordisk No. 970128

**Vol. # 1.10, and page #:** 37-306

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Dosing started on 3 Mar 97.

**GLP compliance:** Yes

**QA - Report** Yes (X) No ( )

**Methods:** The potential toxicity of Liquid Norditropin 10 mg, Degraded was assessed when administered daily by subcutaneous injection at doses of 0.08, 0.8, 8 mg/kg to rats for 3 months. The study also included Liquid Norditropin 10 mg and Norditropin PenSet.

**Dosing:**

- **Species / strain:** SPF Wistar rats from \_\_\_\_\_
- **#/sex/group or time point:** 10M;10F per group
- **Age:** 5 - 6 weeks
- **Weight:** Males - 118 to 143 g; Females - 96 to 124 g
- **Dosage groups in administered units:** Groups 1-6; Group 1 - Vehicle for Liquid Norditropin 10 mg; Groups 2-4 - 0.08, 0.8, 8 mg/kg Liquid Norditropin 10 mg, Degraded; Group 5 - Liquid Norditropin 10 mg - 8 mg/kg; Group 6 - Norditropin, PenSet 24, 8 mg/kg.
- **Route, form, volume, and infusion rate if (i.v.):** Subcutaneously in the dorsal part of the neck at a volume of 1.2 ml/kg.

**Drug, lot # :** Liquid Norditropin 10 mg - 31796022,  
Vehicle - Batch 433-970227-60

**Formulation/vehicle:** The test article was Liquid Norditropin 10 mg, Degraded, Batch 31796022' \_\_\_\_\_ with 10 mg somatropin in 1.5 ml solution. [Degradation was induced by keeping the test article at \_\_\_\_\_

The composition of Liquid Norditropin 10 mg was:  
Somatropin (hGH) \_\_\_\_\_

Phenol  
 Poloxamer 188  
 Mannitol

Positive control: Norditropin, PenSet 24 (freeze dried)  
 Labeled Norditropin 28 IU  
 Batch 67538 and 67586

The composition of the freeze dried preparation after reconstitution with distilled water was:

Somatropin (hGH)

Mannitol

**Observation and times:** Samples of test formulations were taken on the first day of dosing and after 1 month and at end of treatment. [Further samples should have been taken after 2 months' treatment, but this was not done, and a technician was found culpable of having falsified study records to indicate samples had been taken. Terminal samples were frozen and shipped to the sponsor three weeks later.]

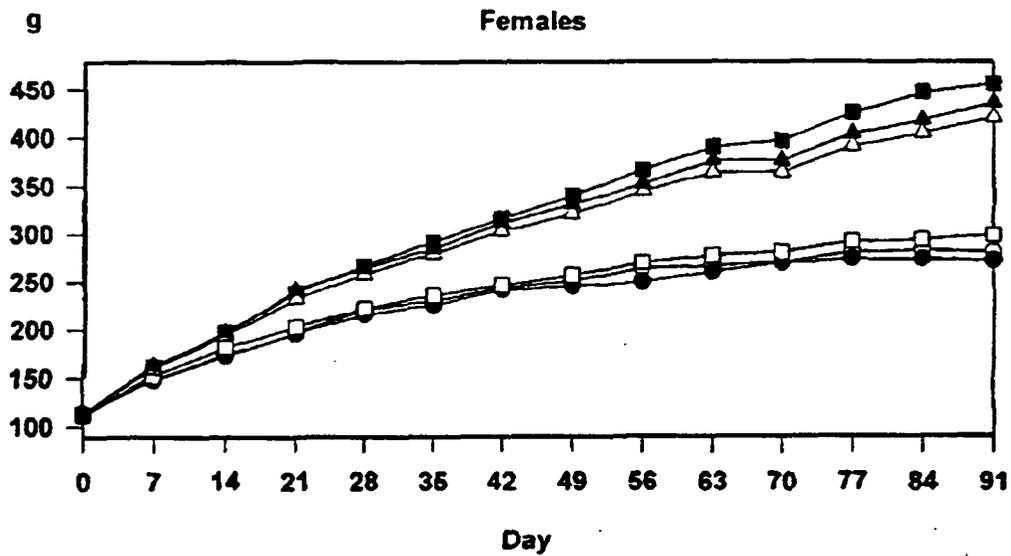
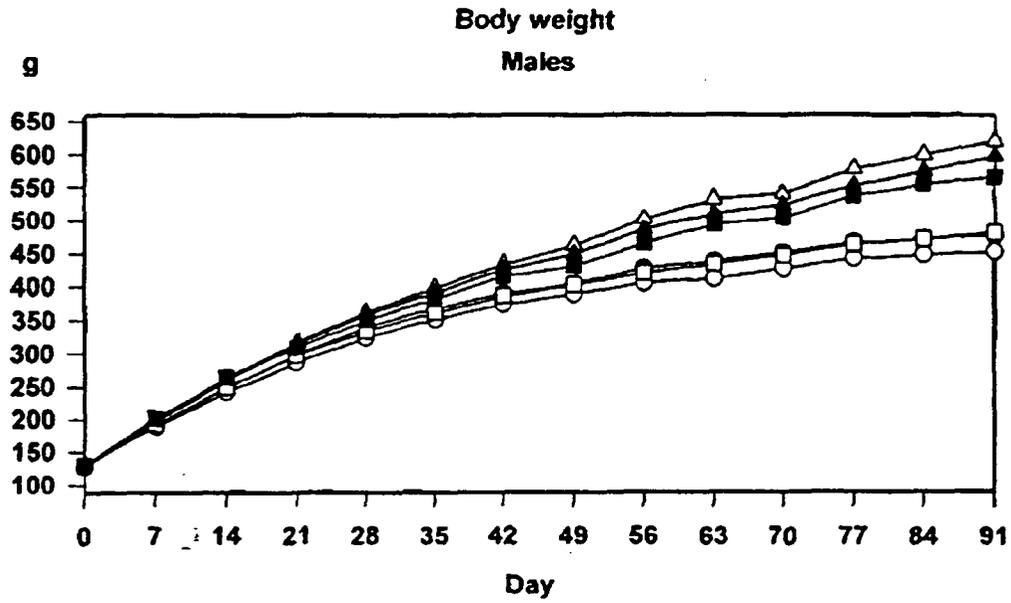
Blood samples for hematology, clinical chemistry and blood glucose were drawn from the orbital venous plexus during CO<sub>2</sub> anesthesia.

- **Clinical signs:** Observation period not stated.
- **Body weights:** On arrival and once weekly during the acclimatization and treatment periods.
- **Food consumption:** Weekly.
- **Ophthalmoscopy:** Before start of dosing. Before termination of dosing all animals in Groups 1 and 4 were re-examined.
- **Hematology:** Samples were drawn from the orbital venous plexus during CO<sub>2</sub> anesthesia during the week before termination of treatment.
- **Clinical chemistry:** Blood samples for clinical chemistry and blood glucose were drawn from the orbital venous plexus during CO<sub>2</sub> anesthesia during the week before termination of treatment.
- **Urinalysis:** Shortly before termination of treatment urine samples from all animals were collected overnight while the rats were in metabolism cages.
- **Organ Weights:** At necropsy.
- **Gross pathology:** At necropsy.
- **Organs weighed:** See histopathology chart on page 20.
- **Histopathology:** All tissues from all control rats (Gp 1) and high dose animals (Gp 4). All tissues from all animals dying or killed during study. All gross lesions and all injection sites from all animals. See histopathology chart on page 20.

#### Results:

- **Clinical signs:** No treatment related clinical signs were seen and there were no unscheduled deaths.
- **Body weights:** Body weights of both sexes and body weight gains were increased from day 7 of the study in Groups 4, 5 and 6 (8 mg/kg/day: Liquid Norditropin 10 mg Degraded, Liquid Norditropin 10 mg, Norditropin, PenSet 24). The lower dose levels of Liquid Norditropin 10 mg, Degraded showed no significant differences compared with controls. Body weight gain of both sexes adjusted group mean values Day 91 (body weight day 0 used as covariant), Groups 1-6 (S.E.M. 12.6) = 368.3, 376.1, 392.4, 512.0, 521.9, 517.0.

Sponsor's Figures Vol. 1.10/62:



- |           |           |
|-----------|-----------|
| ○ Group 1 | ● Group 2 |
| □ Group 3 | ■ Group 4 |
| △ Group 5 | ▲ Group 6 |

- **Food consumption:**

Food consumption was increased from the second week of treatment in Groups 5 and 6 (8 mg/kg/day: Liquid Norditropin, 10 mg and Norditropin, PenSet 24). From 6 weeks on there was a statistically significant increase in Group 4 (8 mg/kg/day, Liquid Norditropin 10 mg, Degraded). Lower dose levels of Liquid Norditropin 10 mg, Degraded showed no consistent effects. The food conversion ratio showed effects of treatment indicating that the effects on body weight and food consumption were directly related. However, some isolated statistically significant differences were noted. Food consumption group mean values per animal per cage (g) Total weeks 1-13 Groups 1-6: Males: 2330.7, 2477.1, 2476.9, 2907.5, 3006.2, 3039.3, respectively; Females: 1891.8, 1776.7, 1745.5, 2464.0, 2161.5, 2352.4, respectively.

- **Ophthalmoscopy:** Since no effects of treatment were seen in Group 4 eyes, eyes of the other treated groups were not examined.

- **Hematology:**

**Males –** Males treated with Liquid Norditropin 10 mg, degraded Liquid Norditropin 10 mg and Norditropin PenSet 24 (Groups 4, 5 and 6) showed statistical reductions in red blood cell count (7.90, 7.79, 7.78 vs 8.77 for controls) and packed cell volume (45.7, 45.2, 46.1 vs 48.4 for controls).

Significantly reduced hemoglobin concentrations for males were seen for Liquid Norditropin 10 mg at 8 mg/kg/day (Group 5; 9.4 vs 10.1 for controls) and Degraded Liquid Norditropin 10 mg at 8 mg/kg/day (Group 4; 9.5 vs 10.1). Male MCV values for Groups 4, 5, 6 were statistically increased (58.0, 58.1, 59.3 vs 55.0 for controls).

Mean male thrombocyte values were significantly increased for Groups 4, 5 and 6 (882, 957, 945 vs 820 for controls).

**Females –** Groups 4, 5, and 6 showed increases in mean corpuscular volume (58.6, 59.1, 58.4 vs 57.2 for controls) and increases in mean white blood cell count (Groups 5 and 6 = 12.1, and 14.1 vs 8.6 for controls) – differences primarily due to increased numbers of lymphocytes which were significantly increased in Groups 4, 5 and 6 (10.5, 10.5, 12.3 vs 7.1 in controls). Females of Groups 4, 5 and 6 also showed increased fibrinogen concentrations (2.90, 2.73, 2.86 vs 2.02).

The numbers of thrombocytes for Groups 4, 5 and 6 were statistically increased (897, 885, 889 vs 805 for controls).

Liquid Norditropin 10 mg, Degraded showed no treatment effects at the lower dose levels.

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- **Clinical chemistry:**

Groups 4, 5 and 6 (8 mg/kg/day: Liquid Norditropin 10 mg, Degraded Liquid Norditropin 10 mg, and Norditropin, PenSet 24) showed a slight significant reduction in circulating chloride concentrations (males: 106.5, 106.9, 106.5 vs 107.8 for controls; females: 106.7, 106.8, 106.3 vs 108.8). Also there was a significant increase in phosphorus concentrations in females of these groups (2.67, 2.50, 2.69 vs 2.15). Protein concentrations showed some variations. The percent Albumin was slightly significantly reduced in females from Groups 5 and 6 (56.3 and 56.4 vs 60.2) and  $\beta$ -globulins were significantly increased in Group 4 and 5 males [13.03 (18.8%), 13.25 (18.7%) vs 11.02 (16.7%)] and females [12.69 (18.4%), 12.22 (17.9) vs 11.19 (15.9%)]. The female A/G ratio was significantly decreased in Groups 5 and 6 (1.30, 1.30 vs 1.52). Other changes appeared to be isolated statistically significant differences.

- **Urinalysis:**

Significantly lower activities of  $\gamma$ -glutamyl transferase were seen for both sexes of Groups 4, 5 and 6 [males: 2.195, 1.709, 2.243, vs 2.732 for controls; females; 1.62, 1.132, 1.137 vs 2.116 for controls]. Urine volumes were significantly increased for Group 5 males (22.2 vs 12.6) and females (18.6 vs 16.0). Other changes appeared to be isolated differences and not associated with treatment.

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**- Organ Weights:**

The mean weights of the majority of organs from rats in Groups 4, 5 and 6 were increased in general in agreement with effects on bodyweight. Liquid Norditropin 10 mg, Degraded showed no effects of treatment at lower doses.

Adjusted mean organ weight calculations showed the only consistent effects of treatment to be increased spleen weights in both sexes of Groups 4, 5 and 6 and male adrenal weights for these groups.

Sponsor's Tables Vol. 1.10/102,103

**Males**

GROUP	BODY WT, g				ADRENALS				BRAIN				HEART				KIDNEYS			
	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p
1	456.0	63.1	10		46.4	8.0	10		2745.3	121.9	10		1404.5	163.1	10		2768.7	355.4	10	
2	478.1	69.4	10		56.7	11.9	10		2183.7	117.4	10		1426.4	175.4	10		2826.0	362.8	10	
3	484.5	25.9	10		56.1	9.3	10		2156.1	85.3	10		1383.5	147.4	10		2863.4	331.5	10	
4	572.4	60.4	10	**	85.3	16.2	10	**	2292.3	93.3	10	**	1640.9	200.9	10	**	3226.6	394.8	10	**
5	625.7	54.4	10	**	96.5	12.2	10	**	2324.2	79.6	10	**	1755.2	187.1	10	**	3386.3	516.9	10	**
6	602.1	52.8	10	**	84.7	21.3	10	**	2310.5	116.2	10	**	1682.6	125.5	10	**	3330.8	376.1	10	**

GROUP	LIVER				LUNGS#				PITUITARY				PROSTATE				SPLEEN			
	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p
1	14850	3557	10		.	.	0		11.8	2.1	10		761.4	128.7	10		728.8	137.4	10	
2	16136	3380	10		.	.	0		12.7	2.4	10		725.6	205.2	10		793.8	133.6	10	
3	16185	1891	10		.	.	0		12.0	1.7	10		646.3	173.9	10		644.9	199.1	10	
4	21232	4461	10	**	.	.	0		11.8	2.9	10		847.5	293.5	10		1111.9	166.6	10	**
5	22503	3782	10	**	.	.	0		11.4	2.0	10		848.9	276.1	10		1166.6	172.2	10	**
6	20573	2464	10	**	.	.	0		13.1	2.5	10		927.3	259.1	10		1124.8	122.4	10	**

GROUP	TESTES				THYMUS				THYROIDES			
	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p
1	3125.8	245.6	10		336.1	61.2	10		20.6	3.9	10	
2	3270.5	298.6	10		337.9	99.6	10		21.0	3.5	10	
3	3330.8	205.7	10		367.0	90.1	10		22.4	4.6	10	
4	3482.0	212.8	10	**	445.6	153.2	10	**	22.5	5.4	10	
5	3546.8	245.4	10	**	491.2	129.0	10	**	25.2	3.9	10	**
6	3591.3	201.2	10	**	427.2	98.7	10	**	24.9	4.5	10	**

\* means p<0.05, versus control group

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

# The weights of the lungs of males were inadvertently not recorded.

Females

GROUP	BODY WT. g				ADRENALS				BRAIN				HEART				KIDNEYS			
	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p
1	284.7	35.4	10		59.4	7.8	10		1996.8	79.2	10		961.5	101.6	10		1708.4	153.3	10	
2	273.9	21.2	10		59.8	8.0	10		1986.9	47.7	10		933.5	77.1	10		1658.5	110.4	10	
3	300.0	88.9	10		63.2	10.4	10		2016.1	98.8	10		984.7	144.6	10		1770.3	271.2	10	
4	466.9	45.1	10	**	78.6	9.6	10	**	2179.8	89.5	10	**	1326.7	175.5	10	**	2267.1	198.3	10	**
5	427.0	84.1	10	**	74.1	21.5	10		2135.3	139.2	10	**	1251.5	290.7	10	**	2227.9	414.4	10	**
6	447.9	87.2	10	**	77.0	16.4	10	**	2105.7	107.1	10	**	1277.8	177.5	10	**	2367.8	400.6	10	**

GROUP	LIVER				LUNGS				OVARIES				PITUITARY				SPLEEN			
	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p
1	8541	741	10		1306.5	134.2	10		107.8	24.1	10		14.7	1.6	10		513.6	79.8	10	
2	8405	1041	10		1252.0	136.5	10		107.8	18.4	10		15.3	2.3	10		477.5	64.6	10	
3	9150	2038	10		1310.8	196.9	10		112.5	26.8	10		14.6	2.2	10		545.5	98.1	10	
4	17132	2303	10	**	1741.8	190.8	10	**	135.1	32.0	10		15.3	1.4	10		881.8	132.1	10	**
5	15250	3566	10	**	1675.7	239.5	10	**	143.5	43.1	10	*	14.4	2.5	10		857.6	178.9	10	**
6	16870	3211	10	**	1666.7	230.5	10	**	138.0	33.6	10		14.4	2.4	10		965.4	128.5	10	**

GROUP	THYMUS				THYROIDS				UTERUS			
	MEAN	S.D	N	p	MEAN	S.D	N	p	MEAN	S.D	N	p
1	300.2	68.7	10		17.0	3.3	10		697.0	141.2	10	
2	301.5	52.5	10		17.3	5.1	10		680.9	122.8	10	
3	329.3	76.6	10		18.6	3.8	10		657.5	142.6	10	
4	507.4	154.9	10	**	22.1	4.3	10		650.8	152.5	10	
5	451.4	98.8	10	**	22.4	6.2	10	**	651.0	186.0	10	
6	562.2	130.2	10	**	21.4	4.1	10	**	583.0	129.6	10	

\* means p<0.05, versus control group  
 \*\* means p<0.01, versus control group  
 S.D. = standard deviation N = number of animals

- **Gross pathology:** Various animals from all groups showed marked subcutaneous hemorrhages at injection sites. There were no differences in incidence and severity between vehicle group animals and rats treated with either degraded or undegraded Liquid Norditropin 10 mg (Groups 2, 3, 4 and 5). The Norditropin, PenSet 24 group (6) showed hemorrhages to be slight and a lower number of animals to be affected. A few rats in all groups showed hemorrhages in the lung, mandibular lymph nodes and thymus possibly related to blood sampling procedures.
- **Organs weighed:** See histopathology chart on page 20.
- **Histopathology:** Mammary glands showed treatment related changes in both sexes in Group 4 (8 mg/kg/day Liquid Norditropin 10 mg, Degraded). Mammary glandular hyperplasia was seen in 7/10 males and in all females. All females in Group 4 were in the same estrous cycle i.e. diestrus. General findings at injection sites were in general subcutaneous hemorrhage, fibrosis, necrosis and inflammatory cell infiltration. There did not appear to be any differences in incidence and severity in vehicle animals and in rats treated with liquid and Norditropin 10 mg, Degraded (Groups 2, 3 and 4) nor in Group 5 rats treated with Liquid Norditropin 10 mg.. The incidence and severity of subcutaneous hemorrhage and the incidence of subcutaneous necrosis were lower in the PenSet 24, (Group 6).

It is reported that the sinusoidal hemorrhages found in the mandibular lymph node represent absorption of blood extravasated from the retrobulbar venous plexus following collection of blood samples. Blood sampling was also possibly responsible for alveolar hemorrhages in the lung and interstitial hemorrhages in the thymus. Other findings were minor and incidental to this age and strain of rats.

**Key Study Findings:** None for Liquid Norditropin 10 mg, Degraded at dose levels of 0.08 and 0.8 mg/kg/day. Effects of treatment were similar to those seen in rats treated with 8 mg/kg/day Liquid Norditropin 10 mg or 8 mg/kg/day Norditropin, PenSet 24 – however, histopathology was not performed for these groups.

**Overall Toxicology Summary:** The parameters assessed showed few adverse effects on male or female rats following subcutaneous administration of Liquid Norditropin 10 mg, Degraded for 3 months at dose levels of 0.08 and 0.8 mg/kg/day.

Liquid Norditropin 10 mg, Degraded administered s.c. at 8 mg/kg/day produced increased weight gain and food consumption. Also seen were a number of hematological and clinical chemistry parameters. Organ weights were increased. Those not directly associated with body weight were male adrenals and the spleen of both sexes. All females were in diestrus and histopathology showed glandular hyperplasia in the mammary glands of both sexes. In general effects of treatment were similar to those seen in rats treated with 8 mg/kg Liquid Norditropin 10 mg or 8 mg/kg/day Norditropin, PenSet 24 - however, histopathology was not done on these groups.

**Note:** The analyzed content was in good correlation with the expected content except for the sample from Group 6 (31 Mar 97) which was too low (5.8 mg/ml vs 6.75 mg/ml expected). The sponsor had no explanation for this finding for the positive control Norditropin PenSet 24 (freeze-dried).

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## Histopathology Inventory for IND

Study	970128 3 mo. S.C.			
Species	Rat			
Adrenals	X*			
Aorta	X			
Bone Marrow smear	O			
Bone (femur)	X			
Brain	X*			
Cecum	X			
Cervix	O			
Colon	X			
Duodenum	X			
Epididymis	X			
Esophagus	X			
Eye	X			
Fallopian tube	O			
Gall bladder	O			
Gross lesions	X			
Harderian gland	O			
Heart	X*			
Hypophysis	O			
Ileum	X			
Injection site	X			
Jejunum	X			
Kidneys	X*			
Lachrymal gland	O			
Larynx	O			
Liver	X*			
Lungs	X* #			
Lymph nodes, cervical	O			
Lymph nodes mandibular	X			
Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity	O			
Optic nerves	O			
Ovaries	X*			
Pancreas	X			
Parathyroid	X*			
Peripheral nerve	O			
Pharynx	O			
Pituitary	X*			
Prostate	X*			
Rectum	X			
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	O			
Skin	X			
Spinal cord	X			
Spleen	X*			

Sternum	X			
Stomach	X			
Testes	X*			
Thymus	X*			
Thyroid	X*			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	X*			
Vagina	X			
Zymbal gland	O			

X = Tissues sampled. \* organ weight obtained.  
 # Due to accidental oversight, lungs of males were not weighed. O = omitted

**SPECIAL TOXICOLOGY STUDIES**

**Study Title:** Liquid Norditropin 10 mg Testing for Local Tissue Irritation after Intramuscular Injection in the Rabbit.

**Study No:** Novo Nordisk No. 960514

**Vol. 1.11 page 1:**

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Dosing took place on 27 Dec 96.

**GLP compliance:** Yes

**QA - Reports** Yes (X) No ( ):

**Methods:** The objective of the study was to assess the potential toxic effect in muscle tissue of an injectable preparation Liquid Norditropin 10 mg administered by intramuscular injection to the right longissimus dorsi muscle of rabbits. Needle: \_\_\_\_\_

The toxicity was quantified in terms of loss of creatine kinase (CK) from injection site muscle tissue.

26 SPF male albino New Zealand White rabbits weighing 1.9 to 2.1 kg were randomly allocated to three groups. Pelleted diet: \_\_\_\_\_

Rabbits were sacrificed 3 days after the injection. Sections of muscle were removed from Groups 1 and 2 and the changed area was isolated by careful dissection from the surrounding muscle tissue. Group 3 animals were examined histologically.

**Dosing:**  
 Group 1: 0.9% NaCl 1 ml  
 Group 2: Liquid Norditropin 10 mg 1 ml  
 Group 3: Liquid Norditropin 10 mg 1 ml  
 And 0.9% NaCl 1 ml

[The rabbits in Group 3 were injected with 1 ml 0.9% NaCl solution in one dorsal longissimus muscle and with 1 ml Liquid Norditropin 10 mg in the other one.]

**Drug Batch No.** 31796022, **TKS No.** 649. **Expiry date** 24 Jun 97.

**Formulation/vehicle:** Liquid Norditropin 10 mg in 1.5 ml cartridges.

The composition of the aqueous solution was:

Somatropin (hGH)	
Phenol	
Poloxamer 188	
Mannitol	

Vehicle: 0.9% NaCl, Batch 96C06S05 - Pharmacia

**Observations and times:** Rabbits were sacrificed 3 days after the injection. Sections of muscle were removed from Groups 1 and 2 and the changed area was isolated by careful dissection from the surrounding muscle tissue. Group 3 animals were examined histologically.

**Results:**

**Macroscopic Findings:** Only Muscle tissue at the injection site was examined.

Group 1 (0.9% NaCl): 5 rabbits showed a slight hemorrhage; 5 rabbits showed no changes.

Group 2 (Liquid Norditropin 10 mg. 1 ml): 1 rabbit showed moderate hemorrhage; 7 rabbits showed slight hemorrhage; 2 rabbits had no changes.

Group 3: 1 rabbit showed moderate hemorrhage and 5 rabbits had slight hemorrhage after injection of Liquid Norditropin 10 mg (1 ml). 2 rabbits had slight hemorrhage and no changes were seen after injection of 0.9% NaCl (1 ml) in 4 rabbits.

**Microscopic Findings:** Findings were mainly caused by the needle and related to the needle canal. Findings in injection site muscle injected with Liquid Norditropin 10 mg or 0.9% NaCl included hemorrhage, inflammation, fibroblast proliferation and myocyte necrosis.

**Muscle Tissue Depleted of CK Activity:** No depletion of CK from injection site muscle tissue was seen after injection of 1 ml Liquid Norditropin 10 mg or 1 ml 0.9% NaCl.

**Summary:**

Liquid Norditropin 10 mg (1 ml) produced slight to moderate hemorrhage macroscopically. Muscle tissue injected with 0.9% NaCl (1 ml) showed slight hemorrhage. Microscopically muscle tissue injected with Liquid Norditropin 10 mg and 0.9% NaCl showed no differences and changes seen were those caused by the needle.

**Key finding(s):** There was no significant difference in relation to CK depletion between Group 1 and Group 2. Neither group showed depletion of CK activity from injection site muscle tissue.

## IMMUNOTOXICOLOGY

**Study title:** Transgenic Human Somatropin Mice Used as an Immunogenicity Model.

**Study No:** 27255 Vol. 1.11/ 21

**Site and testing facility:** Novo Nordisk

**GRP compliance:** NA

**QA – Report:** Yes ( ) No (X):

**Lot and batch numbers:** Human Somatropin, Batch B-hGH,1986 or Porcine Somatropin Batch ?

**Methods:**

- **Species/strain:** F1-hybrids of strain 3.2 and Balb/c mice carrying the somatropin gene were selected for the transgenic group, whereas littermates not having the gene were selected for the non-transgenic groups.
- **Doses employed:** At day 0 mice were immunized subcutaneously with 20 µg of the preparation emulsified in Complete Freund's Adjuvant and boosted at day 21 and 41 with the same preparation, but now emulsified in Incomplete Freund's Adjuvant.
- **Route of Administration:** subcutaneous
- **Rationale:** In the development of Liquid Norditropin, it was expected that the final formulation would contain several naturally occurring degradation products of Somatropin. The sponsor wished to examine whether any of these degradation products could induce an immunological response to Somatropin.
- **Number of animals/sex/dosing group:** 6-8 transgenic or 6-8 non-transgenic animals per group. Sex unknown.



- **Endpoints:** The immune response towards Somatropin was monitored by the immunogenicity model using somatropin transgenic mice. The amount of specific Somatropin-antibodies after three injections of \_\_\_\_\_ was measured in a specific ELISA-assay. Non-transgenic littermates served as positive controls of the immunization protocol.
- **Observations:** \_\_\_\_\_ did not induce an antibody response in the Somatropin-transgenic mice as it did in the non-transgenic littermates.
- **Timing:** . Day 0 and boosted on Days 21 and 41 Blood samples were taken before the first immunization and again ten days after the two booster injections.

**Overall Summary:**

It is expected that the final formulation of Liquid Norditropin will contain several naturally occurring degradation products of Somatropin. A immunogenicity model using somatropin transgenic mice was used to measure the immune response towards Somatropin. The amount of specific Somatropin antibodies was measured in a specific ELISA-assay after 3 injections of \_\_\_\_\_ Positive controls were non-transgenic littermates. \_\_\_\_\_ did not induce an antibody response in Somatropin transgenic mice. An antibody response was induced in non-transgenic littermates.

The following Immunogenicity Studies were conducted by Novo Nordisk according to the methods used in Study 27278 Immunogenicity of \_\_\_\_\_ Tested in Transgenic Somatropin Mice above.

**Immunogenicity Studies:**

Somatropin Degradation Product	Study #	Lot/Batch	Antibody Response Transgenic Mice	AntibodyResponse Non-Transgenic Littermates
_____	27281 Vol. 1.11/46	13 December 1995 JHMe/HHS	Negative	Positive
_____	27283 Vol. 1.11/56	13 December 1995 JHMe/HHS	Negative	Positive
_____	26859 Vol. 1.11/66	GLP FP 980026 3/11-1998 (glas 3+5)	Negative	Positive
_____	27261 Vol. 1.11/76	BhHG-AM-44d/ThC	Negative	Positive
_____	27285 Vol. 1.11/86	BhGH-AM-42h/ThC	Negative	Positive
_____	27291 Vol. 1.11/96	Bwel 01-10-1997/Bwel	Negative	Positive
_____	27288 Vol. 1.11/106	31796022 — — ,B1Ha/KiEb	Negative	Positive

## PHARMACOKINETICS/TOXICOKINETICS

### Norditropin SimpleXx – Nonclinical Pharmacokinetics (Liquid Norditropin) Vol. 1.11/116

A preclinical bioequivalence study was conducted with Liquid Norditropin and lyophilized Norditropin (designated here as Norditropin, and a preclinical bioequivalence study with Liquid Norditropin and degraded Liquid Norditropin, and preclinical pharmacokinetic studies with degradation products of somatotropin which are present in Liquid Norditropin after storage. The latter studies focused on ~~\_\_\_\_\_~~ Doses ranged from 50-100  $\mu\text{g}/\text{kg}$  following i.v. administration and 150-450  $\mu\text{g}/\text{kg}$  following s.c. administration. According to the sponsor, doses in the preclinical studies were ca 3-225 fold higher compared with the recommended clinical dose (ca 2-50  $\mu\text{g}/\text{kg}/\text{day}$ ).

Plasma concentration was determined by an ELISA procedure. Thus, all concentrations and pharmacokinetic parameters cannot be related specifically to somatotropin or degradation products of somatotropin but are related to the total plasma content of somatotropin and degradation products.

The plasma concentration of somatotropin increased rapidly following s.c. adm. And  $C_{\text{max}}$  was seen at 30-45 min after injection ( $t_{\text{max}}$ ). Different studies with different doses did not show dose dependency in  $t_{\text{max}}$ . Following a single s.c. dose,  $t_{1/2}$  varied between 48 and 79 min.

In general, there were no differences between Norditropin and Liquid Norditropin, Liquid Norditropin and degraded Liquid Norditropin, or any of the degradation products (~~\_\_\_\_\_~~) and the reference somatotropin.

Thus it would appear that in the rat the pharmacokinetics of somatotropin and the degradation products of somatotropin were similar.

Pharmacokinetics of reference:  
hGH = human Growth Hormone,

~~\_\_\_\_\_~~  
HT (1.2) = Main peak after ~~\_\_\_\_\_~~ separation of the ~~\_\_\_\_\_~~ forms ~~\_\_\_\_\_~~ in the temperature degraded hGH sample.

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## 2 Tabulated Summary for Nonclinical ADME studies.

Type of study	Species	Strain	Number/ group	Route	Dose level(s) (µg/kg)	Batch No.	Laboratory	Parameters studied	Results	Study no.
Pharmacokinetics after single dose	Rats	Male Wistar	4/time pt. 6/time pt.	IV SC	50 150	N/A	Applicant	$t_{1/2}$ , CL, $V_d$ , $V_{d\beta}$ , AUC(0-∞) and $f$	degradation products of somatropin (r somatropin control and reference somatropin were dosed iv and sc. The pharmacokinetic parameters for the four somatropins were similar. Hence, the data indicates that the pharmacokinetics of the two deamidated somatropins does not differ from somatropin.	940516
Pharmacokinetics after single dose	Rats	Male Wistar	4/time pt.	SC	450	N/A	Applicant	$C_{max}$ , $t_{max}$ , $t_{1/2}$ , AUC and MRT	Liquid Norditropin and degraded Liquid Norditropin were dosed sc. The ratios for AUC, $C_{max}$ and $t_{max}$ were 0.97, 1.2 and 1, respectively. Based on these findings, the conclusion is that Liquid Norditropin and degraded Liquid Norditropin were bioequivalent in the rat.	960492

Type of study	Species	Strain	Number/group	Route	Dose level(s) (µg/kg)	Batch No.	Laboratory	Parameters studied	Results	Study no.
Pharmacokinetics after single dose	Rats	Male Wistar	4/time pt.	IV SC	75 250	N/A	Applicant	$C_{max}$ , $t_{1/2}$ , $Cl_r$ , $V_d$ , $V_{1/2}$ , $t_{max}$ , AUC, MRT and $f$	_____ somatropin control and reference somatropin were dosed iv and sc. The findings for _____ were comparable with the findings for somatropin control and reference somatropin. Hence, the pharmacokinetics of _____ did not differ from somatropin.	970055
Pharmacokinetics after single dose	Rats	Male Wistar	4/time pt.	IV SC	75 250	N/A	Applicant	$C_{max}$ , $t_{1/2}$ , $Cl_r$ , $V_d$ , $V_{1/2}$ , $t_{max}$ , AUC, MRT and $f$	_____ somatropin control and reference somatropin were dosed iv and sc. The findings for _____ were comparable with the findings for somatropin control and reference somatropin. Hence, the pharmacokinetics of _____ did not differ from somatropin.	970056

Type of study	Species	Strain	Number/ group	Route	Dose level(s) ( $\mu\text{g}/\text{kg}$ )	Batch No.	Laboratory	Parameters studied	Results	Study no.
Pharmacokinetics after single dose	Rats	Male Wistar	4/time pt.	IV SC	100 300	N/A	Applicant	$C_{max}$ , $t_{1/2}$ , $CL$ , $V_d$ , $V_{d\beta}$ , $t_{max}$ , AUC, MRT and $f$	Liquid Norditropin and Norditropin® were dosed sc and somatropin were dosed iv. The ratios for AUC, $C_{max}$ and $t_{max}$ were 1.08, 0.94 and 1.00, respectively. Thus, based on these parameters the conclusion is that the two different formulation of somatropin were bioequivalent in the rat.	970069

Sponsor's Tables Vol. 1.11/125

Summary of the pharmacokinetic parameters after intravenous administration:

			Peak	Ref hGH
t <sub>1/2</sub> (min)	10.1	17.2	18.5	12.6
CL (ml/min)	11.5	14.5	16.7	17
V <sub>d</sub> (l)	0.168	0.362	0.444	0.307
V <sub>ss</sub> (l)	0.105	0.191	0.212	0.180
AUC(0-∞) (ng·min/ml)	4332.2	3438.7	2999.5	2949.2

Summary of pharmacokinetic parameters after subcutaneous administration:

			Peak	Ref hGH
t <sub>1/2</sub> (min)	61.6	64.1	76.9	78.7
V <sub>d</sub> (l)	1.5	1.31	3.12	1.97
AUC(0-∞) (ng·min/ml)	8893.3	10561.4	5344.0	8667.2
f (%)	68.4	102.4	59.4	98.0

Sponsor's Table Vol. 1.11/126:

Pharmacokinetic parameter	Liquid Norditropin s.c. administration Dose: 450 µg/kg	Degraded Liquid Norditropin s.c. administration Dose: 450 µg/kg
C <sub>max</sub> (ng/ml)	107.6 ± 14.4	89.7 ± 21.2
t <sub>max</sub> (min)	45	45
t <sub>1/2</sub> (min)	57	58
AUC (ng · min/ml)	15856 (1.9%)*	16304 (2.1%)*
MRT (min)	128	129

\*The figures in brackets are the percentage of the AUC estimated by extrapolation from last data point to infinity.

Sponsor's Table Vol. 1.11/127:

The pharmacokinetic parameters for somatropin following administration of   
 somatropin  peak and pure reference somatropin

PK parameter	I.v. administration. Dose: 75 $\mu\text{g}/\text{kg}$			S.c. administration. Dose: 250 $\mu\text{g}/\text{kg}$		
	<del>          </del>	<del>          </del> peak	Reference	<del>          </del>	<del>          </del> peak	Reference
$C_{max}$ (ng/ml)	-	-	-	62	61	37
$t_{max}$ (min)	-	-	-	30	30	45
$t_{1/2}$ (min)	8	8	8	69	51	59
AUC (ng min/ml)	4974	4982	4543	8500	6957	4966
CL (ml/min/kg)	15	15	17	-	-	-
$Y_r$ ( $\bar{l}/\text{kg}$ )	0.18	0.18	0.19	-	-	-
$V_d$ (l/kg)	0.10	0.10	0.10	-	-	-
MRT (min)	7	7	6	121	100	107
f(%)	-	-	-	51	42	33

Sponsor's Table Vol. 1.11/128:

The pharmacokinetic parameters for somatropin following administration of   
 peak and pure somatropin reference.

PK parameter	I.v. administration. Dose: 75 $\mu\text{g}/\text{kg}$			S.c. administration. Dose: 250 $\mu\text{g}/\text{kg}$		
	<del>          </del>	<del>          </del> peak	Reference	<del>          </del>	<del>          </del> peak	Reference
$C_{max}$ (ng/ml)	-	-	-	39	47	55
$t_{max}$ (min)	-	-	-	45	45	30
$t_{1/2}$ (min)	8	8	8	55	55	64
AUC (ng min/ml)	4604	6092	5787	5175	5851	7129
CL (ml/min/kg)	16	12	13	-	-	-
$V_d$ (l/kg)	0.19	0.14	0.14	-	-	-
$V_d$ (l/kg)	0.11	0.07	0.08	-	-	-

## Sponsor's Tables (2) Vol. 1.11/129:

PK parameter	I.v. administration. Dose: 75 µg/kg			S.c. administration. Dose: 250 µg/kg		
		 peak	Reference		 peak	Reference
MRT (min)	6	6	6	100	96	112
f (%)	-	-	-	34	29	37

Pharmacokinetic parameter	Group 1	Group 2	Group 3
	Somatropin i.v.. administration	Liquid Norditropin s.c. administration	Norditropin® s.c. administration
	Dose: 100 µg/kg	Dose: 300 µg/kg	Dose: 300 µg/kg
C <sub>max</sub> (ng/ml)	-	76.6	81.7
t <sub>max</sub> (min)	-	45	45
t <sub>1/2</sub> (min)	44	49	78

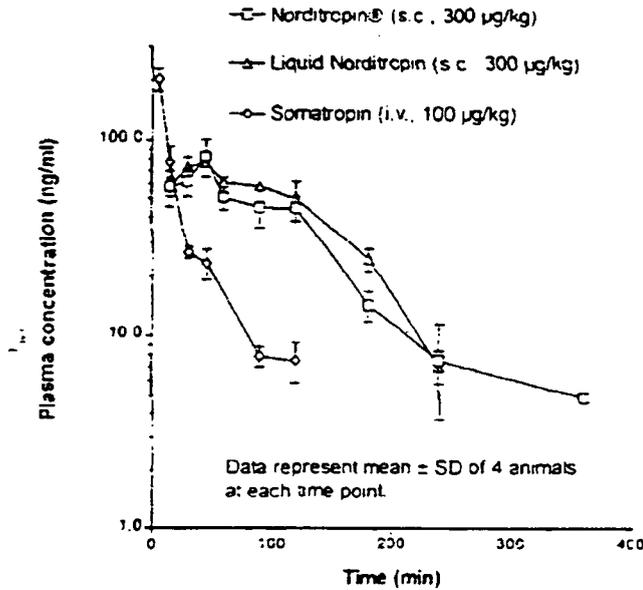
## Sponsor's Table Vol. 1.11/130

Pharmacokinetic parameter	Group 1	Group 2	Group 3
	Somatropin i.v.. administration	Liquid Norditropin s.c. administration	Norditropin® s.c. administration
AUC (ng · min/ml)	5278 (8.8%)*	10588 (4.7%)*	9833 (5.6%)*
CL (ml/min/kg)	18.9	-	-
V <sub>z</sub> (l/kg)	1.2	-	-
V <sub>ss</sub> (l/kg)	0.7	-	-
MRT (min)	36	101	122
f (%)	-	66.9	62.1

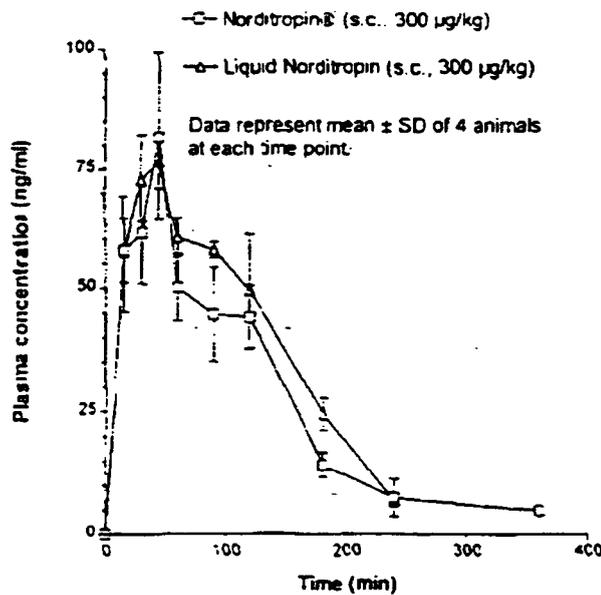
\* The figures in brackets are the percentage of the AUC estimated by extrapolation from last data point to infinity.

Sponsor's Figures Vol. 1.11/237:

**Figure 1. Plasma concentration of Somatropin following s.c. administration of Liquid Norditropin and Norditropin® and i.v. administration of Somatropin to rats (logarithmic scale).**



**Figure 2. Plasma concentration of Somatropin following s.c. administration of Liquid Norditropin and Norditropin (linear scale).**



**POLOXAMER 188**

CAS number: 9003-11-6 MW 7680

Poloxamer 188 is a polymer comprising polyoxypropylene and polyoxyethylene used in a wide range of products due to its

Poloxamer 188 is intended for use as a pharmaceutical excipient in Norditropin SimpleXx™ Cartridges [Somatropin (rDNA origin) for subcutaneous injection]. The expected human load of Poloxamer 188 is in the magnitude of 2.25 mg/patient/day – ca 0.045 mg/kg.

**Acute Subcutaneous Toxicity:**

Mice: — Report 14907; Novo Nordisk Study 970107 Vol. 1.12/29

&amp;

Rats: — Report 14924; Novo Nordisk Study 970108 Vol. 1.12/56

A single 2000 mg/kg dose of Poloxamer 188 into the dorsal scapular region produced no mortality or toxicity other than swelling of the neck.

**TOXICOLOGY****General Comments**

**Study Title:** Poloxamer 188: 4-Week Subcutaneous Toxicity Study in Rats Followed by a 2-Week Recovery Period.

**Study No:** — Report 15773; — < Project 565334; Novo Nordisk 970110

**Vol. #, and page #:** Vol. 1.12 page 83

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 30 Jun 97 Start of Dosing 7 Jul 97

**GLP compliance:** Yes

**QA – Report Yes (X) No ( )**

**Methods:** Rats received a single daily s.c. injection (vol. 2.5 ml/kg) for at least 28 days up to 24 hours before sacrifice. Recovery animals were allowed a 2 week recovery period before sacrifice. Satellite rats each received an administration of [<sup>14</sup>C]-Poloxamer 188 at the appropriate dose level on Days 1 and 25. The target radioactive dose on each occasion was ca 0.4 MBq [<sup>14</sup>C]. On each of the other days of the study each animal received a non-radiolabeled administration of Poloxamer 188 along with the Main study groups.

**Dosing:**

- **Species / strain:** Sprague-Dawley rats
- **#/sex/group or time point:** 10M;10F
- **Age:** 4 weeks old (+ 3 weeks to acclimatize before dosing)
- **Weight:** 54-80 g (on arrival)
- **Satellite groups used for toxicokinetics or recovery:** Toxicokinetics: 6M;6F per group  
Additional 5M;5F allocated to Control and High Dose groups for 2- week recovery period.
- **Dosage groups in administered units:** 0, 10, 100, 500 mg/kg/day
- **Route, form, volume, and infusion rate if (i.v.):** s.c. Vol. 2.5 ml/kg

**Drug, lot #, radiolabel (if applicable), and % purity:** Poloxamer 188 Batch 635353; [<sup>14</sup>C] Poloxamer 188 \_\_\_\_\_, Batch CSL-97-700-11-29

**Formulation/vehicle:** sterile water for injection

**Observation and times:**

- **Clinical signs:** 2x daily
- **Body weights:** weekly
- **Food consumption:** weekly
- **Ophthalmoscopy:** pretrial and week 4
- **Hematology:** week 4 and end of recovery
- **Clinical chemistry:** week 4 and end of recovery
- **Urinalysis:** week 4 and end of recovery

Food Consumption (g.animal<sup>-1</sup>.day<sup>-1</sup>)  
Group Mean Values: Males

Group / Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )		Pretrial	Treatment Period (Days)					Recovery Period (Days)	
		0	7	14	21	28	7	14	
1 (0)	Number	8	8	8	8	8	3	3	
	Mean	29.5	31.0	31.3	32.2	31.6	30.8	31.4	
	SD	3.0	3.3	3.4	3.2	2.8	3.2	2.8	
2 (10)	Number	8	8	8	8	8	0	0	
	Mean	30.2	30.3	32.1	32.4	31.7			
	SD	1.4	1.1	1.1	1.2	0.8			
3 (100)	Number	8	8	8	8	8	0	0	
	Mean	28.6	29.4	31.0	31.2	31.2			
	SD	1.8	2.0	2.2	2.4	2.5			
4 (500)	Number	11	11	11	11	11	3	3	
	Mean	28.7	28.5	28.2	29.0	27.9	29.5	31.2	
	SD	1.8	1.8	1.6	2.2	1.7	3.6	4.5	
	Prob.								

Significantly different from the Control: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001  
No Group 2 or 3 animals in recovery period

- Organ Weights: at necropsy
- Gross pathology: at necropsy
- Organs weighed: Yes. See histopathology inventory chart on page 43.
- Histopathology: Main study control and high dose and injection sites and kidneys from all recovery animals. Kidneys from main study low and intermediate dose groups.
- Toxicokinetics: Days 1 and 25; pre-dose, 0.5, 1, 2, 4, 8, 24 hours

Results:

- Clinical signs: No adverse signs.
- Body Weights: A dose related reduction in body weight was seen for males in all treated groups (sig. in Group 4 males). During recovery, body weight profiles improved and body weight gains were comparable with those of controls. Treated female body weight gains were considered acceptable throughout the Main and Recovery studies.

Sponsor's Table Vol. 1.12/119

Body Weights (g)  
Group Mean Values: Males

Group / Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )		Pretrial		Treatment Period (Days)				Recovery Period (Days)		
		-7	0	7	14	21	28	0	7	14
1 (0)	Number	15	15	15	15	15	15	5	5	5
	Mean	180	247	295	341	374	390	383	412	432
	SD	9	20	28	38	46	52	41	40	45
2 (10)	Number	16	16	16	16	16	16	0	0	0
	Mean	178	243	285	331	368	378			
	SD	13	19	28	31	32	35			
3 (100)	Number	16	16	16	16	16	16	0	0	0
	Mean	179	239	285	324	357	374			
	SD	10	14	17	27	34	34			
4 (500)	Number	21	21	21	21	21	21	5	5	5
	Mean	179	238	278	314	340	352	361	385	406
	SD	8	11	12	16	18	19	27	31	38
	Prob.									

Significantly different from the Control: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001  
No Group 2 or 3 animals in recovery period

- **Food Consumption:** Lower than that of controls for M and F 500 mg/kg animals. Full recovery was evident at the end of the recovery period.

Sponsor's Table Vol. 1.12/121

Food Consumption (g.animal<sup>-1</sup>.day<sup>-1</sup>)  
Group Mean Values: Males

Group / Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )		Pretrial	Treatment Period (Days)					Recovery Period (Days)	
		0	7	14	21	28	7	14	
1 (0)	Number	8	8	8	8	8	3	3	
	Mean	29.5	31.0	31.3	32.2	31.6	30.8	31.4	
	SD	3.0	3.3	3.4	3.2	2.8	3.2	2.6	
2 (10)	Number	8	8	8	8	8	0	0	
	Mean	30.2	30.3	32.1	32.4	31.7			
	SD	1.4	1.1	1.1	1.2	0.8			
	Prob.								
3 (100)	Number	8	8	8	8	8	0	0	
	Mean	28.6	29.4	31.0	31.2	31.2			
	SD	1.6	2.0	2.2	2.4	2.5			
	Prob.								
4 (500)	Number	11	11	11	11	11	3	3	
	Mean	28.7	28.5	28.2	29.0	27.9	29.5	31.2	
	SD	1.6	1.8	1.6	2.2	1.7	3.6	4.5	
	Prob.								

Significantly different from the Control: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001  
No Group 2 or 3 animals in recovery period

Food Consumption (g.animal<sup>-1</sup>.day<sup>-1</sup>)  
Group Mean Values: Females

Group / Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )		Pretrial	Treatment Period (Days)					Recovery Period (Days)	
		0	7	14	21	28	7	14	
1 (0)	Number	8	8	8	8	8	3	3	
	Mean	21.8	21.1	22.6	24.2	24.5	25.4	24.6	
	SD	0.9	1.5	1.6	1.5	1.4	1.8	1.2	
2 (10)	Number	8	8	8	8	7	0	0	
	Mean	21.5	21.1	23.0	24.3	24.5			
	SD	0.8	1.2	1.6	1.5	2.4			
	Prob.								
3 (100)	Number	8	8	8	8	8	0	0	
	Mean	20.8	19.9	21.8	22.6	23.2			
	SD	1.2	0.7	1.1	1.6	1.7			
	Prob.								
4 (500)	Number	11	11	11	11	11	3	3	
	Mean	21.3	20.5	21.6	22.5	22.1	26.4	23.6	
	SD	1.5	1.3	1.7	1.9	1.5	3.9	2.6	
	Prob.								

Significantly different from the Control: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001  
No Group 2 or 3 animals in recovery period

- **Ophthalmoscopy:** no adverse findings
- **Hematology:** Within background control ranges
- **Clinical Chemistry:** Within background control ranges
- **Urinalysis:** Within background control ranges
- **Organ Weights:** Kidney weights were increased in Main study rats receiving 100 and 500 mg/kg/day. Absolute organ weights for males of these two groups, respectively, were 3.26 and 3.16 vs 3.05 g for controls and for females 2.28 and 2.46(sig.) vs 2.19 g for controls. Based on Covariance Analysis with a male body weight of 372 g values for mid and high dose were 3.2 and 3.36 (sig.) vs 2.91 for controls. Covariance Analysis kidney values for female body weight of 246 g were 2.29 (sig.) and 2.49 (sig.) for the mid and high dose vs 2.13 for controls. Following the recovery period (control and high dose only) kidney weights of the high dose were comparable with those of controls
- **Gross pathology:** Only background findings as expected.
- **Histopathology:** Increased severity and incidence of tubular vacuolation in the kidney of males and females on 500 mg/kg and in 100 mg/kg female - at recovery partially reversible in 500 mg/kg/day rats. The incidence of kidney basophilic tubules was reduced in males and females on 500 mg/kg and in 100 mg/kg females compared to controls.

**Main Study - Kidneys**

Dose Level (mg/kg)	Tubular Vacuolation		Basophilic Tubules
<b>Males</b>	<b>Graded</b>		
Vehicle (0)	4/10	minimal	7/10
Low Dose (10)	0		3/10
Mid-Dose (100)	1/10	minimal	3/10
High Dose (500)	8/10	3 mild; 5 minimal	0/10
<b>Females</b>			
Vehicle (0)	4/10	minimal	9/10
Low Dose (10)	4/10	minimal	7/10
Mid-Dose (100)	8/10	4 minimal; 4 mild	2/10
High Dose (500)	10/10	mild	0/10

**Recovery Study - Kidneys [Control and High Dose]**

**Tubular Vacuolation – Males and Females**

Control and high dose males had a similar incidence and severity of vacuolated tubules. However, the appearance in high dose animals 58 and 59 was particularly prominent.

Control female rats had a zero incidence of tubular vacuolation.

High dose females had an incidence of 3/5 with 2 graded as minimal and 1 as mild.

Thus, there was incomplete reversibility in both males and females in the 2-week time period.

**Basophilic Tubules – Males and Females**

Control and high dose recovery rats showed a similar incidence.

**Injection Sites:**

Inflammatory cell infiltration of a chronic type was present at the injection sites of control and high dose rats. The incidence and grades were similar at Injection Site One in both sexes and Injection Site Two in males.

Injection Site Two – The overall incidence of inflammatory cell infiltrate was similar for control and high dose females. However, 7/10 of the high dose group had a moderate infiltrate compared to only 1/10 moderate for control females. [The sponsor attributed this to the repeated injection procedure.]

**Recovery:** Chronic inflammatory cell infiltration was noted at sites of a number of animals in both control and treated animals. [The sponsor did not consider this finding to be Poloxamer 188 treatment related.]

- **Toxicokinetics:** Relationship between dose and systemic exposure (as measured by AUC estimates over the dose range 10 to 500 mg/kg) was broadly linear;  $C_{max}$  (obs.) was sublinearly related to dose on both Days 1 and 25. Both AUC and  $C_{max}$  (obs) appeared unaffected by time of exposure.  $T_{max}$  (obs.) was 0.5 to 1.0 hours after dosing.  $T_{1/2}$  of elimination was 1.52 to 5.15 hours. Clearance (CL/F) ranged from 421.7 to 672.8 ml.h<sup>-1</sup>. Systemic exposure appeared to be slightly lower in females than in males on the same dose.

.Sponsor's Tables 1.12/123,124

Toxicokinetic Studies: Parameter Estimation from Mean Plasma Concentrations vs Time Data: Males

Group/Dose Level (mg kg <sup>-1</sup> day <sup>-1</sup> )		Parameter/(Units)					
		Day 1			Day 25		
		T <sub>max</sub> (obs) (h)	C <sub>max</sub> (obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-8) (h x µg equiv ml <sup>-1</sup> )	T <sub>max</sub> (obs) (h)	C <sub>max</sub> (obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-8) (h x µg equiv.ml <sup>-1</sup> )
2 (10)	Mean	1.00		17.0	0.50		19.0
3 (100)	Mean	0.50		159.1	0.50		170.5
4 (500)	Mean	1.00		685.6	1.00		670.4

Toxicokinetic Studies: Parameter Estimation from Mean Plasma Concentrations vs Time Data: Females

Group/Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )		Parameter/(Units)					
		Day 1			Day 25		
		T <sub>max</sub> (obs) (h)	C <sub>max</sub> (obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-8) (h x µg equiv ml <sup>-1</sup> )	T <sub>max</sub> (obs) (h)	C <sub>max</sub> (obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-8) (h x µg equiv.ml <sup>-1</sup> )
2 (10)	Mean	0.50		14.4	0.50		15.3
3 (100)	Mean	1.00		146.3	0.50		142.1
4 (500)	Mean	1.00		668.3	0.50		609.3*

\* = Last quantifiable plasma concentration was at 4 hours after dosing

**Key Study Findings:**

Absorption was rapid and the relationship between dose and systemic exposure was largely linear although  $C_{max}$  was sublinearly related to dose both on days 1 and 25. Systemic exposure was slightly lower in females than in males. At 500 mg/kg body weight gain (males only) and food consumption were reduced. Kidney weights and renal tubular vacuolation were increased and basophilic tubules were decreased with partial reversibility evident following the 2 week recovery period. 100 mg/kg kidney weights were increased for males and females. Renal tubular vacuolation was increased and basophilic tubules were decreased for females only.

**Overall Toxicology Summary:**

[ $^{14}C$ ] Poloxamer 188 was given to rats subcutaneously for 4 weeks at dose levels of 0, 10, 100 or 500 mg/kg/day. Control and high dose recovery animals were retained for a further 2 week recovery period. Absorption was rapid  $T_{max}$  being 0.5 or 1.0 h after dosing with a  $T_{1/2}$  of elimination of 1.52 to 5.15 hours across dose groups. Systemic exposure showed a largely linear relationship to dose over the range of 10 to 500 mg/kg/day with  $C_{max}$  increasing with dose sublinearly. Consistent increases were seen in both  $C_{max}$  and  $AUC_{(0-8h)}$  for the high dose between days 1 and 25. Compared to males, females showed a small decrease in systemic exposure.

In general there were no adverse clinical signs, ophthalmoscopy findings, hematology, clinical chemistry, urinalysis, findings. Body weight gains were reduced for high dose males. During the recovery period body weight profiles improved. Food consumption for Main study high dose males and females was less than controls. Kidney weights were increased for Main study mid and high dose animals. Kidney weights of high dose animals were comparable with those of controls after the recovery period. Males and females on the high dose and females on the mid dose had an increased severity and incidence of tubular vacuolation in the kidney which was partially reversible in the 500 mg/kg animals following the recovery period. A similar scenario was seen in the reduced incidence of basophilic tubules in the kidney. [For chronic comparison to human exposure, see 13-week s.c. rat toxicity study below page 42.]

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**Study Title:** Poloxamer 188: 13-Week Subcutaneous Toxicity Study in Rats Followed by a 4-Week Recovery Period:

**Study No:** Report 16115; Project 453966; Novo Nordisk 970332

**Vol. #, and page #:** Vol. 1.13 p. 1

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 6 Aug 97; **Experimental Start Date** 22 Sep 97.

**GLP compliance:** Yes

**QA - Report** Yes (X) No ( )

**Methods:** From the start of dosing to Week 6 the dosing site used was the dorsal scapular region, from Weeks 7 to 9 it was the left or right flank and from then until the end of dosing it alternated between the scapular and flank regions as appropriate to allow recovery from any swelling that was evident.

**Dosing:**

- **Species / strain:** Sprague-Dawley — CD@BR) rats
- **#/sex/group or time point:** 20M;20F 15M;15F sacrificed at 13 weeks — remaining kept for 4-week recovery period.
- **Age:** at receipt ca 4 weeks old [acclimated for 2 weeks prior to treatment]
- **Weight:** males ca 83-87 g; females ca 58-62 g

- **Satellite groups used for toxicokinetics or recovery:** ca 5M;5F
- **Dosage groups in administered units:** 0, 10, 100, 500 mg/kg Poloxamer 188  
7 days/week for 13 weeks. Groups 1-4 Controls received sterile water for injection only.
- **Route, form, volume, and infusion rate if (i.v.):** Subcutaneous, Sterile water for injection only. Volume 2.5 ml/kg

**Drug, lot #, radiolabel (if applicable):** Poloxamer 188 Batch 635353

**Vehicle:** Sterile water for injection.

**Observation and times:** Viability – twice daily

- **Clinical signs:** Daily; In addition, all animals received a detailed clinical examination once each week.
- **Body weights:** The weight of each animal was recorded once each week commencing one week pretrial and then daily.
- **Food consumption:** weekly
- **Ophthalmoscopy:** pretreatment, Control and High dose – Week 13 and towards end of recovery period.
- **Hematology:** Blood from 10/sex/group Weeks 6 and 13 and survivors at end of recovery period.
- **Clinical chemistry:** Blood from 10/sex/group Weeks 6 and 13 and survivors at end of recovery period.
- **Urinalysis:** Urine from 10/sex/group Weeks 6 and 13 and survivors at end of recovery period.
- **Gross pathology:** At necropsy
- **Organs weighed:** See Histopathology Inventory chart on page 43.
- **Histopathology:** Control and High dose at week 13 and after the recovery period. Kidneys and injection sites also from Low and Mid-dose groups.

#### Results:

- **Clinical signs:** There were no premature deaths during the dosing period. One 500 mg/kg male was killed in the last week of the recovery period due to poor condition. Transient subcutaneous swellings at or near the injection sites of all animals on 100 or 500 mg/kg Poloxamer 188 kg/day were generally noted up to 6 hours after dosing from 2-3 weeks after the beginning of dosing until the end of the dosing period. [If a s.c. swelling were present, it generally appeared first, at the injection site, 0-2 hours after dosing. It gradually dissipated and had normally disappeared completely by the predose check the following morning.]
- **Body Weights:** Male body weight gain of the 500 mg/kg but not the 100 mg/kg group was slightly reduced over the dosing period. [Body weight gain High Dose 304 g (sig.) vs 340 g for Controls.] Female body weight gain was not affected. Weight gain per week during the recovery period was similar to that achieved by the control group.
- **Food Consumption:** Male food consumption of the 500 mg/kg group was slightly reduced. [Week 13 High Dose 26.7 g/animal/day vs 30.0 g/animal/day for Controls.] Females were unaffected. There were no visual differences in water consumption.
- **Ophthalmoscopy:** No apparent treatment related findings.
- **Hematology:** Red Blood Cell Parameters – minor changes which reached statistical significance included at Week 13: MCH – all male groups [control through high dose = 18.2, 17.7, 17.7, 17.3]; MCV – in males receiving 10 (50.4) and 500 (49.4) mg/kg vs 51.8 for Controls; Platelets – increased in high dose females only at Week 13 (1129 vs 1025 for Controls). Week 6 RBC counts – high dose females [8.36 vs 7.92 for controls];
- **Clinical Chemistry:** ALT levels of 500 mg/kg males Week 13 ( $p < 0.001$ ) 48 vs 71 for controls) and females Week 6 ( $p < 0.05$ ) 48 vs 59 for Controls and Week 13 ( $p < 0.001$ ) 43 vs 88 for Controls were decreased compared to controls. The decrease was still present (non sig.) in males after the recovery period. Week 13 Females showed slight decreases in mid and high dose Na, Cl, TP, Phos values and mid-dose Creat. values.

- **Urinalysis:** pH of males and females of the 500 mg/kg group was decreased (non sig.) and remained decreased [females, (p<0.01), 7.9 vs 8.8 for Controls) after the recovery period. Specific gravity was increased in 500 mg/kg males in Week 13 (p<0.01), 1.069 vs 1.040 for Controls.
- **Organ Weights:** Female kidney and liver weights (Covariance Analysis based on 297 g body weight) were increased in the 100 [kidney (p<0.001), 2.25 vs 2.02 for Controls; liver (p<0.01), 11.46 vs 10.08 for Controls] and 500 [kidney (p<0.001), 2.28 vs 2.02 for Controls; liver (p<0.01), 11.69 vs 10.08 for controls] mg/kg groups. The kidney weight effect was not fully resolved at the end of the recovery period [mid-dose (p<0.01), 2.17 vs 1.98] and [High Dose (p<0.001), 2.25 vs 1.98 for Controls].
- **Gross pathology:**
  - Injection/Treatment Sites: At necropsy injection sites were thickened/swollen/reddened in appearance in 4 males at 100 mg/kg and in both males (12) and females (2) at 500 mg/kg/day.
  - Skin and subcutis: Swollen in 1M;3F on 100 mg/kg and in 2M;3F on 500 mg/kg. Described as gelatinous in 2F on 100 mg/kg and 6F on 500 mg/kg/day.
- **Histopathology:**
  - Kidney – There was an increase in incidence and severity of tubular vacuolation at 500 mg/kg. Similar changes were seen with 100 mg/kg. No evidence of an increase in tubular vacuolation was seen with 10 mg/kg.
    - Recovery – There was a partial reversal of kidney changes after the 4 week recovery period.
    - [Kidney Tubular Vacuolation – This described cytoplasmic vacuolation of the tubules in the cortex and medulla. See table below.]
  - Injection site – there was an increased incidence and/or severity of inflammatory reaction at the injection site on 500 mg/kg. Similar changes were seen with 100 mg/kg. With 10 mg/kg there was an increased incidence and severity of the inflammatory reaction only at the scapular site of males.
  - Mammary tissue subcutis section – site of routine sampling taken with mammary gland: Inflammatory changes were seen at 500 mg/kg.
    - Recovery – the change in subcutaneous tissue became chronic in nature but persisted in most animals.
    - Cellular infiltrates were of a chronic type. There was fibrosis and thickening of subcutaneous tissue.

**Main Study - Kidneys**

Dose Level (mg/kg)	Focal Tubular Vacuolation	Basophillic Tubules
<b>Males</b>	<b>Graded</b>	
Vehicle (0)	1 minimal	5/15
Low Dose (10)	2 minimal	
Mid-Dose (100)	11 10 minimal, 1 mild	
High Dose (500)	2 1 minimal, 1 mild	1/15
<b>Females</b>		
Vehicle (0)	0	4/15
Low Dose (10)	0	
Mid-Dose (100)	7/15 minimal	
High Dose (500)	5/15 4 minimal, 1 mild	1/15

Diffuse tubular vacuolation: in males seen only in 500 mg/kg rats (13/15, minimal in 1, mild in 9, moderate in 3); in females seen only in 500 mg/kg (9/15, minimal in 7 and mild in 2).

**Recovery: 100 mg/kg:**

Males: 3/5 minimal/mild

Females: 2/5 both focal and minimal

**500 mg/kg:**

Males: 5/5 3 minimal/mild, 2 diffuse and minimal

Females: 3/5 focal in 2 and diffuse in 1, minimal grade in all cases

**Injection/Treatment Sites:**

**Inflammatory Changes:**

**Main Study**

Dose Level (mg/kg)	Scapular		Left Flank		Right Flank	
Males	Recovery		Recovery		Recovery	
Vehicle (0)	8/15		2/15		1/15	1/5
Low Dose (10)	11/15	3/5	2/15			1/5
Mid-Dose (100)	15/15	4/5	12/15	3/5	12/15	5/5
High Dose (500)	14/15	2/5	15/15	5/5	15/15	5/5
<b>Females</b>						
Vehicle (0)	7/15		2/15		1/15	
Low Dose (10)	< controls		1/15	1/5		
Mid-Dose (100)	< controls	1/5	10/15	4/5		4/5
High Dose (500)	8/15	2/5	15/15	4/5	15/15	5/5

According to the sponsor, the subcutaneous inflammation seen at sites remote from the injection site in most animals treated with the test material is considered likely to have spread from the injection sites on the flank.

**Scapular:**

**Males:** The severity of scapular inflammation was similar in all groups although a severe change was noted in 2 rats receiving 10 mg/kg.

**Females:** The incidence of the high dose was similar to that of controls but severity was increased in the high dose group. The change was minimal in controls and 4 high dose animals; mild in 3 and moderate in 1 500 mg/kg animal.

**Recovery:** Scapular findings were minimal or mild.

**Left Flank:**

There was an increase in incidence and severity of s.c. inflammation in both males and females receiving 100 and 500 mg/kg Poloxamer 188.

**Males:** Controls and low dose showed change of a minimal or mild grade. Grades for the mid dose males were minimal or mild in 10 and moderate in 2, and for the high dose 8 mild and 7 moderate.

**Females:** Controls showed a minimal grade change. Changes in the high dose were graded as 7 mild and 8 moderate.

**Recovery:** At recovery chronic inflammatory changes were seen in 5/5 males on the high dose, 3/5 on the mid dose and for females 1/5 low dose, and 4/5 each on the mid and high dose. The change was minimal or mild in all but 1 male receiving 100 mg/kg where the change was moderate.

**Right Flank:**

There was an increase in incidence of subcutaneous inflammation in males and an increase in incidence and severity in females.

**Males:** At 100 mg/kg the change was minimal or mild in 9, moderate in 2, and severe in 1. The changes in 500 mg/kg males were mild in 5, and in 10 moderate. The 1 control was graded moderate.

**Females:** 1 control was a mild grade and for the 500 mg/kg group 1 minimal, 7 mild, 6 moderate and 1 severe.

**Recovery:** The change was moderate in 1 male receiving 500 mg/kg and minimal or mild in the others.

**Skin and Subcutis:**

Subcutaneous inflammation was seen only in rats receiving the 500 mg/kg dose (15/15 males and 12/15 females). This occurred at the site of routine sampling, taken with the mammary gland.

**Recovery:** After the recovery period chronic subcutaneous inflammation was still present in 5/5 males and 3/5 females of the high dose group.

**Other Changes:** Mainly inflammatory which were considered by the sponsor to be expected in rats of this age at Inveresk.

**Key Study Findings:**

Under the conditions of study, there were injection site reactions and kidney toxicity at 100 and 500 mg/kg Poloxamer 188. Effects were minimal at the 10 mg/kg site of treatment.

**Overall Toxicology Summary:**

Rats received Poloxamer 188 at doses of 0, 10, 100 and 500 mg/kg subcutaneously for 13 weeks. Male body weight gain and food consumption were slightly less than that of controls. Some changes in RBC parameters and ALT levels were also evident.

Subcutaneous swelling noted at or near the injection sites of all mid and high dose animals were transient on a daily basis, and were generally seen up to 6 hours after dosing from 2-3 weeks until the end of the dosing period. A number of these animals also had skin thickening at the injection sites. These two groups also had an increase in incidence and/or severity of inflammation compared to controls. Chronic inflammatory changes were still apparent after recovery.

The incidence and severity of focal tubular vacuolation of the kidney was increased in 100 and 500 mg/kg rats compared to controls. Males appeared to be affected more notably than females, especially with diffuse change. Although still present after recovery, severity was decreased.

Compared to controls there was a small decrease in the number of rats with basophilic kidney tubules in the high dose group. [The sponsor indicates that this common background finding may be due to chance.]

Only minimal effects were seen at 10 mg/kg. On the basis of body surface area ( $\text{mg}/\text{m}^2$ ) this value is ca 41X that of the expected human load of Poloxamer 188 of 2.25 mg/patient/day (ca. 0.04 mg/kg).

## Histopathology Inventory for IND

Study	4-Week s.c. 970110	13-Week s.c. 970332	4-Week s.c. 970112	13-Week s.c. 970333
Species	Rat	Rat	Dog	Dog
Adrenals	X*	X*	X*	X
Aorta	X	X	X	X*
Bone Marrow smear				
Bone (femur)	X	X	-	
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix				
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X*	X*	X*	X*
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder			X	X
Gross lesions	X	X	X	X
Harderian gland	X	-	-	-
Heart	X*	X*	X*	X*
Hypophysis				
Ileum	X	X	X	X
Injection site	X	X	X	X
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland				
Larynx				
Liver	X*	X*	X*	X*
Lungs	X*	X*	X*	X*
Lymph nodes, cervical				
Lymph nodes SUB mandibular	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves	X	X	-	X (1)
Ovaries	X*	X*	X*	X*
Pancreas	X	X	X*	X*
Parathyroid	X*	X*	X*	X*
Peripheral nerve				
Pharynx				
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Rectum	X	X	X	X
Salivary gland	X*	X*	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X	X	-	-
Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	-	X	-	
Spleen	X*	X*	X*	X*

Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X*	X*
Vagina	X	X	X	X
Zymbal gland				

\* organ weight obtained.

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**Study Title: Poloxamer 188: 4-Week Subcutaneous Toxicity Study in Dogs Followed by a 2-Week Recovery Period****Study No:** Report 15909; Project 565329; Novo Nordisk 970112**Vol. #, and page #:** Vol. 1.14 p. 1**Conducting laboratory and location:****Date of study initiation:** 18 Jun 97; **Start of Dosing:** 15 Jul 97**GLP compliance:** Yes**QA – Report Yes (X) No ( )****Methods:** Poloxamer 188 was administered subcutaneously on a daily basis to Beagle dogs for 4 weeks to assess toxicity followed by a 2-week recovery period to assess the reversibility of any adverse findings.**Dosing:**

- **Species / strain:** Beagle Dog
- **#/sex/group or time point:** 3M;3F An additional 2M;2F allocated to Control and High Dose for a further 2-week recovery period.
- **Age:** 7-10 months [3-week acclimation period]
- **Weight:** Males: 8.0-11.1 kg; Females: 6.8-9.5 kg
- **Satellite groups used for toxicokinetics or recovery:** 2M;2F allocated to Control and High Dose for a further 2 week recovery period.
- **Dosage groups in administered units:** 0, 0.5, 3, 20 mg/kg/day Poloxamer 188 (Groups 1-4)
- **Route, form, volume, and infusion rate if (i.v.):** Subcutaneous 0.2 ml/kg

**Drug, lot #, radiolabel (if applicable),:** Poloxamer 188 Batch 635353<sup>14</sup>C]-Poloxamer 188 Batch CSL-97-700-11-29**Vehicle:** Poloxamer 188 was dissolved in sterile water for injection.**Observation and times:**

- **Clinical signs:** daily
- **Body weights:** weekly
- **Food consumption:** daily
- **Ophthalmoscopy:** pretrial, towards the end of Week 4 and at end of Recovery period.
- **Hematology:** pretrial, towards the end of Week 4 and at end of Recovery period.
- **Clinical chemistry:** pretrial, towards the end of Week 4 and at end of Recovery period.
- **Urinalysis:** pretrial, towards the end of Week 4 and at end of Recovery period.
- **Gross pathology:** at necropsy
- **Organs weighed:** See histopathology inventory chart on page 43.
- **Histopathology:** See histopathology inventory chart on page 43.
- **Toxicokinetics:** Main study animals each received an administration of [<sup>14</sup>C]-Poloxamer 188 at the appropriate dose level on Days 1 and 25. The target radioactivity dose on each occasion was ca 0.4 MBq[<sup>14</sup>C].

Blood samples (ca 2 ml) were collected from all animals on Days 1 and 25 (predose, 0.5, 1, 2, 4, 8, and 24 hours after administration), following administration of [<sup>14</sup>C] Poloxamer 188 for estimation of C<sub>max</sub>(obs.), T<sub>max</sub>(obs.) and AUC(0-t).

**Results:**

- **Clinical signs:** Mortality was unaffected. Thickening of the skin at the injection site was seen during Weeks 3 and 4 in all of dogs given 20 mg/kg/day and common in dogs given 0.5 or 3.0 mg/kg/day, but was not noted in any recovery dogs following completion of dosing. [The sponsor considered these effects to be due method of dosing rather than an effect of treatment with the test material.] A single incidence of edema at the injection site was seen in one 20 mg/kg/day male.  
Fecal Occult Blood samples were negative.
- **Body Weights:** Reported that there were no significant effects of treatment on bodyweight throughout the study.
- **Food Consumption:** Not affected.

- **Ophthalmoscopy:** No apparent treatment-related findings.
- **Hematology:** Not affected.
- **Clinical Chemistry:** Not affected.
- **Urinalysis:** Not affected.
- **Organ Weights:** Male pancreas weights were significantly increased with 3.0 or 20 mg/kg/day but not dose related or in females.
- **Gross pathology:** No treatment-related pathological changes.
- **Histopathology:** Inflammatory cell infiltrates at the injection sites were noted which were reported as similar in nature, extent and severity throughout the groups. [The sponsor considered these effects to be due method of dosing rather than an effect of treatment with the test material.]
  
- **Toxicokinetics:** Mean Tmax(obs.) was 1-4 hours after dosing. Mean Cmax(obs.) showed a sublinear relationship to dose on both Days 1 and 25 over the dose range 3.0 to 20.0 mg/kg/day. Mean Cmax(obs.) for Groups 2, 3, and 4 was 0.30, 1.33 and 5.47 µg equiv.ml for Day 1 Males and 0.31, 1.67 and 6.50 µg equiv.ml for Day 1 Females. Cmax(obs.) increased for all groups between Days 1 and 25.

Mean Tmax(obs.) was seen earlier on Day 25 compared to Day 1 suggesting an increase in the rate of absorption between Days 1 and 25. The mechanism is not clear. Mean Tmax(obs.) on Day 1 was 2.0, 1.7 and 4.0 for Group 2, 3, and 4 Males respectively and 1.7, 2.0, and 3.33 for Group 2, 3, and 4 Females.

The sublinear trend was less obvious with AUC(0-t) on Day 1 and not evident on Day 25. AUC(0-t) was 1.62, 8.09, and 34.1 (h x µg equiv.ml<sup>-1</sup>) for Day 1 Males; and 1.60, 8.10, 40.87 (h x µg equiv.ml<sup>-1</sup>) for Day 1 Females for dose groups 2, 3, and 4 respectively.

Duration of Treatment effects: Mean Cmax(obs.) increased between Days 1 and 25 for both sexes in all groups (especially Group 4). Mean Cmax(obs.) increased from 5.47 to 9.67 µg.ml<sup>-1</sup> in Males and from 6.50 to 10.13 µg.ml<sup>-1</sup> in Females between Days 1 and 25 respectively. Mean Tmax(obs.) was seen earlier on Day 25 compared to Day 1.

Toxicokinetics did not show a gender difference in this study.

The use of radiolabeled Poloxamer 188 on Days 1 and 25 only, precluded any observations regarding accumulation over the period of dosing in this study.



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Poloxamer 188  
 4 Week Subcutaneous Toxicity Study in Dogs Followed by a 2 Week Recovery Period  
 Toxicokinetics: Parameter Estimation from Mean Plasma Concentrations vs Time Data: Males

Group/Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )		Parameter/(Units)					
		Day 1			Day 25		
		Tmax(obs) (h)	Cmax(obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-t) (h x µg equiv.ml <sup>-1</sup> )	Tmax(obs) (h)	Cmax(obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-t) (h x µg equiv.ml <sup>-1</sup> )
2 (0.5)	n	3	3	3	3	3	3
	Mean	2	0.30	1.62	1.67	0.41	1.24
	SD	0	0.02	0.11	0.58	0.05	0.15
	CV (%)	0	6.86	6.95	34.64	11.43	12.15
	90% CI (lower)	2	0.27	1.43	0.70	0.33	0.99
	90% CI (upper)	2	0.34	1.81	2.64	0.49	1.50
3 (3.0)	n	3	3	3	3	3	3
	Mean	1.67	1.33	8.09	1.33	2.20	6.72
	SD	0.58	0.21	1.07	0.58	0.26	0.43
	CV (%)	34.64	15.61	13.17	43.30	12.03	6.41
	90% CI (lower)	0.70	0.98	6.30	0.36	1.76	5.99
	90% CI (upper)	2.64	1.68	9.88	2.30	2.64	7.44
4 (20)	n	3	3	3	3	3	3
	Mean	4.00	5.47	34.10	2.00	9.67	49.87
	SD	0.00	0.38	1.38	0.00	0.98	1.97
	CV (%)	0.00	6.93	4.05	0.00	10.15	3.94
	90% CI (lower)	4.00	4.83	31.78	2.00	8.02	46.56
	90% CI (upper)	4.00	6.10	36.42	2.00	11.32	53.17

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Toxicokinetics: Parameter Estimation from Mean Plasma Concentrations vs Time Data: Females

Group/Dose Level (mg.kg <sup>-1</sup> .day <sup>-1</sup> )		Parameter/(Units)					
		Day 1			Day 25		
		Tmax(obs) (h)	Cmax(obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-t) (h x µg equiv.ml <sup>-1</sup> )	Tmax(obs) (h)	Cmax(obs) (µg equiv.ml <sup>-1</sup> )	AUC (0-t) (h x µg equiv.ml <sup>-1</sup> )
2 (0.5)	n	3	3	3	3	3	3
	Mean	1.67	0.31	1.60	1.17	0.41	1.21
	SD	0.58	0.01	0.09	0.76	0.02	0.04
	CV (%)	34.64	1.84	5.86	65.47	5.04	3.36
	90% CI (lower)	0.70	0.30	1.44	-0.12	0.38	1.14
	90% CI (upper)	2.64	0.32	1.76	2.45	0.45	1.28
3 (3.0)	n	3	3	3	3	3	3
	Mean	2.00	1.67	8.10	1.00	2.43	6.94
	SD	0.00	0.21	0.63	0.00	0.21	0.23
	CV (%)	0.00	12.49	7.79	0.00	8.55	3.27
	90% CI (lower)	2.00	1.32	7.04	1.00	2.08	6.56
	90% CI (upper)	2.00	2.02	9.16	1.00	2.78	7.32
4 (20)	n	3	3	3	3	3	3
	Mean	3.33	6.50	40.87	1.33	10.13	45.57
	SD	1.15	0.36	3.69	0.58	0.55	13.00
	CV (%)	34.64	5.55	9.04	43.30	5.44	28.54
	90% CI (lower)	1.39	5.89	34.65	0.36	9.21	23.69
	90% CI (upper)	5.28	7.11	47.08	2.30	11.06	67.44

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