Key Study Findings:

Thickening of skin at injection sites observed at all dose levels from Weeks 3 or 4 was not noted in recovery animals. Histological findings were restricted to inflammatory cell infiltrates at injection sites. Toxicokinetics showed some effects – see overall summary below.

Overall Toxicology Summary:

Poloxamer 188 was given subcutaneously to Beagle dogs at dose levels up to 20 mg/kg/day for 4-weeks followed by a 2-week recovery period. Thickening of skin at injection sites observed at all dose levels from Weeks 3 or 4 was not noted in recovery animals. Histological findings were restricted to inflammatory cell infiltrates at injection sites. [For chronic comparison to human exposure see 13-week s.c. dog toxicity study below page 51.]

The sponsor believed these findings to be due to the method of dosing and not to drug treatment.

Systemic exposure was increased as measured by AUC(0-t) over the range of 0.3 to 20 mg/kg/day. Mean Tmax(obs.) between 1-4 hours after dosing appeared to be longer with increasing dose on Day 1 only. This might suggest saturation of the absorption process with increasing dose – consistent with a sublinear relationship between dose and systemic exposure. An increase in the rate of absorption of Poloxamer from the site of injection was consistent with an increased Cmax(obs.) between Days 1 and 25 in all groups together with a decrease in Tmax(obs.) during this period. No gender differences were apparent.

TOXICOLOGY

General Comments: Poloxamer 188 is intended for use as a pharmaceutical excipient for administration to man. The objective of this study was to assess the toxicity of Poloxamer 188 in Beagle dogs after daily subcutaneous administration for 13-weeks and to assess the reversibility of any adverse findings during a 4-week recovery period.

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

Study No: [redacted] Report 16135; [redacted] Project 567326; Novo Nordisk 970333

Vol. #, and page #: Vol. 1.15 p. 1

Conducting laboratory and location: [redacted]

Date of study initiation: 22 Sep 97; Start of Dosing: 23 Sep 97.

GLP compliance: Yes

QA – Report Yes (X) No ( )

Methods: Beagle dogs were dosed subcutaneously for 13-weeks with Poloxamer 188 or 13 weeks of treatment followed by a 4-week recovery period.

Dosing:

- **Species / strain:** Beagle Dogs
- **#/sex/group or time point:** 3M;3F s.c. for 13 weeks. An additional 2M;2F from each group were retained for a further 4-week recovery period.
- **Age:** on arrival 3 ½ -5 ½ months old Acclimated ca 3 weeks before dosing.
- **Weight:** Males 6.6-9.3 kg; Females 6.0-8.7 kg
- **Satellite groups used for toxicokinetics or recovery:** 2M;2F from each group for a 4-week recovery period.
- **Dosage groups in administered units:** 0, 0.5, 3.0, 20.0 mg/kg/day Groups 1-4
- **Route, form, volume, and infusion rate if (i.v.):** s.c. Vol. 0.2 ml/kg

Drug, lot #, radiolabel (if applicable): Poloxamer 188 Batch 635353; reported as acceptable level of accuracy.

Formulation/vehicle: Dissolved in sterile water for injection.

Observation and times: Regular intervals throughout each working day

- **Clinical signs:** each working day.
- **Body weights:** weekly
- **Food consumption:** daily
- **Ophthalmoscopy:** pretrial, towards the end of Weeks 7 and 13 and at the end of the recovery period.
- **Hematology:** once pretrial, towards the end of Weeks 7 and 13 and at the end of the recovery period.
- **Clinical chemistry:** once pretrial, towards the end of Weeks 7 and 13 and at the end of the recovery period.
- **Urinalysis:** once pretrial, towards the end of Weeks 7 and 13 and at the end of the recovery period.
- **Fecal analysis for occult blood** – once pretrial, towards the end of Weeks 7 and 13 and at the end of the recovery period.
- **Organ Weights:** See histopathology inventory table on page 43.
- **Gross pathology:** at necropsy
- **Organs weighed:** at necropsy
- **Histopathology:** see histopathology inventory table on page 43.

Results:

- **Clinical signs:** Thickening of the skin at the injection site was seen in most females and males (to a lesser extent) at all dose levels. This finding was not apparent until Week 4 or later and was not noted in any animals by Week 2 of the recovery period. [The sponsor considers these findings to be due to the dosing method rather than a toxicological effect of the drug.]
- **Body Weights:** There were no significant treatment-related effects during the 13-week period. Some unexplained decreases in body weight were seen between weeks 13 and 14 in both males and females. During the recovery period (weeks 14-17) mid and high dose males and high dose females did not gain quite as much as the other groups. Findings, however were non significant.

From Sponsor's various Tables: Vol. 1.15/32,34,35,36

Mean Body Weights (kg)

Group/Dose level ($mg.kg^{-1}.day^{-1}$)	13 Week Male	14 Week Male	Body Weight Gain (kg) Weeks 14-17
1 (0)	10.7	11.5	0.3
2 (0.5)	10.8	11.2	0.3
3 (3.0)	10.6	11.5	0.1
4 (20)	10.4	10.4	0.1

Mean Body Weights (kg)

Group/Dose level ($mg.kg^{-1}.day^{-1}$)	13 Wk Female	14 Wk Female	Body Weight Gain (kg) Weeks 14-17
1 (0)	9.6	9.1	0.2
2 (0.5)	9.5	9.0	0.3
3 (3.0)	10.0	10.8	0.3
4 (20)	10.2	9.5	0.0

- **Food Consumption:** Unaffected by treatment.
- **Ophthalmoscopy:** No apparent treatment-related findings.
- **Hematology:** A significant increase in eosinophils was seen pretrial in Group 2 (0.81 vs 0.40 for controls) and 4 (0.63 vs 0.40 for controls) female animals and increased basophil levels were seen in female Group 4 (0.09 vs 0.05) females. Basophil levels were

significantly increased at Week 6 in Group 4 females receiving 20 mg/kg/day (0.09 vs 0.06); monocyte levels were significantly increased in 0.5 mg/kg males (0.84 vs 0.45) at Week 13. [These changes were not dose related and essentially apparent in one sex.]

- **Clinical Chemistry:** During Week 6, lactase dehydrogenase was significantly increased in males of the 20 mg/kg/day group (148 vs 109 for controls) and alanine aminotransferase was decreased in 20 mg/kg/day females (34 vs 48 for controls). During Week 13, triglycerides were increased in 3.0 mg/kg females (0.36 vs 0.26). [These changes were not dose related and were essentially apparent in only one sex.]
- **Urinalysis:** Not affected by Poloxamer 188 treatment. Pretrial urinary volume was statistically increased in Group 2 males. Week 13 specific gravity was slightly decreased in 0.5 mg/kg females (1.044 vs 1.052 for controls).
- **Fecal analysis for occult blood:** negative
- **Organ Weights:** Following covariance analysis (female body weight standardized to 10.0 mg/kg), kidney and liver weights were statistically increased in 3.0 mg/kg females (kidney 53.56 vs 45.65 and liver 368.80 vs 328.77 for controls) and 20.0 mg/kg females (kidney 51.45 vs 45.65; liver 358.96 vs 328.77); increases in males were non significant.
- **Gross pathology:** Subcutaneous swelling at injection sites was seen in 3 of the 20 mg/kg animals.
- **Histopathology:** Inflammatory cell infiltrates were seen at the injection sites in most dogs. Severity was increased in all high dose dogs.

Key Study Findings: From Week 4 all dose levels showed thickening of the skin at the injection site. This finding was not seen in recovery animals by Week 2 of the recovery period. Histological findings showed inflammatory cell infiltrates at the injection sites.

Overall Toxicology Summary: Poloxamer 188 was administered subcutaneously to Beagle dogs at doses of 0, 0.5, 3.0, 20.0 mg/kg/day for 13 weeks followed by a 4-week recovery period. Thickening of the skin seen at injection sites at all dose levels from Week 4 was not seen in any recovery animals by recovery Week 2. Inflammatory cell infiltrates were seen histologically at the injection sites. [The sponsor considered injection site observations to be due to the dosing method rather than to a toxicological effect of the test material.]

Following covariance analysis, kidney and liver weights were statistically increased in 3.0 and 20.0 mg/kg females. Changes that were not dose related and were apparent in only one sex or the other at various time periods included: increases in eosinophils, basophils, monocytes, lactase dehydrogenase, and triglycerides and a decrease in alanine aminotransferase. No significant changes were reported at the recovery period.

Based on liver and kidney weight increases, 0.5 mg/kg/day appears to be a NOAEL. The 0.5 mg/kg/day dose level is ca 6.8X the daily human load of Poloxamer 188 (2.25 mg/patient/day) on a body surface area (mg/m^2) basis.

REPRODUCTIVE TOXICOLOGY

Study title: Poloxamer 188: Effects on Fertility and Early Embryonic Development to Implantation in Rats:
Study No: [redacted]; Report 15764; [redacted] Project 492062; Novo Nordisk 970334
Site and testing facility: [redacted]
GRP compliance: Yes
QA – Reports Yes (X) No ( ):
Lot and batch numbers: Batch 635353
Protocol reviewed by Division Yes ( ) No (X):

Methods: Rats were dosed by subcutaneous injection in the scapular region, using left and right sides on alternate days using a constant dose volume of 2.5 ml/kg. Males were dosed for 4 weeks prior to mating, then throughout the mating period until termination after the majority of females had been killed. Females were dosed for 2 weeks prior to mating, then throughout the mating period until Day 6 of gestation. Animals were examined daily.

- **Species/strain:** Sprague-Dawley Rats [[redacted] CD strain (outbred albino)]
  Rats were ca 6 weeks of age and weighed ca 160 g (males) or ca 140 g (females) on arrival on 2 Sep 97. They were acclimated 13 days for males and 27 days for females prior to treatment.
- **Doses employed:** 0, 10, 100, 500 mg/kg Poloxamer 188 (Groups 1-4) in Sterile Water for Injection.
- **Route of Administration:** subcutaneous
- **Study Design:** Males – treated for 7 weeks overall, beginning 4 weeks prior to mating – killed after 7 weeks of treatment. Females – treated from 2 weeks prior to mating until Day 6 of Gestation (Day 0 = day of detection of mating) – killed on Day 14 (92/96 rats), 15 (2 rats) or 16 (1 rat) of gestation. The remaining female (control 114) mated late into the second week of pairing and was killed on Day 8 of gestation.
  First Day of Treatment: Males 15 Sep 97; Females 29 Sep 97.
- **Number of animals/sex/dosing group:** 24M;24F per group
- **Parameters and endpoints evaluated:** Clinical signs of toxicity, body weight and food consumption. At necropsy, rats were given a detailed post mortem examination and the reproductive organs were preserved. Selected reproductive organ weights were recorded for males and pregnancy outcome was evaluated for females.
- **Statistical evaluations:** Organ weight data were analyzed for homogeneity of variance using the 'F-max' test (Hartley, 1950). If the variances appeared homogeneous, the data were analyzed by analysis of variance and by analysis of covariance using the terminal body weight as the single covariant (Snedecor and Cochran, 1980). Treatment means were compared using an F-protected Least Significant Difference procedure. Where the variance appeared heterogeneous, log or square root transformations were used in an attempt to stabilize the variances. If the variances remained heterogeneous, then the non-parametric Kruskal-Wallis test (Hollander and Wolfe, 1973) was used.

Results:

- **Clinical signs:** Subcutaneous lumps and/or sagging skin were noted at or near the injection sites of all rats receiving 500 mg/kg Poloxamer 188 per day and in 7 rats receiving 100 mg/kg/day. These findings were transient, on a daily basis. They were generally noted up to 6 hours after dosing from 1-3 weeks after the beginning of dosing until the end of the dosing period. A small number of 100 and 500 mg rats also had skin thickening at the injection sites. There was also a slight increase in the number of females with partial hair loss in the 500 mg/kg group which might have been related to the transient injection site signs.
  One high dose male had a gelatinous area at the injection site at necropsy.
- **Mortality:** There were no premature deaths during the study.

- **Body Weight:** Male body weight gain was slightly reduced at 500 mg/kg and marginally reduced at 100 mg/kg. [Weight Gain Weeks 0-7 as a % of Control – Groups 2-4 = 99, 94, 90%.] Female body weight gain of the high dose group was slightly reduced during gestation. [Female Weight Gain Days 0-13 as % of Control - Groups 2-4 = 100, 106, 89%.]
- **Food consumption:** At 500 mg/kg male and female food consumption was slightly reduced. For males this was seen throughout the 4 week period of food consumption measurement. [Total Food Consumed Weeks 1-4 as % of Control – Groups 2-4 = 101, 98, 91%.] For females there was a very small reduction in food consumption from Week 1 of treatment, continuing through gestation. [Total Food Consumed Days 0-13 as % of Control – Groups 2-4 = 101, 101, 93%.]

(- Fertility in Males)

- **In-life observations:** Mating performance was similar in all groups.
  Male Fertility Index (Groups 1-4) = 92, 100, 96, 96%.
- **Terminal and Necroscopic evaluations:** The following organs were fixed and weighed: testes, epididymides, seminal vesicles and coagulating gland, prostate gland, and pituitary gland (not weighed!). Male organ weights were considered to have been unaffected by treatment.

(- Fertility and Early Embryonic Development in Females)

- **In-life observations:** There was no evidence of treatment effects on mating performance, fertility indices, or pregnancy.
  Female Fertility Index (Groups 1-4) = 96, 100, 96, 100%.
- **Terminal and Necroscopic evaluations:** The following organs were fixed: ovaries, uterus, cervix and vagina (non-pregnant animals only), and pituitary gland. There was no evidence of treatment effects on the number of implants, incidence of intra-uterine mortality or uterus weight.

(- Embryo-fetal Development) - See Table below.

(Prenatal and postnatal development, including maternal function) - See Table below.

Summary and Evaluation:

The effects of Poloxamer 188 on fertility and early embryonic development to implantation were studied in rats at doses of 0, 10, 100, 500 mg/kg. There were injection site reactions and mild parental toxicity at 100 and 500 mg/kg/day Poloxamer 188. [Transient subcutaneous lumps and/or sagging skin were noted at or near the injection sites of all rats receiving 500 mg/kg and in 7 on 100 mg/kg.] Parents showed reduced weight gain at 100 and 500 mg/kg/day.

Based on body surface area (mg/m^2) the NOAEL for parent rats of 10 mg/kg/day is about 41X that of the expected human load of Poloxamer 188 of 2.25 mg/patient/day (ca 0.04 mg/kg).

Litter parameters showed no adverse effects at doses up to 500 mg/kg/day which, based on body surface area (mg/m^2), would be ca 2027X that of the expected human load of Poloxamer 188.

Sponsor's Table Vol. 1.15 p. 264

Poloxamer 188
Effects on Fertility and Early Embryonic Development to Implantation in Rats
Pregnancy Performance

	Group/Dose Level (mg Poloxamer 188.kg^{-1}.day^{-1})			
	1 (0)	2 (10)	3 (100)	4 (500)
Number of animals mated	24	24	24	24
Number pregnant	23	24	23	24
Pregnancy Frequency as %	96	100	96	100
Total corpora lutea gravidatis	370	381	374	361
Total number of implants	350	364	366	353
Pre-implantation loss as %	5	4	2	2
Total live implants (%)	329 (94)	342 (94)	355 (97)	335 (95)
Total dead implants (%)	21 (6)	22 (6)	11 (3)	18 (5)
Total early embryonic deaths (%)	21 (6)	22 (6)	11 (3)	18 (5)
Total late embryonic deaths (%)	0	0	0	0
Mean corpora lutea gravidatis	16.1 ± 2.4	15.9 ± 1.9	16.3 ± 1.5	15.0 ± 1.7
Mean implants	15.2 ± 2.3	15.2 ± 2.6	15.9 ± 1.3	14.7 ± 1.9
Mean live implants	14.3 ± 2.8	14.3 ± 3.0	15.4 ± 1.3	14.0 ± 2.2
Mean dead implants	0.9 ± 1.4	0.9 ± 1.1	0.5 ± 0.6	0.8 ± 1.2
Mean early embryonic deaths	0.9 ± 1.4	0.9 ± 1.1	0.5 ± 0.6	0.8 ± 1.2
Mean late embryonic deaths	0	0	0	0
Mean total uterus weight (g)	16.5 ± 3.2	17.0 ± 4.6	16.8 ± 1.7	15.3 ± 2.4

Means are given ± Standard Deviation

Cursory Review Only:

Poloxamer 188: Preliminary Developmental Toxicity Study in Rats: [redacted] Vol. 1.15/350. [redacted] Research Report 15399; [redacted] Project 492020; Novo Nordisk 970335. Q.A. – Present.

Mated Sprague-Dawley rats (6/group) were dosed by subcutaneous injection once daily over Days 6-16 inclusive of gestation, where Day 0 was the day of detection of mating. Dose levels were 0, 10, 100, 250 and 500 mg/kg/day Poloxamer 188. Sacrifice was on Day 20 of gestation.

Clinical profile, body weight performance and food consumption were not affected up to 500 mg/kg/day. The percent preimplantation loss was slightly greater in the treated animals being 8, 12, 9, 13, 13 Control through high dose.

The sponsor indicates that a suitable series of dose levels for use in a full developmental toxicity study would be 0, 10, 100 and 500 mg/kg/day Poloxamer 188.

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Sponsor's Table Vol. 1.15/371

Poloxamer 188
Preliminary Developmental Toxicity Study in Rats
Pregnancy Performance and Foetal Weight (g)

	Group/Dose Level (mg Poloxamer 188.kg^{-1}.day^{-1})				
	1 (0)	2 (10)	3 (100)	4 (250)	5 (500)
Number of animals mated	6	6	6	6	6
Number pregnant	6	6	6	6	6
Number of premature decedents	0	0	0	0	0
Number of decedents pregnant	0	0	0	0	0
Number pregnant at Day 20 necropsy	6	6	6	6	6
Pregnancy Frequency as %	100	100	100	100	100
Total corpora lutea gravidatis	91	90	92	102	86
Total number of implants	84	79	84	89	75
Pre-implantation loss as %	8	12	9	13	13
Total live implants (%)	78 (93)	77 (97)	81 (96)	85 (96)	73 (97)
Total dead implants (%)	6 (7)	2 (3)	3 (4)	4 (4)	2 (3)
Total early embryonic deaths (%)	6 (7)	2 (3)	3 (4)	4 (4)	2 (3)
Total late embryonic deaths (%)	0	0	0	0	0
Total foetal deaths (%)	0	0	0	0	0
Mean corpora lutea gravidatis	15.2 ± 1.7	15.0 ± 2.4	15.3 ± 1.4	17.0 ± 1.8	14.3 ± 1.0
Mean implants	14.0 ± 1.5	13.2 ± 1.6	14.0 ± 0.6	14.8 ± 1.6	12.5 ± 3.3
Mean live implants	13.0 ± 1.7	12.8 ± 1.5	13.5 ± 0.5	14.2 ± 1.8	12.2 ± 3.1
Mean dead implants	1.0 ± 0.6	0.3 ± 0.5	0.5 ± 0.8	0.7 ± 0.5	0.3 ± 0.5
Mean early embryonic deaths	1.0 ± 0.6	0.3 ± 0.5	0.5 ± 0.8	0.7 ± 0.5	0.3 ± 0.5
Mean late embryonic deaths	0	0	0	0	0
Mean foetal deaths	0	0	0	0	0
Mean total uterus weight (g)	77 ± 10	80 ± 10	85 ± 7	88 ± 10	75 ± 17
Mean litter mean foetal weight (g)	3.79 ± 0.21	3.94 ± 0.17	4.02 ± 0.23	3.85 ± 0.07	3.99 ± 0.20

Means are given ± Standard Deviation

REPRODUCTIVE TOXICOLOGY

Study title: Poloxamer 188: Developmental Toxicity Study in Rats
Study No: [redacted] Report 15805; [redacted] Project 492036; Novo Nordisk 970337
Site and testing facility: [redacted]
GRP compliance: Yes
QA – Reports Yes (X) No ( ):
Lot and batch numbers: Poloxamer 188 Batch 635353
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- **Species/strain:** Mated Female Sprague-Dawley Rats
- **Doses employed:** 0, 10, 100, and 500 mg/kg/day Poloxamer 188. Groups 1-4. Vol. 2.5 ml/kg
- **Route of Administration:** subcutaneous injection once daily
- **Study Design:** Mated female Sprague-Dawley rats (20 per group) were dosed subcutaneously once daily over Days 6-16 inclusive of gestation where Day 0 was the day of detection of mating. Sacrifice was on Day 20. The status of each implantation was recorded and the viable fetuses were examined externally and weighed. Fetuses were examined for soft tissue and skeletal abnormalities. First Day of Treatment: 22 Sep 97. [500 mg/kg Poloxamer 188 was given as the maximum practicable dose level for repeat s.c. dosing in rats, taking into account such issues as solubility of the test material and maximum practicable dose volume.
- **Number of animals/sex/dosing group:** 20 mated females per group. Rats were ca 9 weeks of age and weighed ca 200 g on arrival on 19 Sep 97.
- **Parameters and endpoints evaluated:** Animals were monitored during gestation for clinical signs of toxicity, body weight and food consumption. The animals were sacrificed on Day 20 of gestation and the status of each implantation was recorded and the viable fetuses were examined externally and weighed. Half of the fetuses from each uterus were fixed in alcohol for examination for soft tissue abnormalities followed by examination for skeletal abnormalities, while the remaining half were fixed in Bouin's fluid for detailed analysis of soft tissue abnormalities using a freehand sectioning technique.
- **Statistical evaluations:** According to the sponsor, no formal statistical analyses were considered necessary, interpretation of the data being by inspection of the individual and group values.

Results:

- **Clinical signs:** Transient subcutaneous lumps followed by sagging skin were noted at or near the injection sites of all 500 mg/kg rats and in two of the 100 mg/kg rats only during the latter part of the treatment period. A subcutaneous lump generally appeared first at the injection site 1-2 hours after dosing and then gradually turned into an area of sagging skin near the injection site 3-6 hours after dosing and then disappeared completely by the following morning. These findings were not apparent until the latter part of the treatment period (Day 12 to 14 of gestation, corresponding to the 7^{th} to 9^{th} days of dosing) and did not persist beyond the dosing period. No abnormalities were detected at the injection sites at necropsy.
- **Mortality:** none
- **Body Weight:** unaffected
- **Food consumption:** unaffected
- **(- Fertility in Males)** See Table below.
- **(- Fertility and Early Embryonic Development in Females)** See Table below.
  - **In-life observations:** There was no evidence of any effect of treatment on pregnancy performance.

- **Terminal and Necroscopic evaluations:** No abnormalities were detected at the injection sites at necropsy. The pale kidney with pelvic dilation seen in one low dose rat was considered to be incidental.

(- Embryo-fetal Development) See Table below.

- **In-life observations:** There were no signs of embryo-fetal toxicity at any dose level.
- **Terminal and Necroscopic Evaluations:** See table below.
- **Offspring:** The incidences and types of fetal abnormalities and variants, including the skeletal ossification parameters did not provide any indication of an effect of treatment.

Summary and Evaluation: 20 mated female rats per group received 0, 10, 100, and 500 mg/kg Poloxamer 188 by s.c. injection once daily Days 6-16 of gestation. Rats were monitored during gestation for clinical signs of toxicity, bodyweight and food consumption and were sacrificed on Day 20 of gestation. The status of each implantation was recorded and viable fetuses examined externally and weighed. Fetal examination included soft tissue and skeletal observations.

At 100 and 500 mg/kg maternal effects were confined to transient local reactions at the injection sites. The only apparent fetal effects were a slight increase in hemorrhage affecting the head (Control through High dose) of 0, 3 (2%), 5 (2%) 3 (3%) and intra-abdominal hemorrhage of 1 (1%), 2 (2%), 2 (2%) and 4 (3%). 10 mg/kg was without any effect on the dam or fetus. This dose is ca 41X that of the expected clinical load of Poloxamer 188 (2.25 mg/patient/day) on a body surface area (mg/m^2) basis.

Sponsor's Table Vol. 1.16/26

Poloxamer 188
Developmental Toxicity Study in Rats
Pregnancy Performance and Foetal Weight (g)

	Group/Dose Level (mg Poloxamer 188.kg^{-1}.day^{-1})			
	1 (0)	2 (10)	3 (100)	4 (500)
Number of animals mated	20	20	20	20
Number pregnant	18	19	18	19
Number of premature decedents	0	0	0	0
Number of decedents pregnant	0	0	0	0
Number pregnant at Day 20 necropsy	18	19	18	19
Pregnancy Frequency as %	90	95	90	95
Total corpora lutea gravidatis	266	279	261	277
Total number of implants	237	263	242	254
Pre-implantation loss as %	11	6	7	8
Total live implants (%)	230 (97)	254 (97)	231 (95)	244 (96)
Total dead implants (%)	7 (3)	9 (3)	11 (5)	10 (4)
Total early embryonic deaths (%)	7 (3)	9 (3)	8 (3)	10 (4)
Total late embryonic deaths (%)	0	0	2 (1)	0
Total foetal deaths (%)	0	0	1 (0 4)	0
Mean corpora lutea gravidatis	14 7 ± 1.9	14.7 ± 1.8	14.5 ± 2 3	14.6 ± 2.3
Mean implants	13.2 ± 2.7	13.8 ± 1.6	13.4 ± 3.1	13.4 ± 1.9
Mean live implants	12.8 ± 2.6	13.4 ± 1.7	12.8 ± 3.1	12.8 ± 1 9
Mean dead implants	0.4 ± 0.7	0.5 ± 0.8	0.6 ± 0.7	0 5 ± 0.8
Mean early embryonic deaths	0.4 ± 0.7	0.5 ± 0.8	0.4 ± 0.6	0.5 ± 0.8
Mean late embryonic deaths	0	0	0.1 ± 0.3	0
Mean foetal deaths	0	0	0.1 ± 0.2	0
Total live male foetuses (%)	123 (53)	112 (44)	125 (54)	124 (51)
Total live female foetuses (%)	107 (47)	142 (56)	106 (46)	120 (49)
Live foetal sex ratio (male:female)	1.0.87	1:1.27	1:0.85	1:0.97
Mean total uterus weight (g)	78 ± 15	80 ± 11	78 ± 17	78 ± 10
Mean litter mean foetal weight (g)	3.78 ± 0.27	3.77 ± 0.19	3.80 ± 0.30	3.79 ± 0.23

Means are given ± Standard Deviation

Incidence of Fetuses (Litters): Groups 1-4

Total number examined viscerally:	230 (18),	254 (19),	231 (18),	244 (19)
Number with minor visceral abnormality/variant:	20 (10),	18 (9),	24 (13),	16 (10)
Hemorrhage affecting head:	0	3 (2),	5 (2),	3 (3)
Intra-abdominal hemorrhage:	1 (1),	2 (2),	2 (2),	4 (3)
Number with minor skeletal abnormality/variant:	2 (2),	1 (1),	3 (2),	2 (2)

There were no fetuses with major abnormalities.

Cursory Review Only:

Poloxamer 188: Dose Range-Finding Study in Rabbits Preliminary to a Developmental Toxicity Study: [redacted] Vol. 1.16/68. [redacted] Report 15532; [redacted] Project 492041; Novo Nordisk 970336. Q.A. Present. Batch 635353. Rabbits were ca 4-5 months old and weighed ca 3.25 kg on arrival.

The study was conducted in 2 phases. The first phase used unmated rabbits and was designed to estimate the maximum tolerated dose. The second phase used mated rabbits and was designed to enable selection of dose levels for use in a developmental toxicity study.

For the unmated phase, 2 groups of 2 per group female New Zealand White rabbits were dosed by subcutaneous injection once daily with 100 or 200 mg/kg Poloxamer 188 per day for 12 days. Rabbits were monitored for clinical signs of toxicity, body weight and food consumption. [Treatment period 20-31 Aug 97.]

For the mated phase, 6 per group mated female New Zealand White rabbits were dosed subcutaneously with 0, 10, 50, and 200 mg/kg/day (Vol. 1 ml/kg) Poloxamer 188 Days 6-18 inclusive of gestation. Rabbits were monitored during gestation for signs of toxicity, bodyweight and food consumption. They were sacrificed on Day 22 of gestation and pregnancy outcome was evaluated principally by assessing intra-uterine death, fetal weight and any externally visible fetal abnormalities. [Treatment period 15-27 Sep 97.]

Results:

The clinical profile, bodyweight and food consumption were not obviously affected. There were no apparent treatment-related effects on pregnancy, fetal weight or external fetal appearance. The percent preimplantation loss was increased in treated groups being 15, 30, 17, 30% Control through High dose (see table below).

The sponsor concluded that dose levels of 0, 10, 100 and 200 mg/kg/day Poloxamer 188 would be a suitable series of dose levels for a full developmental study.

Sponsor's Table Vol. 1.16/92

Poloxamer 188
Dose Range Finding Study in Rabbits Preliminary to Developmental Toxicity Study
Mated Phase
Pregnancy Performance and Foetal Weight

	Group/Dose Level (mg Poloxamer 188.kg^{-1}.day^{-1})			
	5 (0)	6 (10)	7 (50)	8 (200)
Number of animals mated	6	6	6	6
Number pregnant	4	6	6	6
Number of premature decedents	0	0	0	0
Number of decedents pregnant	0	0	0	0
Number pregnant at Day 22 necropsy	4	6	6	6
Pregnancy Frequency as %	67	100	100	100
Total corpora lutea gravidatis	27	53	59	54
Total number of implants	23	37	49	38
Pre-implantation loss as %	15	30	17	30
Total live implants (%)	22 (96)	26 (70)	45 (92)	35 (92)
Total dead implants (%)	1 (4)	11 (30)	4 (8)	3 (8)
Total early embryonic deaths (%)	1 (4)	9 (24)	4 (8)	1 (3)
Total late embryonic deaths (%)	0	1 (3)	0	2 (5)
Total foetal deaths (%)	0	1 (3)	0	0
Mean corpora lutea gravidatis	9.0 ± 0.0	8.8 ± 1.7	9.8 ± 1.3	9.0 ± 2.0
Mean implants	7.7 ± 1.2	6.2 ± 2.9	8.2 ± 1.5	6.3 ± 1.0
Mean live implants	7.3 ± 1.5	4.3 ± 2.5	7.5 ± 1.4	5.8 ± 1.5
Mean dead implants	0.3 ± 0.6	1.8 ± 1.7	0.7 ± 0.8	0.5 ± 0.8
Mean early embryonic deaths	0.3 ± 0.6	1.5 ± 1.4	0.7 ± 0.8	0.2 ± 0.4
Mean late embryonic deaths	0	0.2 ± 0.4	0	0.3 ± 0.5
Mean foetal deaths	0	0.2 ± 0.4	0	0
Mean total uterus weight (g)	181 ± 33	133 ± 43	166 ± 54	173 ± 28
Mean litter mean foetal weight (g)	6.11 ± 0.79	6.04 ± 0.69	6.11 ± 0.84	6.69 ± 0.90

REPRODUCTIVE TOXICOLOGY

Study title: Poloxamer 188: Developmental Toxicity Study in Rabbits: Vol. 1.16/105
Study No: — Report 15806; — Project 492057; Novo Nordisk 970338.
Site and testing facility: [redacted]
GRP compliance: Yes
QA – Reports Yes (X) No ( ):
Lot and batch numbers: Poloxamer 188 Batch 635353
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- **Species/strain:** mated New Zealand White rabbits
- **Doses employed:** 0, 50, 100, and 200 mg/kg/day Poloxamer 188
- **Route of Administration:** subcutaneous (Alternating left, mid-, right scapular areas)
- **Study Design:** Groups of 20 mated female New Zealand White rabbits were dosed subcutaneously with 0, 50, 100, 200 mg/kg/day Poloxamer 188 over Days 6-18 of gestation (Day 0 = day of mating). Rabbits were monitored during gestation for clinical signs of toxicity, body weight and food consumption with sacrifice on Day 29 of gestation. The status of each implantation was recorded and the viable fetuses were examined for soft tissue abnormalities, weighed and fixed in alcohol for subsequent examination for skeletal abnormalities. First Day of Treatment: 10-12 Nov 97 and 17-19 Nov 97.
- **Number of animals/sex/dosing group:** 20 mated female rabbits per group. Rabbits were 4-5 months of age and weighed ca 3.0 kg on arrival. Acclimation was 3-5 days prior to treatment.
- **Parameters and endpoints evaluated:**). Rabbits were monitored during gestation for clinical signs of toxicity, body weight and food consumption with sacrifice on Day 29 of gestation. Pregnancy performance, fetal weight, and type and distribution of fetal abnormalities and variants were evaluated.
- **Statistical evaluations:** According to the sponsor, no formal statistical analyses were considered necessary, interpretation of the data being by inspection of the individual and group values.

Results:

- **Clinical signs:** There were no treatment-related clinical observations or necropsy findings.
- **Mortality:** 4 premature decedents: (killed due to condition)
  - One Control group rabbit was killed due to poor condition on Day 26 of gestation following a lengthy period of very reduced food consumption.
  - One Group 2 (low dose) aborted on day 25.
  - Two Group 3 (intermediate dose) aborted, one on Day 21 and one on Day 27.
- **Body Weight:** Not considered to have been affected by treatment.
- **Food consumption:** Not affected by treatment.

(- Fertility and Early Embryonic Development in Females)

- **In-life observations:** No evidence was seen of any treatment effect on pregnancy performance.
- **Terminal and Necroscopic evaluations:** No apparent effects on intra-uterine death or fetal weight.

(- Embryo-fetal Development)

- **In-life observations:** No apparent effects on fetal weight.
- **Terminal and Necroscopic evaluations:** The type and distribution of fetal abnormalities and variants, including skeletal ossification showed no apparent treatment-related findings.

See Table below.

Summary and Evaluation:

Mated female New Zealand White rabbits (20 per group) were dosed by subcutaneous injection once daily over Days 6-18 of gestation at dose levels of 0, 50, 100 and 200 mg/kg/day Poloxamer 188. Rabbits were monitored during gestation for clinical signs of toxicity, bodyweight and food consumption followed by sacrifice on Day 29 of gestation. The status of each implantation was recorded and viable fetuses were examined for soft tissue and skeletal abnormalities.

There were no apparent treatment-related clinical observations or necropsy findings and body weight and food consumption were not adversely affected. No apparent treatment-related effects were seen in pregnancy performance, fetal weight or type and distribution of fetal abnormalities and variants. One 100 mg/kg fetus had multiple visceral and skeletal abnormalities. In general findings appeared to be isolated or without treatment-related effects.

The sponsor identified 200 mg/kg/day Poloxamer 188 (the maximum level tested) as a level that was without maternal or fetal effects.

Based on body surface area (mg/m^2) the NOAEL (200 mg/kg/day) without maternal or fetal effects in rabbits is about 1622X that of the expected human load of Poloxamer 188 of 2.25 mg/patient/day (ca. 0.04 mg/kg).

Sponsor's Table Vol. 1.16/129

Poloxamer 188
Developmental Toxicity Study in Rabbits
Pregnancy Performance and Foetal Weight (g)

	Group/Dose Level (mg Poloxamer 188.kg^{-1}.day^{-1})			
	1 (0)	2 (50)	3 (100)	4 (200)
Number of animals mated	20	20	20	20
Number pregnant	17	17	17	17
Number of premature decedents	1	1	2	0
Number of decedents pregnant	1	1	2	0
Number pregnant at Day 29 necropsy	16	16	15	17a
Pregnancy Frequency as %	85	85	85	85
Total corpora lutea graviditatis	158	141	145	146
Total number of implants	131	116	118	136
Pre-implantation loss as %	17	18	19	7
Total live implants (%)	109 (83)	101 (87)	106 (90)	114 (84)
Total dead implants (%)	22 (17)	15 (13)	12 (10)	22 (16)
Total early embryonic deaths (%)	18 (14)	14 (12)	8 (7)	18 (13)
Total late embryonic deaths (%)	1 (1)	1 (1)	3 (3)	1 (1)
Total foetal deaths (%)	3 (2)	0	1 (1)	3 (2)
Mean corpora lutea graviditatis	9.9 ± 1.3	8.8 ± 2.5	9.7 ± 2.0	9.1 ± 1.9
Mean implants	8.2 ± 2.3	7.3 ± 3.0	7.9 ± 1.7	8.5 ± 2.2
Mean live implants	6.8 ± 2.2	6.3 ± 2.8	7.1 ± 1.5	7.1 ± 2.3
Mean dead implants	1.4 ± 1.4	0.9 ± 1.0	0.8 ± 0.9	1.4 ± 1.0
Mean early embryonic deaths	1.1 ± 1.3	0.9 ± 1.0	0.5 ± 0.7	1.1 ± 0.8
Mean late embryonic deaths	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.4	0.1 ± 0.3
Mean foetal deaths	0.2 ± 0.4	0	0.1 ± 0.3	0.2 ± 0.8
Total live male foteuses (%)	45 (41)	44 (44)	60 (57)	56 (49)
Total live female foetuses (%)	64 (59)	57 (56)	46 (43)	58 (51)
Live foetal sex ratio (male:female)	1:1.42	1:1.30	1:0.77	1:1.04
Mean total uterus weight (g)	395 ± 110	385 ± 147	416 ± 78	428 ± 111
Mean litter mean foetal weight (g)	38.1 ± 5.1	39.8 ± 4.2	38.7 ± 3.8	37.9 ± 4.4

Means are given ± Standard Deviation
Premature decedent values excluded below double line
a = Includes Animal 79 (with no live foetuses)

REPRODUCTIVE TOXICOLOGY

Study title: Poloxamer 188: Effects on Pre- and Post-Natal Development and on Maternal Function in Rats: Vol. 1.16/172
Study No: [redacted] Report 16137; [redacted] Project 492078; Novo Nordisk 970339
Site and testing facility: [redacted]
GRP compliance: Yes
QA – Reports Yes (X) No ( ):
Lot and batch numbers: Batch 635353
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- **Species/strain:** Sprague-Dawley rats [redacted] CD strain (outbred albino) about 9 weeks of age and 230 g on arrival
- **Doses employed:** 0, 10, 100,and 500 mg/kg/day Poloxamer 188
- **Route of Administration:** subcutaneous injection
- **Study Design:** Mated female Sprague-Dawley rats were randomized into 4 treatment groups each containing 24 rats. These rats were dosed by subcutaneous injection once daily from Day 6 of gestation, when Day 0 was the day of detection of mating until the day before termination at or after weaning of their litters on Day 24 of lactation. Dose levels were 0, 10, 100 and 500 mg/kg/day Poloxamer 188. F_0 dams were monitored for signs of toxicity particularly in terms of ability to bear and nurse their litters. During the lactation period and thereafter, the F_1 offspring were monitored for normal growth, development of various other physical and functional characteristics, and for their reproductive capacity. On Day 21 of lactation 1M;1F were selected for each litter where available. At Day 24 of lactation selected animals were rehoused for further testing. After weaning of the selected pups, the F_0 mother and remaining F_1 pups were sacrificed and examined (included thoracic and abdominal contents; cranial contents were also examined for F_1 animals). Termination of F_1 parents and their F_2 offspring occurred after the majority of litters had reached Day 14 of lactation, and all litters had reached Day 4 of lactation.
- **Number of animals/sex/dosing group:** 24 females per group
- **Parameters and endpoints evaluated:** Rats were tested for effects on pre-and post-natal development and on maternal function following daily subcutaneous injection of the material to dams from the time of implantation through lactation..
- **Statistical evaluations:** None. Interpretation was based on inspection of the individual and group values.

Results:

- **Clinical signs:** Local injection site reactions (transient lumps and/or sagging skin) were observed for all 500 mg/kg/day rats and for occasional 100 mg/kg/day rats and one Control rat.
- **Mortality:** No premature decedents at any dose level for the F_0 group.
- **Body Weight:** Mean bodyweight gain of the 500 mg/kg/day F_0 group were slightly lower than that of controls 320 $\pm$ 32 vs 338 $\pm$ 24 for controls) during the first 2 weeks of lactation. Bodyweight performance during lactation was similar to Controls for the 10 and 100 mg/kg/day groups.
- **Food consumption:** Mean food consumption of the 500 mg/kg/day F_0 group was slightly less than controls (ca 87% of controls) during the first 2 weeks of lactation. Food consumption of the 10 and 100 mg/kg/day animals was similar to Controls.

(- Fertility and Early Embryonic Development in Females)

- **In-life observations:** There were no premature decedents at any dose level. The mean duration of gestation was similar in all groups. Mean body weight gain and

food consumption of the 500 mg/kg/day group were slightly lower than that of controls during the first 2 weeks of lactation.

(- Embryo-fetal Development)

- In-life observations:

F_1 Litter Size and Survival: The incidence of litters in which all pups died was 1, 1, 5 and 2 at 0, 10, 100 and 500 mg/kg, respectively. The mean number of pups born was similar for all groups. Mean pup survival was marginally lower than controls at 500 mg/kg and similar to controls for lower levels.

F_1 Litter and Pup Weights: At 500 mg/kg mean pup weights of males and females were slightly lower than Controls throughout the lactation period. Mean litter weights were also reduced. In general mean litter weights of the lower doses were similar to Controls. Differences were due to litter size.

F_1 Preweaning and Functional Development: In general mean ages for attainment of physical developmental landmarks were similar as were functional development parameters.

Abnormalities of F_1 Pups: Isolated abnormalities were not considered to be Poloxamer 188 treatment related.

- Terminal and Necroscopic evaluations:

- Offspring: [See tables at end of study.]

F_1 Post-Weaning Clinical Observations and Necropsy Findings:

No clinical observations or necropsy findings were considered to be treatment-related. However, hair loss was seen in 1, 5, 5, 7 of the F_1 generation control to high dose, respectively. One high dose animal died. According to the sponsor, death was due to swelling/fusion of the hind foot.

F_1 Body Weight Performance: F_1 animals showed no obvious differences in bodyweight performance between groups – including that of females during gestation and lactation. Although not statistically significant bodyweights (g) during lactation of the two higher doses (363 $\pm$ 28; 364 $\pm$ 39) were not quite as large as controls (375 $\pm$ 23).

F_1 Assessment of Sexual Maturity: Mean ages of vaginal opening and preputial separation of the 100 and 500 mg/kg groups were slightly greater than that of Controls. Minor differences in age might have been a reflection of the slightly lower mean body weights of these animals.

F_1 Post-Weaning Functional Development: The Rota-Rod, Open Field and Y-Maze tests did not indicate any adverse treatment-related effects.

F_1 Reproductive Performance:

There were no apparent indication of treatment-related effects on F_1 reproductive performance. See tables at end of study.

(- Prenatal and postnatal development, including maternal function)

- In-life observations: Food consumption was not recorded for F_1 rats. Pup survival and mean pup weight at 500 mg/kg/day were slightly lower than that of Controls – there was an associated reduction in mean litter weight.

Summary and Evaluation: Mated Sprague-Dawley rats were dosed by s.c. injection once daily from Day 6 of gestation at dose levels of 0, 10, 100, and 500 mg/kg/day Poloxamer 188 until weaning (ca Day 24 of lactation). F_0 dams and F_1 offspring were monitored. There was a local reaction at the injection site consisting of transient lumps and/or sagging skin in all 500 mg/kg/day rats, for occasional 100

mg/kg/day rats and for one Control rat. Mean body weight gain and food consumption were slightly lower than control for the 500 mg/kg/day group during the first 2 weeks of lactation. At 500 mg/kg pup survival and mean pup weights of the 500 mg/kg/day group and mean litter weights were slightly lower than controls. There were no other indications of any effects of treatment on the performance of the F_1 generation. The F_1 and F_2 rats were not dosed directly. 10 mg/kg/day appears to be a no adverse effect level. This dose level is 41X the human dose of Poloxamer 188 (2.25 mg/patient/day) based on body surface area (mg/m^2).

Sponsor's Table Vol. 1.16/207

Poloxamer 188
Effects on Pre- and Post-Natal Development and on Maternal Function in Rats
F_0 Generation, F_1 Production
Duration of Gestation and Overall Litter Performance

	Group/Dose Level (mg Poloxamer 188.kg^{-1}.day^{-1})			
	1 (0)	2 (10)	3 (100)	4 (500)
Number Pregnant	24	22	24	23
Duration of Gestation (Days)				
21	13	9	14	15
22	10	12	10	8
23	1	0	0	0
Mean Duration	21.5	21.6	21.4	21.3
Number of females producing a live litter	24	20	24	23
Gestation index as %	100	91	100	100
Mean number of implant sites[a] per pregnancy ± standard deviation	13.4 ± 2.0	13.4 ± 1.6	13.8 ± 1.8	13.9 ± 2.7
Mean total number of pups[a] born	12.7 ± 1.7	11.9 ± 1.6	12.8 ± 1.6	12.3 ± 2.2
Mean number of live pups[a] per litter ± standard deviation.				
Day 0 of lactation	12.4 ± 1.9	11.8 ± 1.7	12.7 ± 1.6	12.1 ± 2.0
Day 1 of lactation	12.0 ± 2.2	11.5 ± 1.6	12.3 ± 1.9	11.5 ± 2.8
Day 4 of lactation	11.7 ± 2.3	11.0 ± 1.5	12.2 ± 2.0	10.9 ± 2.9
Day 7 of lactation	11.7 ± 2.2	11.0 ± 1.5	12.2 ± 2.0	10.8 ± 2.9
Day 14 of lactation	11.5 ± 2.3	11.0 ± 1.5	12.2 ± 2.0	10.8 ± 2.9
Day 21 of lactation	11.5 ± 2.3	11.0 ± 1.5	12.2 ± 2.0	10.8 ± 2.9

a = Excludes litters where all pups died

Sponsor's Table Vol. 1.16/208

Poloxamer 188
Effects on Pre- and Post-Natal Development and on Maternal Function in Rats
F_1 Generation
Survival Indices

		Group/Dose Level (mg Poloxamer 188.kg^{-1} day^{-1})			
		1 (0)	2 (10)	3 (100)	4 (500)
Birth Index	Mean Litter Index (%)	92	85	90	90
	Number Losing >2 pups	3	6	3	7
	Number of Litters	23	22	24	23
Live Birth Index	Mean Litter Index (%)	98	95	95	98
	Number Losing >1 pup	2	1	5	1
	Number of Litters	24	21	24	23
Viability Index Days 0-4	Mean Litter Index (%)	90	94	76	83
	Number Losing >3 pups	2	2	4	4
	Number of Litters	24	20	24	23
Lactation Index Days 4-21	Mean Litter Index (%)	98	100	100	99
	Number Losing >1 pup	1	0	0	0
	Number of Litters	23	20	19	21
Overall Survival Index Birth-21	Mean Litter Index (%)	87	89	75	81
	Number Losing >4 pups	2	2	5	4
	Number of Litters	24	21	24	23

Sponsor's Table Vol. 1.16/223

Poloxamer 188
Effects on Pre- and Post-Natal Development and on Maternal Function in Rats
F_2 Generation
Survival Indices

		Group/Dose Level (mg Poloxamer 188.kg^{-1}.day^{-1})			
		1 (0)	2 (10)	3 (100)	4 (500)
Birth Index	Mean Litter Index (%)	92	93	92	93
	Number Losing >2 pups	5	1	4	3
	Number of Litters	23	19	19	19
Live Birth Index	Mean Litter Index (%)	99	99	99	99
	Number Losing >1 pup	1	1	1	0
	Number of Litters	23	19	19	19
Viability Index Days 0-4	Mean Litter Index (%)	93	89	94	94
	Number Losing >3 pups	1	2	2	1
	Number of Litters	23	19	19	19
Lactation Index Days 4-14	Mean Litter Index (%)	99	98	99	98
	Number Losing >1 pup	22	18	18	19
	Number of Litters	22	18	18	19
Overall Survival Index Birth-14	Mean Litter Index (%)	91	86	91	91
	Number Losing >4 pups	22	18	18	19
	Number of Litters	23	19	18	19

F_1 Generation, F_2 Production: From various Sponsor's Tables Vol. 1.16/221,222, 224

	Group/Dose Level (mg Poloxamer 188.kg^{-1}. Day^{-1})			
	1 (0)	2 (10)	3 (100)	4 (500)
Male Fertility Index (%)	100	95	95	100
Female Fertility Index (%)	100	95	100	100
Gestation Index as %	100	100	100	95
Mean total number of pups[a] born	13.8 ± 3.4	14.4 ± 3.7	14.1 ± 2.6	14.9 ± 2.1
Mean number live pups[a] per litter : Day 0 of lactation	13.5 ± 3.3	14.3 ± 3.6	13.8 ± 2.5	14.8 ± 2.0
Day 4 of lactation	13.2 ± 3.2	13.6 ± 3.7	12.9 ± 2.6	13.8 ± 1.9
Day 14 of lactation	13.1 ± 3.1	13.3 ± 3.6	12.7 ± 2.6	13.6 ± 1.9
Group Mean Litter Weight g				
Day 1	87 ± 18	92 ± 25	83 ± 14	91 ± 15
Day 4	123 ± 24	129 ± 34	116 ± 21	125 ± 23
Day 14	343 ± 51	344 ± 89	329 ± 51	336 ± 38
Mean Pup Weight (g)				
Males: Day 1	6.8 ± 0.9	6.8 ± 0.6	6.3 ± 0.7	6.5 ± 0.7
Day 14	28.0 ± 5.0	26.5 ± 3.4	26.7 ± 3.3	25.5 ± 1.9
Females: Day 1	6.4 ± 0.7	6.4 ± 0.6	6.0 ± 0.7	6.0 ± 0.7
Day 14	26.5 ± 4.7	25.5 ± 2.9	25.9 ± 3.2	24.4 ± 2.0

[a] = Excludes litters where all pups died ± = Standard deviation

GENETIC TOXICOLOGY

Study Title: Testing for Mutagenic Activity With Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100 And Escherichia coli WP2uvrA
Study No: [redacted] Report 14554; [redacted] Project 759591; Novo Nordisk 970104
Study Type: Ames Test
Volume # 1.17 and Page # 2 :
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 11 Dec 96; 17 Jan 97
GLP Compliance: Yes
QA – Reports Yes (X) No ( ): Report and Standard Operating Procedure audited.
Drug Lot Number: Batch 635353
Study Endpoint: See criteria for positive results below.
Methodology: Procedures used are based on the method of Ames et al. (1975). Tests were conducted using the pre-incubation method, on agar plates in the presence and absence of an Aroclor 1254 induced rat liver preparation and co-factors (S9 mix) required for mixed-function oxidase activity. Positive control compounds demonstrated the sensitivity of the assay and the metabolizing potential of the S9 mix. The study was performed to comply with Japanese MHW Guidelines on toxicity Studies (YakuShin 1 No 24, 11 Sep. 1989).

- **Strains/Species/Cell line:** Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100 and Escherichia coli WP2uvrA
- **Dose Selection Criteria:** Concentrations of 156.25, 312.5, 625, 1250, 2500 and 5000 µg per plate.
  - **Basis of dose selection:** A toxicity test was performed using TA 100 only, in the presence and absence of S9 mix, to establish suitable dose levels for the mutation tests. One plate of each of the following concentrations of Poloxamer 188 was used: 0.1, 1, 10, 100, 1000 and 5000 µg per plate. No toxicity to the bacteria was observed and there was no precipitation of test material.
  - **Range finding studies:** See basis of dose selection.
- **Metabolic Activation System:** S9 mix from livers of Male Fischer 344 rats injected once i.p. with Aroclor 1254 (diluted in corn oil to a concentration of 200 $mg.ml^{-1}$) at a dosage of 500 $mg.kg^{-1}$. The S9 mix sterility was checked with every experiment.
- **Controls:**
  - **Vehicle:** Ultra-pure water was used to dissolve and dilute Poloxamer 188.
  - **Negative Controls:** Ultra-pure water was used as the vehicle control.
  - **Positive Controls:** 2-aminoanthracene (2-AAN), methyl methanesulphonate (MMS), N-ethyl-N-nitro-N-nitrosoguanidine (ENNG), 9-Aminoacridine (9AA) and 2-Nitrofluorene (2-NF)

    The positive control substances, except methyl methanesulphonate, were dissolved in dimethylsulphoxide. Methyl methanesulphonate was dissolved in sterile, ultra-pure water.
  - **Comments:** The inducer was a polychlorinated biphenyl mixture, Aroclor 1254. Male Fischer 344 rats were used to prepare the S9.
    plates with 2% glucose used in the Ames test were prepared in-house using purified agar.
- **Exposure Conditions:**
  - **Incubation and sampling times**

  - **Doses used in definitive study:** Concentrations of 156.25, 312.5, 625, 1250, 2500 and 5000 µg per plate.
  - **Study design:** Ames Test
- **Analysis:**
  - **No. slides/plates/replicates/animals analyzed:** Triplicate plates were prepared for each bacterial strain and dose level in both the presence and absence of S9 mix. Vogel-Bonner Medium E agar plates with 2% glucose used in the Ames test were prepared in-house using purified agar.
  - **Counting method;**
  - **Cytotoxic endpoints:** Toxicity to bacteria; precipitation
  - **Genetic toxicity endpoints/results:** See criteria for positive results.
  - **Statistical methods:** None?
- **Criteria for Positive Results:** A significant mutagenic response was recorded if there was: **i)** for S.typhimurium strains TA 1535, TA 1537 and TA 98 and for E coli at least a doubling of the mean concurrent vehicle control values at some concentration of the test substance and, for S. typhimurium strain TA 100, a 1.5 fold increase over the control value. If the mean colony count on the vehicle control plates was less than 10, then a value of 10 was assumed for assessment purposes. In such cases a minimum count of

20 was required before a significant mutagenic response was identified. **ii)** a dose related response, although at high dose levels this relationship could be inverted because of, for example, (1) toxicity to the bacteria generally, (2) specific toxicity to the mutants and (3) inhibition of foreign compound metabolizing enzymes where mutagens require metabolic activation by the liver. **iii)** a reproducible effect in independent tests.

Results:

- **Study Validity:** Reported that all tests were acceptable according to the study criteria except the second test with TA 98; the culture used was contaminated. The test was repeated and met required criteria.
- **Study Outcome:** No precipitation was seen and there was no toxicity to the bacteria. Poloxamer 188 was not mutagenic to Salmonella typhimurium or Escherichia coli when tested in ultra-pure water up to a predetermined maximum limit.

Summary: Poloxamer 188 was tested for mutagenic activity in Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and Escherichia coli WP2 uvrA at concentrations ranging from 156.25 to 5000 µg per plate. Tests were conducted using the pre-incubation method on agar plates in the presence and absence of an Aroclor 1254 induced rat liver preparation and co-factors (S9 mix) required for mixed-function oxidase activity. Positive controls demonstrated the sensitivity of the assay and the metabolizing potential of the S9 mix.

There was no toxicity to bacteria and no precipitation. No mutagenic activity was observed in any of the 5 bacterial strains, either activated or non-activated. It was concluded that Poloxamer 188 was not mutagenic in this system.

GENETIC TOXICOLOGY

Study Title: Poloxamer 188: Chromosomal Aberrations Assay with Human Peripheral Lymphocyte Cultures in vitro.
Study No: [redacted] Report 15028; [redacted] Project 759607; Novo Nordisk 970103
Study Type: Chromosomal Aberrations Assay for evaluation of clastogenic potential
Volume # 1.17 and Page #: 50
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 29 Nov 96/29 Apr 97
GLP Compliance: Yes
QA – Reports Yes (X) No ( ): Report and Standard Operating Procedure audited.
Drug Lot Number: Batch 635353
Study Endpoint: See Genetic toxicity endpoints below.
Methodology:

- **Strains/Species/Cell line:** human peripheral blood lymphocyte cultures
- **Dose Selection Criteria:**
  - **Basis of dose selection:** For the first experiment 9 dose levels, covering a wide concentration range were tested. The highest dose was 5 $mg.ml^{-1}$, the maximum allowable concentration. Subsequent dose levels were "halving" dilutions of this dose. Poloxamer 188 was non toxic and non clastogenic, therefore in the second experiment, the dose levels selected were the same as the highest 4 concentrations used in the first test.
- **Metabolic Activation System:** Post-mitochondrial supernatant fraction obtained from the livers of adult male rats treated with Aroclor 1254 (S9) and a NADPH-generating system.
- **Controls:**
  - **Vehicle:** Culture Medium: RPMI 1640 medium with 25 mM HEPES buffer and supplemented with the antibiotic minocycline. For the growth and treatment period this medium was supplemented with 15% fetal bovine serum. For washing, serum free medium was used.
  - **Positive Controls:** cyclophosphamide [0.01-0.05 $mg.^{ml-}1$] and mitomycin C [0.00001-0.0005 $mg.ml^{-1}$]
  - **Comments:** The study was conducted incorporating 2 independent tests.
- **Exposure Conditions:**
  - **Incubation and sampling times:** See study design below.
  - **Doses used in definitive study:** Poloxamer 188 was tested at concentrations ranging from 0.02 to 5 $mg.ml^{-1}$. Poloxamer 188 was considered non-toxic in this assay. Poloxamer 188 was non toxic and non clastogenic, therefore in the second experiment, the dose levels selected were the same as the highest 4 concentrations used in the first test.
  - **Study design:** Cultures established ca 48 hours before testing were treated for 5 hours in the presence or 25 hours in the absence of S9 mix. Cultures were harvested at 29 hours (Test 1) or 53 hours (Test 2) post treatment.

    The lymphocyte mitogen, phytohemagglutinin (PHA), was supplied in freeze dried form and reconstituted to 4.5 $mg.ml^{-1}$. Lymphocyte cultures were established by adding whole blood (10%) to tissue culture medium (88%) containing phytohemagglutinin (PHA – 2%). Aliquots of the suspension, 5 ml (Test 1) or 10 ml (Test 2) were dispensed into tissue culture tubes. Cells were incubated at 37°C, the pH of the culture medium was 6.8-7.2.

    Culturing the cells in medium containing colcemid for 3 hours accumulated cells in metaphase; the stage of cell division at which chromosomes can be examined using light microscopy. Five slides per culture were made. These were also examined for mitotic indices and signs of cellular necrosis.

    A further assessment of polyploidy was also made using approximately 300 metaphase cells.

- **Analysis:**
  - **No. slides/plates/replicates/animals analyzed:** From 2-4 slides per culture, up to 50 metaphase cells per slide, a total of 100 metaphase cells per culture were examined where possible.
  - **Counting method:** [redacted] microscope
  - **Cytotoxic endpoints:** The following 2 parameters are generally taken together to judge toxic levels of a test material. A dose level was considered to be toxic if the mitotic indices were less than 60% of the mean vehicle control values. If however, mitotic indices are greater than 60%, then dose levels can be considered toxic if there are consistent changes to cell morphology on the slides.

    Poloxamer 188 was non toxic with one exception, to human peripheral blood lymphocytes in both the presence and absence of S9 mix. The exception was seen in Test 1, in the presence of S9 mix, in one culture treated with 0.156 $mg.ml^{-1}$. As this was not seen in the duplicate culture, or was not dose related, the sponsor deemed the response sporadic.
  - **Genetic toxicity endpoints:** The results for test material and positive control treated cultures are evaluated by comparison with the concurrent vehicle control cultures and with historical negative control data. A negative response was recorded if responses from the test material treated cultures are within the 95% confidence limits for the historical negative control data. The response of a single dose was classified as significant if the percent of aberrant cells is consistently greater than the 99% confidence limits for the historical negative control data or greater than double the frequency of an elevated vehicle control culture, if appropriate. A test was positive if the response in at least one acceptable dose level is significant by the criterion described above.

    A test material was positive if both Test 1 and 2 were positive, as described above or if the second test was positive after the first test gave indications of activity. These indications may be suspicious levels of aberrant cells (between 95% and 99% confidence limits). Experiments that met in part the criteria for a positive response, or marginally met all the criteria, were classed as inconclusive.
  - **Statistical methods:** (?)
- **Criteria for Positive Results:** See Genetic toxicity endpoints above.

Results:

- **Study Validity:** Enzymatic activity of each batch of S9 was characterized by testing selected pre-mutagens in an Ames test with TA 1538. The test material was freely soluble in RPMI 1640 medium and osmolality of the culture medium was not affected. No precipitation was noted in cultures treated with Poloxamer 188. The following criteria were fulfilled: there was no evidence of contamination; the results of vehicle and positive control cultures were typical; the test material had 3 acceptable dose levels for assessment.
- **Study Outcome:** All cultures treated with Poloxamer 188 had levels of structural aberrations within the 95% confidence for a negative response. An extra assessment of polyploidy was carried out in the cultures harvested at 48 hours. All cultures had levels of numerical aberrations within the 95% confidence limits of a negative response. Thus, Poloxamer 188 was considered not clastogenic when tested for such effects in vitro with human peripheral blood lymphocytes.

Summary:

The objective of this study was to determine the potential of Poloxamer 188 to induce chromosomal aberrations in human peripheral lymphocyte cultures in vitro. S9 mix (prepared from rat liver) provided an exogenous metabolic activation system for increasing the detection capability of the test system for pre-mutagens. Poloxamer 188 was tested at concentrations ranging from 0.02 to 5 $mg.ml^{-1}$. Poloxamer 188 was considered non-toxic in this assay. Poloxamer 188 was non toxic and non clastogenic, therefore in the second experiment, the dose levels selected were the same as the highest 4 concentrations used in the first test.

All cultures treated with Poloxamer 188 had levels of structural aberrations within the 95% confidence for a negative response. An extra assessment of polyploidy was carried out in the cultures harvested at 48 hours. All cultures had levels of numerical aberrations within the 95% confidence limits of a negative response. Thus, Poloxamer 188 was considered not clastogenic when tested for such effects in vitro with human peripheral blood lymphocytes.

APPEARS THIS WAY
ON ORIGINAL

GENETIC TOXICOLOGY

Study Title: Poloxamer 188: Mouse Lymphoma Mutation Assay
Study No: [redacted] Report 14906, [redacted] Project 759612, Novo Nordisk Study 970105
Study Type: Mouse Lymphoma Mutation Assay
Poloxamer 188 was assayed for mutagenic potential in the mouse lymphoma L5178Y cell line, clone 3.7.2.C, scoring for forward mutations at the thymidine kinase locus: tk^+tk^- to tk^-tk^-.
Volume # 1.17 and Page #: 105
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 29 Nov 96/31 Mar 97
GLP Compliance: Yes
QA – Reports Yes (X) No ( ): Short term study not individually inspected. Processes involved are inspected at intervals according to a predetermined schedule. Report has been audited.
Drug Lot Number: Poloxamer 188 Batch 635353
Study Endpoint: The objective of the study was to determine the potential of the test material to induce forward mutations at the tk^+tk^- locus of mouse lymphoma L5178Y cells.

Methodology:

- **Strains/Species/Cell line:** Cells were from the tk^+tk^- -3.72.C mouse lymphoma L5178Y cell line.
- **Dose Selection Criteria:**
  - **Basis of dose selection:** A preliminary cytotoxicity test showed that Poloxamer 188 was not toxic at the preset maximum concentration of 5000 $\mu g.ml^{-1}$. Initial toxicity tests were performed in the absence and presence of S9 mix.
  - **Range finding studies:** A preliminary cytotoxicity test showed that Poloxamer 188 was not toxic at the preset maximum concentration of 5000 $\mu g.ml^{-1}$.
- **Test Agent Stability:** The test material formulations were prepared within 1 hour of dosing. Chemical analysis of the test material formulations was not carried out.
- **Metabolic Activation System:** S9 Mix - Arochlor 1254-induced S9 enzymes (the supernatant of the post-mitochondrial 9000 G fraction) were prepared from the livers of adult, male Fischer rats, as described by Ames et al. (1975).
- **Controls:**
  - **Vehicle:** Culture Medium: The basic culture medium (F_0P) was Fischer's medium supplemented with [redacted]

    The medium used during treatment was F_0P supplemented with [redacted]

    For colony formation, cloning medium was used, consisting of F_0P supplemented with [redacted] for selection of tk^-tk^- cells, cloning medium was supplemented with [redacted]
  - **Negative Controls:** Vehicle control cultures were included.
  - **Positive Controls:** Positive controls used in the absence of S9 mix were: 250 $\mu g.ml^{-1}$ ethyl methanesulphonate (EMS), a large colony inducer; and 10 $\mu g.ml^{-1}$ methyl methanesulphonate (MMS), which usually induces greater numbers of small colonies. In the presence of S9 mix, 3-methylcholanthrene (3-MC), a large and small colony inducer, was used at a concentration of 2.5 $\mu g.ml^{-1}$.

- **Exposure Conditions:**
  - **Incubation and sampling times:** [redacted] On Day 2 cell counts were determined. Expression of genetic damage was determined by performing 2 parallel cloning assays: the viability assay and the mutant selection assay.
  - **Doses used in definitive study:**
    The concentrations of Poloxamer 188 tested were as follows ($\mu g.ml^{-1}$):
    Assay 1 (in the absence of S9 mix): 625, 1250, 2500 and 5000
    Assay 2 (in the presence of S9 mix): 625, 1250, 2500 and 5000
    Assay 3 (in the absence of S9 mix): 150, 500, 1500 and 5000
    Assay 4 (in the presence of S9 mix): 150, 500, 1500 and 5000
  - **Study design:** This study was designed to comply with OECD Guideline No. 476, Directive 97/302/EEC Part B and Addendum 9 of the US EPA Pesticide Assessment Guidelines Subdivision F, Series 84-2. Two principal methods of performing the mouse lymphoma assay exist; the soft agar cloning method and the microwell method. [redacted] uses the agar cloning method. [It is reported that the laboratory has performed a comparison of tests (with other compounds!) and has obtained similar results with the 2 methods.]
- **Analysis:**
  - **No. slides/plates/replicates/animals analyzed:** Duplicate cultures were carried through the experiments for each treatment point. [Viability-testing 3 plates.]
  - **Counting method:** [redacted] image analyzer - [redacted]
  - **Cytotoxic endpoints:** Relative total growth (RTG) of treated groups
  - **Genetic toxicity endpoints/results:** 2 categories of genetic damage: Chemicals inducing point mutations or slight structural chromosomal damage in the region of the TK locus result in cells that otherwise grow normally. Chemicals inducing large chromosomal aberrations about either side of the TK locus result in cells that have reduced rates of growth in soft agar cloning medium. Colony size distribution patterns are able therefore to determine whether chemicals are causing small or large scale chromosomal damage.
    Poloxamer 188 was assessed for mutagenic activity at concentrations of 625, 1250, 2500 and 5000 $\mu g.ml^{-1}$ in the absence and presence of S9 mix. Absence of S9 mix (Assay 1): No significant increase in mutant fraction was obtained at any dose level of Poloxamer 188. Presence of S9 mix (Assay 2): Increases in mutant fraction up to 1.7 fold were obtained over the vehicle control values across the range of concentrations of Poloxamer 188. A second assay was carried out with an increased spread of dose levels.
    Concentrations of: 150, 500, 1500 and 5000 $\mu g.ml^{-1}$. Absence of S9 mix (Assay 3): an increase of 1.6-fold over the vehicle control was obtained at 500 $\mu g.ml^{-1}$. No similar increases were observed at higher or lower concentrations. Presence of S9 mix (Assay 4): No evidence of mutagenic activity. Separation of the 2 colony sizes was well defined in Assays 1 and 2, showing the presence of both small type colonies (indicative of chromosomal aberrations) and large type colonies (indicative of small deletions and/or point mutations). In Assays 3 and 4 the separation of the 2 populations was less clear, although the shapes of the histograms indicated the presence of both colony types.
  - **Statistical methods:** ANOVA and t-test
- **Other:** Negative Response: A negative response was recorded if responses from the test material were not significantly higher than those of the vehicle control. The provision was that the chemical was tested to pre-set limits that included either a reduction of

relative total growth to 20%, or precipitation of the test material, or a maximum acceptable dose of 5000 $\mu g.ml^{-1}$.

- **Criteria for Positive Results:** The response at a single dose was classified as significant if the cloning efficacy was at least 10% of the current vehicle and the mean mutant fraction of the 2 duplicate cultures was at least 1.7 fold higher than the mean control value [McGregor, et al. (1988)]. An experiment was positive if the response in at least the highest acceptable dose was significant by the criterion described above and was associated with an increase in mutant numbers or (but preferably and) an upward trend in the remaining doses. A test material was positive if 2 positive experiments out of 2 were recorded within the same activation condition. In addition, a test material was classified as positive in one or other activation condition if the following occurred: the first experiment indicated that the test material was positive, but did not meet the necessary criteria due to the lack of results from a critically toxic concentration. The second experiment (conducted over a focused dose range) gave an unequivocal positive.

  Inconclusive Response: Experiments that met in part the criteria for a positive response, or marginally met all the criteria, were classed as inconclusive.

Results: There was no toxicity at the preset maximum concentration of 5000 $\mu g.ml^{-1}$. Solvent control values were within the normal ranges experienced in this laboratory and reported in the literature with the L5178Y cell line. The high mutant fractions obtained with EMS, MMS and 3-MC were within the normal ranges for this laboratory. 3-MC (which is not mutagenic in the absence of S9 mix) proved the efficacy of the S9.

- **Study Outcome:**

  The sponsor concluded that Poloxamer 188 is not mutagenic in mouse lymphoma L5178Y cells, in either the absence or the presence of S9 mix, when tested to the preset maximum concentration of 5000 $\mu g.ml^{-1}$. [Although some increases in mutant fraction were observed in some Poloxamer 188 treated cultures, there was no evidence of a reproducible dose-related mutagenic effect in either the absence or presence of S9 mix.]

Summary:

Poloxamer 188 was assayed for mutagenic potential in the mouse lymphoma L5178Y cell line, clone 3.7.2.C, scoring for forward mutations at the thymidine kinase locus: tk^+tk^- to tk^-tk^-. Tissue culture medium was the solvent. Tests were conducted both in the absence and in the presence of a post-mitochondrial supernatant fraction obtained from the livers of adult, male rats. It is reported that the assay was designed to be consistent with the current guidelines of the EC, OECD and US EPA.

Poloxamer 188 was not toxic at the preset maximum concentration of 5000 $\mu g.ml^{-1}$.

Final concentrations of Poloxamer 188 in the treatment medium ranged between 150 and 5000 $\mu g.ml^{-1}$ in the absence and presence of S9 mix. Positive control cultures were included and duplicate cultures were used for each treatment point. Vehicle controls were tested in quadruplicate.

Although increases in mutant fraction were seen in some Poloxamer 188-treated cultures there was no evidence of a reproducible mutagenic effect in either the absence or presence of S9 mix. The sponsor thus concluded that Poloxamer 188 is not mutagenic in mouse lymphoma L5178Y cells tested up to a concentration of 5000 $\mu g.ml^{-1}$.

House Lymphoma in the Absence of S9 Mix
Data Summary
(Assay 1)

Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth (Vehicle Mean = 12.6)	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} $\frac{200\ (MC)}{(VC)}$	Fold Increase Over Control (Vehicle Mean = 98.8)
FdP	(4 ml added)		76	15.3	11.6	92		84	
			88	13.1	11.5	91		109	
			77	17.9	13.8	109		105	
			78	17.4	13.6	108		97	
Ethyl methanesulphonate	250		59	14.9	8.8	70ϕ		371	3.8
			77	11.8	9.1	72		338	3.4
Methyl methanesulphonate	10		49	8.0	3.9	31		345	3.5
			54	8.2	4.4	35		308	3.1
Poloxamer 188	625		78	13.9	10.8	86		144	1.5
			88	12.8	11.3	90		83	0.8
	1250		81	12.2	9.9	78		139	1.4
			80	13.7	11.0	87		85	0.9

* = Cloning efficiency x growth in suspension
ϕ = The method of calculating this value is detailed in Appendix 12b

Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth (Vehicle Mean = 12.6)	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} $\frac{200\ (MC)}{(VC)}$	Fold Increase Over Control (Vehicle Mean = 98.8)
Poloxamer 188	2500		80	16.3	13.0	103		73	0.7
			91	13.3	12.1	96		119	1.2
	5000		76	12.0	9.1	72		87	0.9
			77	14.9	11.5	91		119	1.2

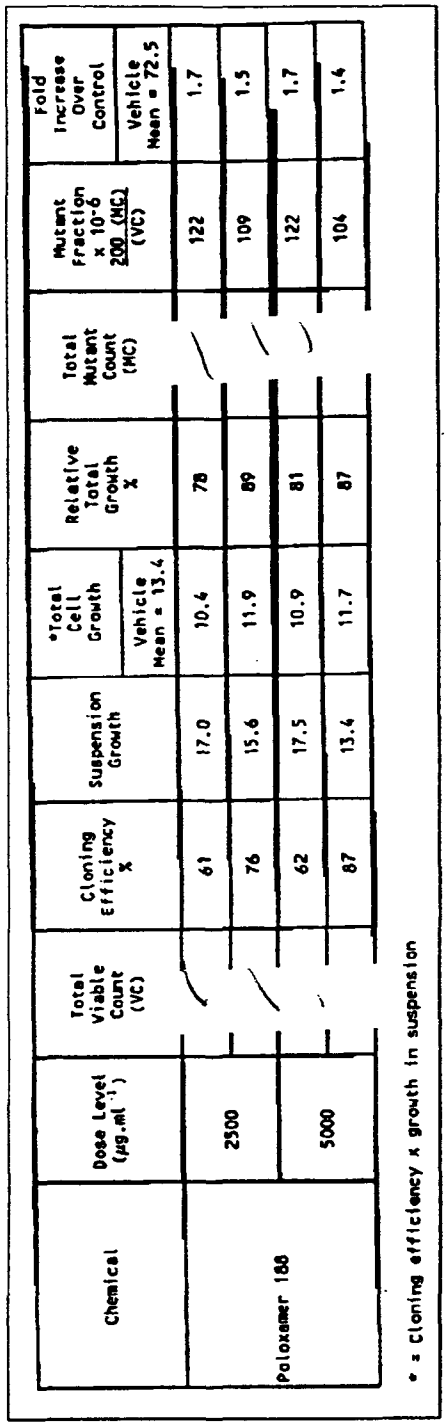
* = Cloning efficiency x growth in suspension

Mouse Lymphoma in the Presence of S9 Mix (FLI 083)
Data Summary
(Assay 2)

Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth (Vehicle Mean = 13.4)	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} $\frac{200\ (MC)}{(VC)}$	Fold Increase Over Control (Vehicle Mean = 72.5)
FOP	(4 ml added)		78	17.9	14.0	104		83	
			82	16.3	13.4	100		59	
			81	15.8	12.8	96		77	
			70	19.1	13.4	100		71	
3-Methylcholanthrene	2.5		43	11.1	4.8	36		568	7.8
			49	10.6	5.2	39		545	7.5
Poloxamer 188	625		76	15.2	11.6	87		91	1.3
			71	13.9	9.9	74		99	1.4
	1250		66	16.9	11.2	84		80	1.1
			77	13.3	10.2	76		107	1.5

* = Cloning efficiency x growth in suspension

Assay 2 continued:



Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth (Vehicle Mean = 13.4)	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} $\frac{200\ (MC)}{(VC)}$	Fold Increase Over Control (Vehicle Mean = 72.5)
Poloxamer 188	2500		61	17.0	10.4	78		122	1.7
			76	15.6	11.9	89		109	1.5
	5000		62	17.5	10.9	81		122	1.7
			87	13.4	11.7	87		104	1.4

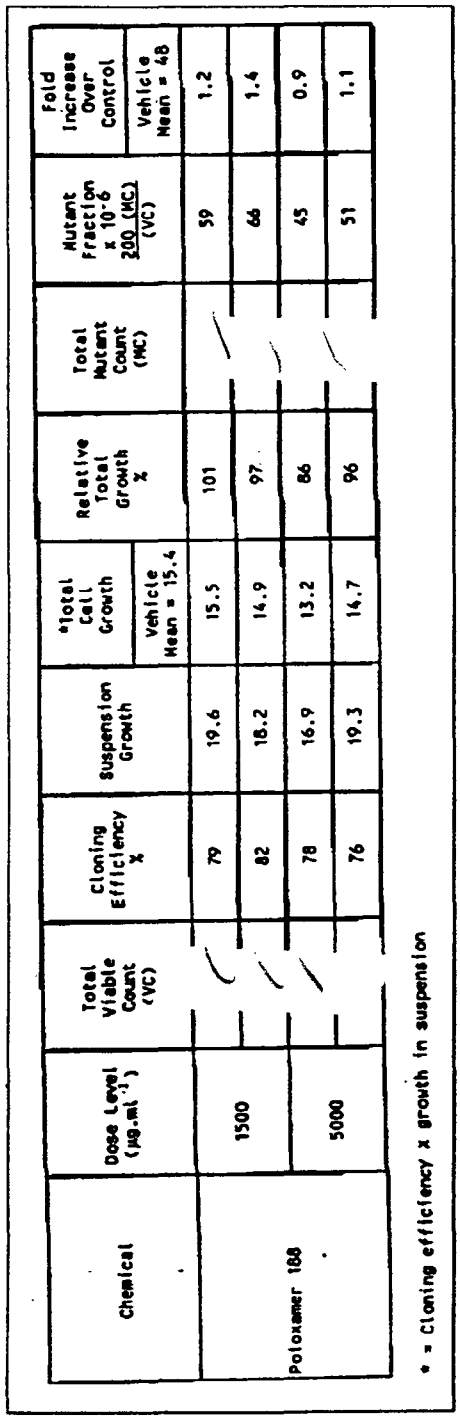
* = Cloning efficiency x growth in suspension

House Lymphoma in the Absence of S9 Mix
Data Summary
(Assay 3)

Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth (Vehicle Mean = 15.4)	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} 200 (MC) (VC)	Fold Increase Over Control (Vehicle Mean = 48)
FoP	(4 ml added)		71	19.2	13.6	89		33	
			72	19.8	14.3	93		50	
			82	18.6	15.3	100		59	
			89	20.4	18.2	119		50	
Ethyl methanesulphonate	250		39	14.2	5.5	36		435	9.5
			45	18.1	8.1	53		409	8.5
Methyl methanesulphonate	10		24	12.6	3.0	20		414	8.6
			35	13.1	4.6	30		243	5.1
Poloxamer 188	150		66	19.7	13.0	85		32	0.7
			73	20.0	14.6	95		55	1.1
	500		66	19.9	13.1	85		79	1.6
			80	18.5	14.8	96		76	1.6

* = Cloning efficiency x growth in suspension

Assay 3 continued:



Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth (Vehicle Mean = 15.4)	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} $\frac{200\ (MC)}{(VC)}$	Fold Increase Over Control (Vehicle Mean = 48)
Poloxamer 188	1500		79	19.6	15.5	101		59	1.2
			82	18.2	14.9	97		66	1.4
	5000		78	16.9	13.2	86		45	0.9
			76	19.3	14.7	96		51	1.1

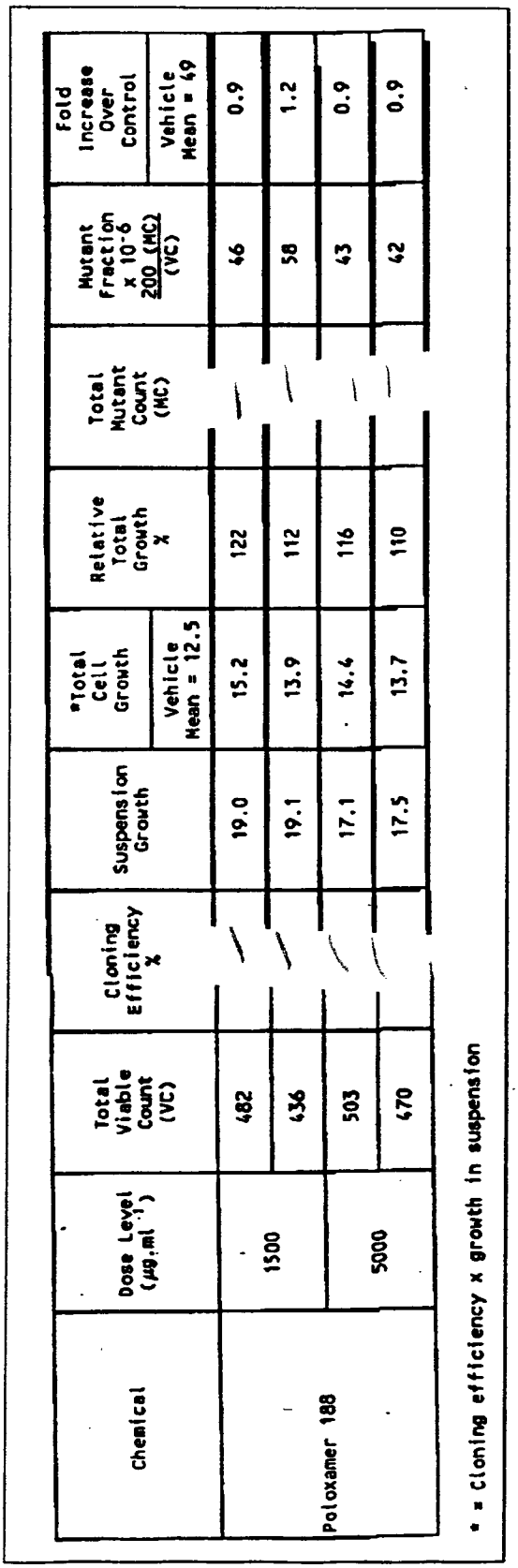
* = Cloning efficiency x growth in suspension

Mouse Lymphoma in the Presence of S9 Mix (FLI 083)
Data Summary
(Assay 4)

Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Visible Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth (Vehicle Mean = 12.5)	Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} 200 (MC) (VC)	Fold Increase Over Control (Vehicle Mean = 49)
FOP	(4 ml added)		62	18.2	11.3	91		51	
			67	15.7	10.5	84		51	
			76	20.6	15.7	126		44	
			72	17.1	12.3	99		50	
3-Methylcholanthrene	2.5		34	13.4	4.6	37		321	6.6
			28	12.9	3.6	29		429	8.8
Poloxamer 188	150		65	20.4	13.3	107		33	0.7
			72	17.6	12.7	102		43	0.9
	500		62	22.4	13.9	112		56	1.1
			70	18.1	12.7	102		34	0.7

* = Cloning efficiency x growth in suspension

Assay 4 continued:



Chemical	Dose Level ($\mu g.ml^{-1}$)	Total Viable Count (VC)	Cloning Efficiency %	Suspension Growth	*Total Cell Growth		Relative Total Growth %	Total Mutant Count (MC)	Mutant Fraction x 10^{-6} $\frac{200\ (MC)}{(VC)}$	Fold Increase Over Control
						Vehicle Mean = 12.5				Vehicle Mean = 49
Poloxamer 188	1500	482		19.0	15.2		122		46	0.9
		436		19.1	13.9		112		58	1.2
	5000	503		17.1	14.4		116		43	0.9
		470		17.5	13.7		110		42	0.9

* = Cloning efficiency x growth in suspension

GENETIC TOXICOLOGY

Study Title: Poloxamer 188: Micronucleus Test in Bone Marrow of CD-1 Mice
Study No: [redacted] Report 14785; [redacted] Project 759628; Novo Nordisk 970106
Study Type: In vivo genotoxic (clastogenic) potential
Volume # 1.17 and Page #: 171
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 29 Nov 96/28 Feb 97
GLP Compliance: Yes
QA – Reports Yes (X) No ( ): Short term study not individually inspected. Processes involved are inspected at intervals according to a predetermined schedule. Report has been audited.
Drug Lot Number: Poloxamer 188 Batch 635353
Study Endpoint: The analysis indicated that a positive response is to be suspected if the total numbers of micronuclei within any one sample group, of a given number of mice, exceed those shown in the table below (next page).
Methodology:

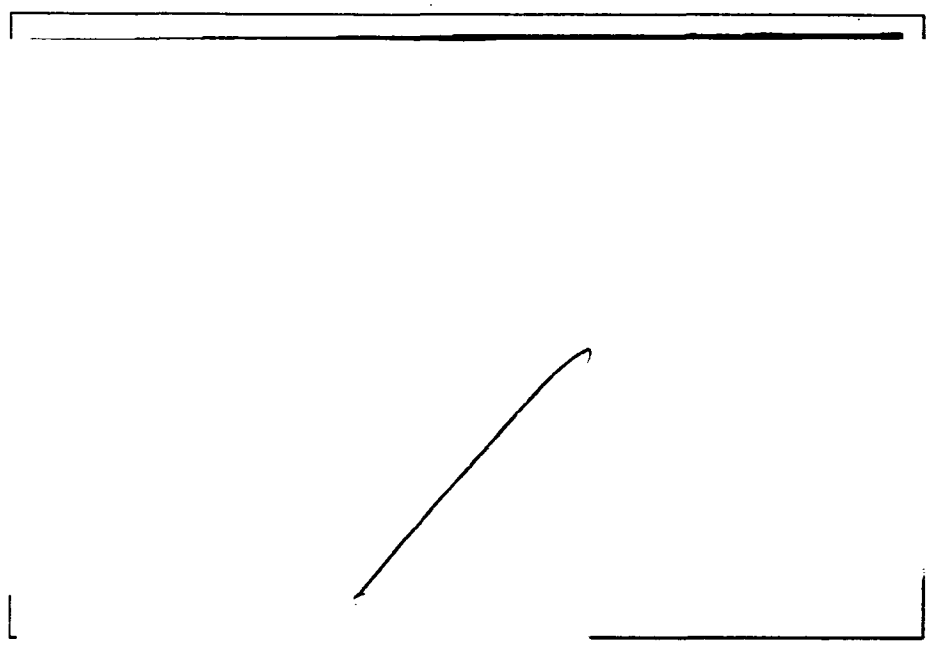
- **Strains/Species/Cell line:** Male and Female CD-1 mice 5M;5F regular assessment base.
- **Dose Selection Criteria:** Dose volume 10 $ml.kg^{-1}$ body weight s.c.
  - **Basis of dose selection:** Limit toxicity test: 2M;2F CD-1 mice were dosed s.c. at 0 h + 24 h with 2000 mg Poloxamer $188.kg^{-1}.day^{-1}$. Mice were observed for clinical signs or mortality at frequent intervals. Surviving mice were killed on Day 4.
  - **Range finding studies:** A limited toxicity study was undertaken to confirm the non-toxicity of Poloxamer 188 at the maximum recommended dose of 2000 $mg.kg^{-1}.day^{-1}$
- **Test Agent Stability:**
- **Controls:**
  - **Vehicle:** water for irrigation
  - **Positive Controls:** Cyclophosphamide (CPH) 50 $mg.kg^{-1}$
- **Exposure Conditions:**
  - **Incubation and sampling times:** CD-1 mice were dosed s.c. at 0 h and 24 h with test or control materials, then femur marrow samples were taken 24 h later.
  - **Doses used in definitive study:** 2000 mg Poloxamer $188.kg^{-1}$. The dose volume used for both the control and test material treated animals was a constant 10 $ml.kg^{-1}$ body weight.
  - **Study design:**. The test is reported to follow recommendations published by the US Environmental Protection Agency Gene-Tox Program and the Japanese collaborative Study Group for Micronucleus Testing.

    CD-1 mice were dosed s.c. at 0 h and 24 h with test (2000 mg Poloxamer 188 per kg) or control materials, then femur marrow samples were taken 24 h later. Two slides were prepared from each tube/animal. Two thousand (2000) polychromatic erythrocytes (PCE) per animal were scored for micronuclei and the frequency of micronucleated cells (MN-PCE) determined. The PCE/NCE ratio, a measure of any induced systemic toxicity, was determined by counting a minimum total of 1000 erythrocytes (PCE + NCE) per marrow preparation. Scored micronuclei were assigned on the basis of size into small or large categories.

- **Analysis:**
  - **No. slides/plates/replicates/animals analyzed:** Number of Mice Used: Limit Toxicity test: 4 males; Micronucleus Test: Vehicle Control 5M;5F; Test Material 10M;10F; Positive Control 5M. Two slides were prepared from each tube/animal.

- **Counting method:** [redacted] microscope
- **Cytotoxic endpoints:** Mice were observed for clinical signs or mortality at frequent intervals in a limit toxicity test.
- **Genetic toxicity endpoints/results:** Assuming a mean frequency of MN-PCE of 0.128% per mouse, Salamone et al. (1980) were able to calculate statistical criteria for a negative or positive micronucleus response. The analysis indicated that a positive response is to be suspected if the total numbers of micronuclei within any one sample group, of a given number of mice, exceed those shown in the table below:

Sponsor's Table Vol. 1.17/188.



The background micronucleus incidence in vehicle control dosed CD-1 mice, treated with distilled water, corn oil or 0.5% carboxymethylcellulose, is 0.121% in the laboratory conducting this study.

- **Statistical methods:** (?) Assuming a mean frequency of MN-PCE of 0.128% per mouse, Salamone et al. [in J Ashby and F de Serres (eds.), Proceedings of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity, Elsevier, Amsterdam, 1980, pp. 686-697] were able to calculate statistical criteria for a negative or positive micronucleus.
- **Criteria for Positive Results:** See Genetic toxicity endpoints/results above.

Results:

- **Study Validity:** The positive control (50 mg cyclosphosphamide.kg^{-1}) induced large increases in bone marrow micronuclei.
- **Study Outcome:**

  Toxicity Study: No deaths occurred following exposure to 2000 mg Poloxamer 188.kg^{-1}.day^{-1} Swellings were observed in the shoulder and dorsal thoracic region following treatment.

  Micronucleus Test: The only clinical sign seen with test material mice was a swelling in the dorsal neck region.

  Vehicle Control Group: The numbers of micronucleated bone marrow polychromatic erythrocytes (MN-PCE) in mice dosed with the vehicle averaged 0.07%. This MN-PCE frequency conformed to the established in-house control range for vehicle treated CD-1 mice (0.00-0.24% per 10 mice).

  Positive Control Group: 50 mg cyclophosphamide.kg^{-1}, induced large increases in bone marrow micronuclei. The mean MN-PCE frequency for the mice was 1.43%. An evident increase in the number of MN-NCE was also seen.

  Test Material Group: There was no indication that Poloxamer 188 induced bone marrow micronuclei in treated mice. The highest MN-PCE frequency recorded for the test material was in the females where an incidence of 0.04% was observed.

Summary:

The in vivo genotoxic potential of Poloxamer 188 was evaluated in a micronucleus test in bone marrow erythrocytes of young, male and female CD-1 mice following a 0 h + 24 h subcutaneous dosing and 48 h sampling regimen at a single dose level. A limit toxicity study was performed to confirm the non-toxicity of Poloxamer 188 at the maximum recommended dose of 2000 mg.kg^{-1}.day^{-1}.

One group of CD-1 mice were dosed at 0 h and 24 h s.c. with the test material at the maximum recommended concentration of 2000 mg.kg^{-1}.day^{-1}. Bone marrow samples were taken 48 h after the initial 0 h dose. Two control groups of CD-1 mice were also dosed s.c. with either the 10 ml water for irrigation.kg^{-1}.day^{-1} vehicle, or the positive control, 50 mg cyclophosphamide.kg^{-1}.day^{-1}.

Mice treated with the vehicle alone showed normal background levels of micronuclei; those dosed with cyclophosphamide showed substantial increases in the numbers of bone marrow micronuclei.

No micronucleus induction was seen in the bone marrow erythrocytes of mice dosed with 2000 mg Poloxamer 188.kg^{-1}.day^{-1}.

PHARMACOKINETICS/TOXICOKINETICS

Poloxamer 188: Vol. 1.17/201; 239,290

To support the subcutaneous route of administration two single studies (one in rats and one in dogs) have been performed with Poloxamer 188. Both studies investigated 3 doses of Poloxamer 188, 5, 50 and 500 mg/kg in the rat and 0.5, 5 and 50 mg/kg in the dog. The plasma concentration of Poloxamer 188 was based on total radioactivity following administration of [^{14}C]-Poloxamer 188.

An analytical method for the determination of Poloxamer 188 in water solutions has been developed and validated. The procedure was found to be satisfactory over ranges of ca 1 mg.ml^{-1} to ca 200 mg.ml^{-1} Poloxamer 188 in the formulations.

Non-radiolabeled Poloxamer 188: Batch 635353

Carbon-14 labeled Poloxamer 188 Batch CSL-97-700-11-29. Specific activity 0.26 MBq/mg

Male Rat (Sprague-Dawley): [redacted] Project 159737 dtd. March 1998. [Q.A – Present]

Dose levels : 5, 50, 500 mg/kg

Other studies:

Additional pharmacokinetic data has been obtained in the 28-day toxicology studies in rats (10, 100 and 500 mg/kg) and dogs (0.5, 3 and 20 mg/kg) where plasma samples were taken at days 1 and 25 for toxicokinetics following administration of [^{14}C]-Poloxamer 188 on these days only.

Comments: Reported that at the lowest doses only [^{14}C]-Poloxamer 188 was administered and it would not be feasible to get reliable pharmacokinetics and excretion data if the dose was reduced further using the present techniques.

Summary:

RAT: Excretion and plasma kinetics of total radioactivity were investigated in male rats following a single s.c. administration of [^{14}C]-Poloxamer 188 at doses of 5, 50, 500 mg/kg.

Radioactivity was extensively and rapidly absorbed from the s.c. dosing site with peak plasma concentrations seen at 2 hours post dose. Less than 1% of the dose remained at the injection site by 24 hours post dose.

More than 83% of the total dose was excreted after 24 hours. Most of the administered radioactivity was recovered in the urine with means of 86.4% for the 5 mg/kg dose, 83.1% for the 50 mg/kg dose and 74.1% of the dose for the 500 mg/kg dose over a 96 hour period.

Fecal radioactivity accounted for 3.5% of the dose at 5 mg/kg, 6.8% of the dose at 50 mg/kg and 14.7% at 500 mg/kg over 96 hours. The carcass contained 2-3% of the dose. Greater than [redacted] was recovered at all levels.

Plasma radioactivity was highest at 2 hours, with a mean of 2.27 µg equiv/ml at 5 mg/kg, 22.26 µg equiv/ml at 50 mg/g and 164.75 µg equiv/ml at 500 mg/kg. Concentrations of radioactivity initially decreased to a mean of 0.64, 6.32, and 107.80 µg equiv/ml at 6 hours and further to 0.15, 0.56 and 9.32 µg equiv/ml at 24 hours post low, mid- and high dose, respectively. By 96 hours post dose, the concentrations of total radioactivity had decreased to 0.05, 0.14 and 1.19 µg equiv/ml.

Radioactivity was rapidly absorbed from the injection site and rapidly eliminated from plasma at all dose levels, with greater than 83% of the total administered dose excreted by 24 hours post dose.

5 mg/kg: $AUC_{0\text{-}96h}$ = 19.8 µg equiv.h/ml

Elimination $t_{1/2}$ from plasma ca 3 h for the period 2-12 h

ca 46 h for the 24-96 h period

50 mg/kg: $AUC_{0\text{-}96h}$ = 131 µg equiv.h/ml
Elimination $t_{1/2}$ from plasma ca 2.7 h for the period 2-12 h
ca 38 h for the 24-96 h period

500 mg/kg: $AUC_{0\text{-}96h}$ = 1796 µg equiv.h/ml
Elimination $t_{1/2}$ from plasma ca 6 h for the period 2-12 h
ca 25 h for the 24-96 h period

Dose proportionality was seen indicating linear kinetics of radioactivity of Poloxamer 188 in rats up to the dose of 500 mg/kg.

DOG: Male Beagles age ca 4-6 mos., ca 10 kg. —— Project and Study 159742
[Q.A. - present.] Study initiation 12 Mar 97.
Dose levels: 0.5, 5, 50 mg/kg

Summary:

DOG: Total radioactivity excretion and plasma kinetics were studied in male beagles following a single s.c. administration of [^{14}C]-Poloxamer 188 at doses of 0.5, 5, and 50 mg/kg.

Radioactivity was rapidly absorbed and eliminated at all three dose levels with at least 68% of the dose recovered within 24 hours. Urine was the main route of excretion with recovery means of 73.9% at 0.5 mg/kg, 87.0% at 5 mg/kg and 89.3% at 50 mg/kg. Fecal radioactivity accounted for 3.8% at 0.5 mg/kg, 2.2% at 5 mg/kg and 1.9% at 50 mg/kg.

Total recovery at the low dose level was 84.6 ± 6.8%. At the two higher dose levels recovery in each animal was >93%. The proportion of the dose remaining at the injection sites after 120 h was small means being 0.06% at 5 mg/kg, 0.16% at 5 mg/kg and 0.03% at the 50 mg/kg level.

The highest mean plasma concentrations of total radioactivity were seen 2 hours post dose at the 0.5 mg/kg (0.391 µg equiv/ml) and 5 mg/kg (2.33 µg equiv/ml) The highest mean plasma concentrations for the 50 mg/kg dose (15.1 µg equiv/ml) was seen at 4 hours.

By 8 hours peak concentrations had declined steadily to 0.181 µg equiv/ml at 0.5 mg/kg, 1.13 µg equiv/ml at 5 mg/kg and 11,8 µg equiv/ml at the 50 mg/kg level. Concentrations continued to fall to 0.084 µg equiv/ml at 0.5 mg/kg, 0.41 µg equiv/ml at 5 mg/kg and 4.9 µg equiv/ml at 50 mg/kg at 24 hours. And at 120 hours values were 0.044 µg equiv/ml (0.5 mg/kg), 0.06 µg equiv/ml (5 mg/kg) and 0.4 µg equiv/ml (50 mg/kg).

Based on $AUC_{(0\text{-}120\ h)}$, linearity was indicated with values at the 5 and 50 mg/kg; radioactivity of the 50 mg/kg being ca 10 times the 5 mg/kg value. The terminal half life at these two levels were similar (54 h compared to 59 h). However, at the 0.5 mg/kg level the half life of total radioactivity was higher ca 90 h which influenced the calculated AUC value which was about twice the expected value when compared to the 5 and 50 mg/kg dose values.

0.5 mg/kg: $AUC_{0\text{-}120\ h}$ = 10.35 µg equiv/h/ml
Elimination $t_{1/2}$ from plasma ca 21 h for the 4-24 h period
ca 91 h for the 48-120 h period

5 mg/kg: $AUC_{0\text{-}120\ h}$ = 39.72 µg equiv/h/ml
Elimination $t_{1/2}$ form plasma ca 9 h for the 4-24 h period
ca 54 h for the 48-120 h period

50 mg/kg: $AUC_{0\text{-}120h}$ = 372.4 µg equiv/h/ml
Elimination $t_{1/2}$ from plasma ca 15 h for the 4-72 h period
ca 59 h for the 72-120 h period

Labeling: See Summary section below.

OVERALL SUMMARY AND EVALUATION

Norditropin® SimpleXx™ is a new liquid dosage form of Novo Nordisk's lyophilized Somatropin (rDNA origin) for subcutaneous injection which is currently marketed as Norditropin. Liquid Norditropin is the working name used to describe the proprietary Norditropin®SimpleXx™. 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.

Norditropin SimpleXx is a colorless, sterile solution with a pH value of [redacted] The active substance, somatropin, is formulated in a histidine [redacted] with mannitol as a [redacted] Poloxamer 188 to [redacted] phenol as a [redacted] and [redacted] water for injection of 1.5 ml.

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 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.

The Liquid Norditropin product will use the same Somatropin drug substance as the lyophilized product, however the final liquid product will have different excipients. The liquid product also has a somewhat different degradation profile.

The principal degradation pattern (mainly dependent on the pH of the solution) is dominated by [redacted] at [redacted] and by [redacted] formation at [redacted] forms are also formed at the chosen pH-value [redacted]) in Liquid Norditropin.

The development criteria for Liquid Norditropin were that the [redacted] degradation products in Liquid Norditropin at the end of shelf life should be biologically similar to somatropin and Norditropin. The degradation profiles of the two somatropin formulations, Norditropin and Liquid Norditropin, were not the same at the end of their shelf lives, the major differences being a lower content of [redacted] and an increased content of [redacted] in the liquid formulation. Similar contents of all the [redacted] forms were seen for the two somatropin formulations. [redacted] degradation product [redacted] at the end of shelf life) was not present in Norditropin.

With the exception of [redacted] all [redacted] degradation products and the degraded Liquid Norditropin possessed biological properties comparable to somatropin as estimated by biological activity (weight gain assay in hypophysectomized rats), binding affinity to the somatropin receptor, immunogenicity in transgenic mice, and pharmacokinetics in animals. [redacted] produced an increased estimate of the activity measured by weight gain in rats.

[redacted] of the [redacted] degradation products – [redacted] – showed decreased biological activity. The contents of these products were all minimized in Liquid Norditropin compared to the lyophilized Norditropin.

The main excipient, Poloxamer 188, discussed later, was reported to have no influence upon the two- and three-dimensional structure or biological activity of somatropin.

Pharmacologically Norditropin SimpleXx, degraded Norditropin SimpleXx and Norditropin behaved almost identically in all studies indicating that the vehicle for Norditropin SimpleXx and the degradation products do not alter the safety pharmacology profile from that of Norditropin.

Norditropin SimpleXx and degraded Norditropin SimpleXx had no effects on the cardiovascular and respiratory systems in rats. The minor anti-diuretic effect of these two substances in rats and the dose dependent retention of sodium and chloride ions was comparable to that seen with the same doses of Norditropin and is well known for somatropin. The only CNS effect seen in mice was a temporary slight reduction in spontaneous activity observed during the first 30 minutes after dosing of 8 mg/kg Norditropin SimpleXx.

Pharmacokinetics of Norditropin SimpleXx were studied in male Wistar rats following i.v. and s.c. administration. Norditropin SimpleXx, degraded Norditropin SimpleXx and Norditropin in general showed bioequivalence. Pharmacokinetic parameters for Norditropin SimpleXx, degraded Norditropin SimpleXx, Norditropin and the [redacted] degradation products [redacted]

peak) were in general comparable with reference somatropin. The pharmacokinetics of somatropin did not appear to depend on the formulation or storage of Norditropin SimpleXx as tested.

Toxicology for Norditropin (approved NDA 19-721) has been supplemented with a single dose study in mice and a 3-month repeated dose study in rats performed with degraded Norditropin SimpleXx. In addition no immune response was observed in the transgenic mouse following immunization with the degraded Liquid Norditropin.

Acutely in mice there were no clinical signs or deaths following a single s.c. dose of 67 or 133 mg/kg of Liquid Norditropin 10 mg, Forcibly Degraded.

A 3 month study was carried out in rats with subcutaneous dose levels of 0.08 and 0.8 mg/kg/day Liquid Norditropin 10 mg, Degraded. The parameters assessed showed few adverse effects on male or female rats. Liquid Norditropin 10 mg, Degraded administered s.c. at 8 mg/kg/day produced increased weight gain and food consumption. Also seen were some alterations in 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).

The drug was also tested in rabbits for damage to muscle tissue. 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 specific differences and changes seen were those caused by the needle.

POLOXAMER 188:

Poloxamer 188 (a polymer comprising polyoxypropylene and polyoxyethylene) 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 (doses reported to be in the range of ca 0.002 to 0.05 mg/kg/day).

Although Poloxamer 188 is used in a wide range of products due to except for information submitted with this NDA, little or no subcutaneous data seems to be available.

The sponsor has tested Poloxamer 188 in single and repeated dose studies (3 months duration) including toxicokinetic studies, reproduction studies and in 3 in vitro and one in vivo mutagenicity studies. Animal species have included mouse, rat, rabbit and dog.

Acute toxicity studies at subcutaneous doses up to 1000 mg/kg produced no deaths or signs in mice or rats.

A 4-week toxicity study in rats with a 2-week recovery period produced an increase in kidney weights at 500 and 100 mg/kg/day which consisted of changes in tubular vacuolation at 500 mg/kg/day in males and females and at 100 mg/kg/day in females. A similar scenario was seen in the reduced incidence of basophilic tubules in the kidney. Body weight gains reduced for high dose males improved during the recovery period. There were also injection site reactions. Alterations were partly reversible. The NOEL appeared to be 10 mg/kg/day [roughly 41 times the daily clinical exposure of 2.25 mg/patient/day of Poloxamer 188 on a surface area (mg/m^2) basis].

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.

A 13-week study plus a 4-week recovery period was carried out in rats at 0, 10, 100, 500 mg/kg. 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. Injection site reactions were reported to be minimal at 10 mg/kg.

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 – this is uncertain.] Only minimal effects were seen at 10 mg/kg which is about 41X the daily load of Poloxamer 188 (ca 2.25 mg/patient/day) on a body surface area (mg/m^2) basis.

A 4-week subcutaneous toxicity study followed by a 2-week recovery period was conducted in dogs at doses of 0, 0.5, 3, 20 mg/kg. Thickening of skin at injection sites observed at all dose levels from Weeks 3 or 4 was not noted in recovery animals. Histological findings were restricted to inflammatory cell infiltrates at injection sites. The sponsor believed these findings to be due to the method of dosing and not to drug treatment – this is unexplainable. [For chronic comparison to human exposure see 13-week s.c. dog study below.]

Systemic exposure was increased as measured by AUC(0-t) over the range of 0.3 to 20 mg/kg/day. Mean Tmax(obs.) between 1-4 hours after dosing appeared to be longer with increasing dose on Day 1 only. This might suggest saturation of the absorption process with increasing dose – consistent with a sublinear relationship between dose and systemic exposure. An increase in the rate of absorption of Poloxamer from the site of injection was consistent with an increased Cmax(obs.) between Days 1 and 25 in all groups together with a decrease in Tmax(obs.) during this period. No gender differences were apparent.

A 13-week subcutaneous toxicity study followed by a 4-week recovery period was carried out in dogs at doses of 0, 0.5, 3, 20 mg/kg/day. Thickening of the skin seen at injection sites at all dose levels from Week 4 was not seen in any recovery animals by recovery Week 2. Inflammatory cell infiltrates were seen histologically at the injection sites. [The sponsor considered injection site observations to be due to the dosing method rather than to a toxicological effect of the test material. The validity of this conclusion is uncertain.]

Following covariance analysis, kidney and liver weights were statistically increased in 3.0 and 20.0 mg/kg females. Changes that were not dose related and were apparent in only one sex or the other at various time periods included: increases in eosinophils, basophils, monocytes, lactase dehydrogenase, and triglycerides and a decrease in alanine aminotransferase. No significant changes were reported at the recovery period. Considering liver and kidney weight increases, 0.5 mg/kg/day would appear to be a NOAEL. The 0.5 mg/kg dose level is ca 6.8X the daily load of Poloxamer 188 (2.25 mg/patient/day) on a body surface area (mg/m^2) basis.

The effects of Poloxamer 188 on fertility and early embryonic development were studied in rats at doses of 0, 10, 100, 500 mg/kg. There were injection site reactions and mild parental toxicity at 100 and 500 mg/kg/day Poloxamer 188. [Transient subcutaneous lumps and/or sagging skin were noted at or

near the injection sites of all rats receiving 500 mg/kg and in 7 on 100 mg/kg.] Parents showed reduced weight gain at 100 and 500 mg/kg/day. Litter parameters showed no adverse effects at doses up to 500 mg/kg Poloxamer 188 or about 2027 times the human dose on a surface area (mg/m^2) basis. However, the 10 mg/kg dose was in general without injection site or parental effects, being ca 41X the dose of Poloxamer 188 on a mg/m^2 basis.

Mated female rats were dosed subcutaneously once daily with 0, 10, 100 and 500 mg/kg Poloxamer 188 Days 6-16 of gestation. Sacrifice was on Day 20 of gestation. 10 mg/kg was without any effect on the dam or fetus. At 100 and 500 mg/kg maternal effects were confined to transient local reactions at the injection sites. The only apparent fetal effects were a slight increase in hemorrhage affecting the head (Control through High dose) of 0, 3 (2%), 5 (2%) 3 (3%) and intra-abdominal hemorrhage of 1 (1%), 2 (2%), 2 (2%) and 4 (3%). If one considers only the lack of local reactions at injection sites, the 10 mg/kg level which is without effect on the dam or fetus, is about 41X that of the expected load of Poloxamer 188 of 2.25 mg/patient/day based on mg/m^2.

A preliminary study in rats at 0, 10, 100, 250, 500 mg/kg/day Poloxamer 188 showed the percent of preimplantation loss to be slightly greater (not reported as sig.) in treated groups.

Mated female rabbits were dosed subcutaneously with 0, 50, 100, 200 mg/kg/day Poloxamer 188 Days 6-18 of gestation with sacrifice on Day 29. There were no apparent treatment-related clinical observations or necropsy findings and body weight and food consumption were not adversely affected. No apparent treatment-related effects were seen in pregnancy performance, fetal weight or type and distribution of fetal abnormalities and variants. One 100 mg/kg fetus had multiple visceral and skeletal abnormalities. In general findings appeared to be isolated or without treatment-related effects. The sponsor identified 200 mg/kg/day Poloxamer 188 (the maximum level tested) as a level that was without maternal or fetal effects. On a body surface area (mg/m^2) basis this dose is about 1622X that of the expected human load of Poloxamer 188 of 2.25 mg/patient/day.

A preliminary study in rabbits at 0, 10, 50, 200 mg/kg/day Poloxamer 188 showed the percent preimplantation loss to be increased (not reported as sig.) in treated groups.

Subcutaneous doses of 0, 10, 100 and 500 mg/kg Poloxamer 188 were administered to mated female rats from Day 6 of gestation until weaning (ca Day 24 of lactation) in order to study the effects of pre- and post-natal development on maternal function. F_0 dams and F_1 offspring were monitored. There was a local reaction at the injection site consisting of transient lumps and/or sagging skin in all 500 mg/kg/day rats, for occasional 100 mg/kg/day rats and for one Control rat. Mean body weight gain and food consumption were slightly lower than control for the 500 mg/kg/day group during the first 2 weeks of lactation. At 500 mg/kg pup survival and mean pup weights and mean litter weights were slightly lower than controls. There were no other indications of any effects of treatment on the performance of the F_1 generation. The F_1 and F_2 rats were not dosed directly. 10 mg/kg/day appears to be a no effect level (roughly 41 times the daily clinical exposure on a mg/m^2 basis). There was a general lack of fetal effects at 100 mg/kg which on a surface area basis is ca 405X the clinical exposure for Poloxamer 188 on a daily basis.

Genetic Toxicology of Poloxamer 188 was studied in the Ames test, chromosomal aberration assay in human lymphocytes, test for forward mutations in mouse lymphoma cells, and mouse micronucleus assay.

The Ames test showed no toxicity to bacteria or precipitation. No mutagenic activity was observed in any of the 5 bacterial strains, either activated or non-activated at concentrations up to 5000 μg/plate. It was concluded that Poloxamer 188 was <u>not mutagenic</u> in this system.

A chromosomal aberration assay in human lymphocytes was tested with Poloxamer 188 concentrations ranging from 0.02 to 5 $mg.ml^{-1}$. All cultures treated with Poloxamer 188 had levels of structural aberrations within the 95% confidence for a negative response. An extra assessment of polyploidy was carried out in the cultures harvested at 48 hours. All cultures had levels of numerical aberrations within the 95% confidence limits of a negative response. Thus, Poloxamer 188 was considered not clastogenic when tested for such effects in vitro with human peripheral blood lymphocytes

A mouse lymphoma mutation assay was conducted to assay Poloxamer 188 for mutagenic potential in the mouse lymphoma L5178Y cell line scoring for forward mutations at the thymidine kinase locus: $tk^{+}tk^{-}$ to $tk^{-}tk^{-}$. Poloxamer 188 was not toxic at the preset maximum concentration of 5000 $\mu g.ml^{-1}$.

Final concentrations of Poloxamer 188 in the treatment medium ranged between 150 and 5000 $\mu g.ml^{-1}$ in the absence and presence of S9 mix. Positive control cultures were included and duplicate cultures were used for each treatment point. Vehicle controls were tested in quadruplicate.

Although increases in mutant fraction were seen in some Poloxamer 188-treated cultures (1.7 over that of vehicle – the sponsor considered 2 duplicate cultures of at least 1.7 fold higher than controls in the same activation condition as positive) there was no evidence of a reproducible or dose-related mutagenic effect in either the absence or presence of S9 mix. The sponsor thus concluded that Poloxamer 188 is not mutagenic in mouse lymphoma L5178Y cells tested up to a concentration of 5000 $\mu g.ml^{-1}$. The incidence of mutant fractions is less than the generally accepted factor of 2-fold over that of controls. Thus, we agree with the not mutagenic finding in this system.

The in vivo genotoxic potential of Poloxamer 188 was evaluated in a micronucleus test in bone marrow erythrocytes of young, male and female CD-1 mice following a 0 h + 24 h subcutaneous dosing and 48 h sampling regimen at a single dose level. A limit toxicity study was performed to confirm the non-toxicity of Poloxamer 188 at the maximum recommended dose of 2000 $mg.kg^{-1}.day^{-1}$.

One group of CD-1 mice was dosed at 0 h and 24 h s.c. with the test material at the maximum recommended concentration of 2000 $mg.kg^{-1}.day^{-1}$. Bone marrow samples were taken 48 h after the initial 0 h dose. Two control groups of CD-1 mice were also dosed s.c. with either the 10 ml water for irrigation.$kg^{-1}.day^{-1}$ vehicle, or the positive control, 50 mg cyclophosphamide.$kg^{-1}.day^{-1}$.

Mice treated with the vehicle alone showed normal background levels of micronuclei; those dosed with cyclophosphamide showed substantial increases in the numbers of bone marrow micronuclei.

No micronucleus induction was seen in the bone marrow erythrocytes of mice dosed with 2000 mg Poloxamer 188.$kg^{-1}.day^{-1}$.

Further pharmacokinetics of Poloxamer 188 were examined in rats and dogs. Poloxamer 188 was rapidly absorbed from the site of injection in rats (t_{max}: 0.5-2 h) and dogs (t_{max}: 1-4 h) with more than 83% of the total administered dose in rats and at least 68% in dogs being excreted by 24 hours post dosing. Less than 1% of the dose was found at the site of injection 96 hours after dosing in the rat. The plasma exposure increased with the dose and the findings indicated linear pharmacokinetics of Poloxamer 188 in the dose range of 5-500 mg/kg in the rat and 0.3-50 mg/kg in dogs. Exposure was slightly lower in female rats compared with male rats but similar for male and female dogs. The majority of radioactivity was recovered in the urine – this is reported to be in accordance with previous findings showing that Poloxamer 188 is primarily excreted unchanged in the urine in both rats, rabbits and humans following intravenous administration. In this study findings in rats for [^{14}C]-Poloxamer 188 at days 1 and 25 were similar indicating that the pharmacokinetics of Poloxamer 188 did not change with time.

Safety of Poloxamer 188:

The excipient, Poloxamer 188, at [redacted], was added to Norditropin®SimpleXx™ to [redacted]. The expected human load of Poloxamer 188 is in the magnitude of 2.25 mg/patient/day which would be ca 0.04 mg/kg. It is reported that for many years

poloxamer has been used in medicine for i.v. and topical administration, as well as a food additive. Thus a limited s.c. safety program including bridging toxicity studies, effect on reproduction, genotoxic potential, and absorption and excretion were performed in various animal species. Poloxamer 188 is rapidly absorbed from the injection site. From the toxicological studies carried out at doses several hundred times that found in the clinical formulation, it would appear that Poloxamer 188 would not be expected to result in toxicity in humans when used as an excipient in Norditropin®SimpleXx™. Repeated dose toxicity studies up to and including 13-week studies in rats and dogs showed reaction at the injection site and renal lesions at high doses. Such changes were in general reversible. It would be expected that Poloxamer 188 would be excreted unchanged in the urine within a few hours after administration and generally within 24 hours. Slight reactions might occur at the injection site following repeated administration. However, clinically this would be self-limiting and changing the injection site regularly should lessen or eliminate any such problem. Mutagenicity studies with Poloxamer 188 were negative. Based on the previous uses of Poloxamer 188 and the bridging studies conducted under this NDA the carcinogenicity potential of Poloxamer 188 would appear to be minimal and thus carcinogenicity studies with this excipient are at this time deemed unnecessary.

Relevant Issues Pertaining to Norditropin®SimpleXx™:

The pharmacology/toxicology of growth hormone is well known. However, relevant issues for the to be marketed product, Norditropin®SimpleXx™, include possible degradation, effect of the excipient Poloxamer 188, local tolerance, anti hGH response, glucose intolerance, stimulation of tumor growth and safety of interchangeable dose cartridges.

1) Pharmacologically Norditropin®SimpleXx™, Degraded Norditropin®SimpleXx™ and Norditropin behaved almost identically in all studies indicating that the vehicle for Norditropin®SimpleXx™ and the degradation products do not adversely alter the safety pharmacology profile from that of Norditropin.

2) Studies presented in this submission as well as prior use of Poloxamer 188 would indicate that this excipient at doses in the range of 0.04 mg/kg would not be expected to pose a clinical problem.

3) An irritation study in rabbits showed hemorrhage following 0.9% NaCl and liquid Norditropin to be similar with changes probably produced by the needle. Neither group showed creatine kinase activity depletion from the muscle.

4) The possibility of development of antibodies to somatropin has been discussed in the labeling.

5) The possibility of glucose intolerance requiring adjustment of antidiabetic medications is taken up in the labeling.

6) Possible stimulation of tumor growth or active malignancy has been covered in the labeling.

7) One of the main concerns is safety due to the interchangeability of the dose cartridges. All of the dose cartridges appear to be able to be used in any of the injection pens even though they are color coded. This could pose a problem of over dosing (or under dosing) if the cartridge and pen are not matched. All disciplines (including Devices) are aware of this problem. The final decision regarding solution of the problem of mismatched pens and cartridges will be up to the Medical Officer.

Labeling: [Pharmacology Section - **As submitted by the sponsor.**]

Carcinogenesis, Mutagenesis, Impairment of Fertility: [redacted]

Pregnancy: Pregnancy Category C. [redacted]

Nursing Mothers:

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when is administered to a nursing woman.

To be conveyed to the sponsor:

Labeling Changes: [Pharmacology Section – According to CFR 201.57]

In order to be more factual (since Norditropin and Norditropin®SimpleXx™ are not identical) Labeling should be changed per CFR 201.57 to read as follows:

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity, mutagenicity and fertility studies have not been conducted with

Pregnancy Category C: Animal reproduction studies have not been conducted with Norditropin®SimpleXx™. It is also not known whether can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity, should be given to a pregnant woman only if clearly needed.

Nursing Mothers:. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when is administered to a nursing woman.

CONCLUSIONS and RECOMMENDATION: AP

/S/
David H. Hertig
Pharmacologist

cc: Original NDA 21-148; HFD-520 NDA 21-148; HFD-345;
HFD-510 RSteigerwalt; DHertig; CKing
Recommendation: AP

Team leader concurs
/S/