TNO report
V98.797

Oral embryotoxicity/teratogenicity study with trehalose in New Zealand White rabbits

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Project number:
471005

At the request of:
Hayashibara Company Ltd.
2-3-Shimoishii, 1-Chome
Okayama 700, Japan

Study director:
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Number of pages:
224

Summary

1. Trehalose was fed in the diet to pregnant New Zealand White rabbits (16 animals per dose group) from days 0-29 of gestation. The concentrations in the diet were 0, 2.5, 5 and 10%. On gestation day 29 the dams were sacrificed and macroscopically examined. Reproductive organs were weighed and fetuses were examined after Caesarian section. The viscera of the fetuses of all groups and the skeletons of the fetuses of the control, mid- and high-dose groups were examined.

2. On the basis of the results obtained in this study it was concluded that: trehalose, when administered in the diet did not induce maternal nor developmental toxicity at concentrations of 2.5, 5 and 10%.

3. No statistically significant differences in mean body weights, body weight changes and food consumption were observed amongst the control and the groups fed trehalose in the diet. It was noticed that some animals ate or drank sparingly for several days. The test substance intake during the gestation period ranged from 0.21 $\pm$ 0.05 - 0.77 $\pm$ 0.03 , 0.48 $\pm$ 0.11 - 1.34 $\pm$ 0.11 and 1.04 $\pm$ 0.14 - 2.82 $\pm$ 0.23 (mean $\pm$ standard error of the mean) g trehalose/kg body weight/day for the low-, mid- and high-dose group, respectively.

4. Daily clinical observations of all animals during the gestation period did not reveal any remarkable differences in the appearance, general condition or behaviour amongst the dosing and control groups.
   One animal in the high-dose ate and drank sparingly for several days and was sacrificed on gestation day 16. Another animal in the high-dose group was found dead on gestation day 28. At necropsy, it was found that both animals had a hairball in the stomach.

5. Few macroscopic findings at scheduled sacrifice were observed and are considered common in rabbits.

6. At Caesarian section, 12 of 16 females in the control group, 12 of 16 females in the low-dose group, 14 of 16 females in the mid-dose group and 10 of 13 females in the high-dose group were pregnant. One rabbit in the high-dose group experienced an early delivery.
   The female fecundity index was 75, 75, 87 and 81% for the control, low-, mid- and high-dose groups, respectively. The gestation index was 100% for all the groups.
   There were no statistical differences in the number of corpora lutea, implantations, live and dead fetuses, early and late resorptions, the pre- and post implantation loss, or in the sex ratio of the fetuses between the control group and the groups fed trehalose in the diet .
   No remarkable differences in gravid and empty uterus weight, ovary weight, carcass weight and net weight change (body weight gain from day 0 to day 29 of gestation minus gravid uterine weight) were observed between the control

group and the groups fed trehalose in the diet, except for a statistically significant increase in the mean weight of the empty uterus of the animals of the high-dose group. This was not considered treatment-related, and was likely related to the increased number of fetuses in the high-dose group (8.4 in the high-dose group versus 6.5 in the control group).

7. No significant differences in the mean fetal body weight and placenta weights were observed between the control group and the groups fed trehalose in the diet. External observations of the fetuses and placentas at Caesarian section did not reveal any remarkable findings which could be related to treatment. One fetus in the control group had proboscis and ectopic eyes.
   Fetal examination revealed no treatment-related differences in soft tissues. In the treatment groups examined (mid-and high-dose), no treatment-related skeletal variations were noted on a litter basis.

Contents

Statement of GLP compliance

We, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Nutrition and Food Research Institute, and that the study was carried out under our supervision.
The study was carried out in accordance with the OECD Principles of Good Laboratory Practice.

[signed] 06-07-1999

Ir D.H. Waalkens-Berendsen
(Study director) Date

[signed] 06-07-1999

Drs H.H. Emmen
(Management) Date

Quality Assurance Statement

On:	Oral embryotoxicity/teratogenicity study with trehalose in New Zealand White rabbits
Report Number:	V98.797
Date :	6 July 1999

The protocol, amendment 1 and amendment 2 were inspected on 13 January 1998, 14 April 1998 and 14 August 1998, respectively
The experimental phase of this study was inspected by the Quality Assurance Unit of TNO Nutrition and Food Research as follows:

Date of inspection:	Date of report:
8 April 1998	8 April 1998
21 April 1998	21 April 1998
22 April 1998 (Analysis)	22 April 1998
7 May 1998	7 May 1998

This report was audited as follows:

Dates of audit:	Date of report:
16-18, 22 September 1998	22 September 1998
21-25 September 1998	25 September 1998
30 October 1998	30 October 1998
1 July 1999	1 July 1999

I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all inspections were reported to the study director and the management on the dates indicated.

[signed]	06-07-1999
M.W. van Marwijk (Quality Assurance Unit)	Date:

GLP compliance monitoring unit statements

STAATSTOEZICHT OP DE VOLKSGEZONDHEID
VETERINAIRE HOOFDINSPECTIE

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF
GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 88/320/EEC the conformity with the OECD Principles of GLP was assessed on 22-26 April, 15 May and 9 September 1996 at

TNO Nutrition & Food Research Institute
Toxicology Division
Utrechtseweg 48, P.O. Box 360
3700 AJ Zeist, The Netherlands

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Toxicity; Mutagenicity; Environmental Toxicity on aquatic and terrestrial organisms; Behaviour in water, soil and air; Residues; Effects on mesocosms and natural ecosystems; Analytical and clinical chemistry; Drug metabolism; Pharmacokinetics.

Rijswijk, 10 September 1996

Th. Helder, DVM

Ministry of Health, Welfare and Sport
State Supervisory Public Health Service
Veterinary Public Health Inspectorate
GLP Section

VHI 4

STAATSTOEZICHT OP DE VOLKSGEZONDHEID

VETERINAIRE HOOFDINSPECTIE

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 88/320/EEC the conformity with the OECD Principles of GLP was assessed on 10-13 March 1998 at

TNO Nutrition and Food Research Institute
Analytical Sciences Division
Utrechtseweg 48, P.O. Box 360
3700 AJ ZEIST, The Netherlands

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following area of expertise: Analytical and Clinical Chemistry.

Rijswijk, 24 April 1998

R.W.A. Jaspers, PhD
Ministry of Health, Welfare and Sport
State Supervisory Public Health Service
Veterinary Public Health Inspectorate
GLP Monitoring Unit

Testing facility

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1 Introduction

At the request of Hayashibara Company Ltd., 2-3-Shimoishii, 1-Chome, Okayama 700, Japan, the possible effects of the test substance on the pregnant female rabbit and the development of the embryo and fetus subsequent to exposure of the female from fertilization (gestation day 0) to Caesarian section shortly before term (gestation day 29) were investigated.

2 Experimental

The study was conducted according to the protocol approved by the Sponsor, entitled "Protocol for an oral embryotoxicity/teratogenicity study with trehalose in New Zealand White rabbits (P 471005)" approved by the Study Director on 16 December 1997 and Amendments 1 and 2 dated 25 March 1998 and 11 August 1998, respectively.
The protocol has been drafted in accordance with the OECD Guideline for Testing of Chemicals 414, adopted 12 May 1981
The study was carried out in accordance with the OECD Principles of Good Laboratory Practice.

The following time schedule was used:

a. arrival of the animals	: 26 November 1997 and 18 March 1998 (see section 2.2.1)
b. experimental start date	: 7 April 1998
c. termination in-life part	: 8 May 1998

2.1 Test substance

Test substance name	: α,α-trehalose
Chemical name	: α-D-glucopyranosyl-D-glucopyranoside
CAS.reg.no.	: 99-20-7
Batch number	: 7L111
TNO internal reference no.	: 980020
Purity	: 99%
Molecular formula	: $C_{12}H_{22}O_{11}.2H_2O$
Molecular weight	: 378.33
Appearance	: white crystalline powder
Package	: paper bags in cartons
Quantity received	: 8 bags of 20 kg each
Storage conditions	: at ambient temperature
Date of receipt	: 31 December 1997
Expiry date	: 10 December 1999

Characterization and stability analysis for the test substance as supplied has been conducted by the sponsor.

2.2 Test system

2.2.1 Characterization of the test system

The study was conducted with rabbits. The rabbit was used because this species is considered a suitable species for this type of study.
Sixty female New Zealand White rabbits CRL:KBL(NZW)BR were obtained from a colony maintained under SPF-conditions at Broekman Institute B.V., Someren, The Netherlands and arrived at 26 November 1997. Ten extra female New Zealand White rabbits were obtained from a colony maintained under SPF-conditions at Charles River Deutschland, Sulzfeld, Germany and arrived at 18 March 1998. The rabbits obtained from Charles River originated from the same colony as the rabbits obtained from Broekman Institute B.V.
At the commencement of the study the female rabbits of both groups were at least 4.5 months old.

2.2.2 Animal allocation

Upon arrival, the rabbits of both groups were quarantined (in room 15.21) and checked for overt signs of ill health and anomalies. During the quarantine period serological examinations were conducted in a random sample (blood was taken from 4 rabbits obtained from Broekman Institute B.V. and from 2 rabbits obtained from Charles River). After the results of serology indicated an acceptable microbiological status the animals were transferred to the animal room.
The 60 rabbits obtained from Broekman Institute B.V. were transferred to animal room 06.25 on 25 February 1998 for acclimatization. The ten extra rabbits obtained from Charles River were transferred to this room on 27 March 1998.
One day before the start of the study, 64 healthy animals were allocated to the various groups by computer randomization on the basis of body weight. The rabbits were allocated to the four experimental groups such that the proportion between the rabbits obtained from Broekman Institute B.V. and from Charles River was the same in all groups (14 rabbits of Broekman Institute B.V. and 2 rabbits of Charles River per group; see Appendix 1). At randomisation one of the rabbits (old number 105) displayed abnormal behaviour and was replaced by another female (old number 91). The remaining females were considered as reserve animals; they were kept in the animal room for monitoring during the study and discarded at the end of the study.

2.2.3 Identification of the test system

During the acclimatization period, the animals were identified by their cage number. Following allocation to the groups, one day before the start of the study, the individual rabbits were identified by using odd integers for animal identification numbers, which were written with a felt pen in the ears. Each cage was provided with a card showing the colour code, animal identification number, group letter and study number.

2.3 Experimental conditions

2.3.1 Animal maintenance

2.3.1.1 Environment

The rabbits were housed under conventional conditions in animal room 06.25. The room was ventilated with about 10 air changes per hour and maintained at a temperature of 19 $\pm$ 3 ^{0}C. From 22-29 April 1998 and from 4 May - 10 May 1998 the temperature exceeded 22 ^{0}C several times. A maximum of 24 ^{0}C was reached on 10 May 1998. Relative humidity was 30 - 70 %, except for short periods during cleaning activities when the upper limit for humidity was exceeded. Lighting was artificial by fluorescent tubes with a sequence of ca. 12 hours light and 12 hours dark. However, from 29-30 April 1998 light was on during all the night.

2.3.1.2 Caging

Upon arrival the rabbits were housed individually in stainless steel cages (64x47x36 cm) with wire mesh floors and fronts.

2.3.2 Feed and drinking water

The rabbits were provided with feed and water *ad libitum* from arrival until the end of the study.
The feed was provided as pellets in stainless steel feeders. The feed in the feeders was refreshed once per week and topped up when necessary.
During the quarantine and acclimatization period, the rabbits were fed a closed formula diet obtained from SDS (Special Diets Services, Witham, England). The diet (batch no. 4014) was analysed by the supplier for nutrients and contaminants (see Annex 1.1 for the certificate of analysis).
During the study, the rabbits were fed a modified diet. The feed was provided by the supplier as a powder (batch no. 4262; see Annex 1.2 for the certificate of analysis). Ten percent pregelatinized potato starch (Paselli WA 4, AVEBE, Foxhol, the Netherlands; see Annex 1.3 for certificate of analysis) was added to the powdered diet formulation of the control group, mechanically mixed, and subsequently pelleted using 5% (w/w) tap water as a binding agent. The water was largely evaporated during and after pelleting. Trehalose was substituted for the potaoe starch at concentrations of 2.5, 5.0 and 10% for the low-, mid- and high dose groups, respectively. Furthermore, the diet was supplemented with D-L-methionine (0.1%; Merck 500986), vitamin B6 (40 mg/kg; Merck 501260) and vitamin B12 (50 μg/kg; Merck 500619).
Due to technical problems, preparation of the diets took up several days (between 1 and 9 April 1998). Furthermore, two different moulds were used giving pellets of different size (diets of the control, low- and mid-dose groups larger pellets (ø ca. 8 mm), diets of the mid- and high-dose groups smaller pellets (ø ca. 4 mm). From 9 April 1998 until the end of the study the animals of mid-dose group were fed the smaller pellets).
Just after preparation of the diets, samples for analysis were taken and stored in a freezer at <-18 ^{0}C pending analysis. Diets were stored at <-18 ^{0}C for a maximum of 5.5 weeks.
The drinking water (tap-water) was supplied in polypropylene bottles which were

cleaned about weekly and filled up when necessary. Tap water suitable for human consumption (quality guidelines according to Dutch legislation based on the EEC Council Directive 80/778/EEC, see Annex 2) was supplied by N.V. Waterleiding bedrijf Midden-Nederland (WMN). Results of the routine physical, chemical and microbiological examination of drinking water as conducted by the supplier are made available to TNO Nutrition and Food Research Institute. In addition, the supplier periodically (twice per year) analyzed water samples taken at the premises of TNO Nutrition and Food Research Institute in Zeist for a limited number of physical, chemical and microbiological variables. The results of the most recent analysis are given in Annex 2.

2.4 Experimental procedures

2.4.1 Artificial insemination

Before insemination the females were intravenously injected in the ear vein with luteinizing hormone (Pregnyl, Organon, Oss, The Netherlands; 1 ml of a solution containing 50 IU/ml in 0.9% NaCl solution).
The female rabbits were inseminated with 1.0 ml of a pooled semen sample, of proven fertile males, obtained daily during the insemination period from Broekman Institute B.V., Someren, The Netherlands.
The day of insemination (7, 8 and 9 April 1998) was considered day 0 of gestation.

2.4.2 Administration of the test substance

The oral route was used because this is the route of human exposure. The test substance was administered in the diet starting on the day of fertilization (gestation day 0) and continuing to Caesarian section (gestation day 29). The test substance was incorporated in the diet as indicated in sections 2.3.2 and 2.4.3.

2.4.3 Dose levels and groups

The study comprised four groups of 16 artificially inseminated female rabbits: viz. 3 test groups receiving different levels of trehalose in the diet and one control group. The various groups are characterized in the scheme presented below:

Group	Treatment	Colour code	Dietary[1] supplement (%)		No. of inseminated females
			Trehalose	Pregelatinized potato starch	
A	control	white	0	10	16
B	low-dose	blue	2.5	7.5	16
C	mid-dose	green	5	5	16
D	high-dose	red	10	0	16

[1]: diet used was SDS standard rabbit diet. The diet was supplemented with D-L-methionine (0.1%), vitamin B6 (40 mg/kg) and vitamin B12 (50 μg/kg).

2.4.4 Observations, analyses and measurements

2.4.4.1 Test substance analyses in the carrier

Analyses to determine the content, homogeneity and stability of the test substance in the carrier were conducted in all diets using HPLC method.

Before analyzing samples for the study, the method was validated to conform with the following criteria:

- linearity: the correlation coefficient of the calibration curves should be greater than or equal to 0.996;
- selectivity: no peak should be found in the blank carrier with a retention time of 95-105% of that of the test substance;
- repeatability: the relative standard deviation in the percentage recovery and the retention time when the recovery test is performed 3 times at each concentration used in the study should be less than 10 and 2% respectively;
- recovery: the recovery of the test substance from the carrier should be between 80 and 110% at all concentrations used in the study.

Samples of the diets were taken just after preparation and stored in a freezer at -18 ^{0}C pending analysis (see also 2.3.2). The stability of the test substance under (simulated) experimental conditions was demonstrated by analyzing samples with (low-, mid- and high dose) and without (control) the test substance on the day these samples were thawed (this day was considered to be t=0), after storage of thawed samples at ambient temperature in the animal room in an open container for 7 days and after storage for about 8 weeks in a closed container in a freezer at <-18 ^{0}C . To determine the homogeneity of the test substance in the diet, 5 samples were taken from 5 different locations in the feed container from each dose level (low-,

mid- and high-dose) and analyzed. Only 1 control sample was taken and analyzed.

2.4.4.2 Clinical signs

Females were observed each morning from the start of the study and, if necessary, handled to appraise physical condition. Signs of ill health and reaction to treatment as well as mortality were recorded. On working days, all cages were checked again late in the afternoon for dead or moribund animals to minimize loss of tissue from the study. During weekends and holidays only one check per day was carried out.

2.4.5 Body weight

Body weights of the rabbits were recorded on days -1 (one day before the start of the study for randomization purposes) and on days 0, 7, 14, 21 and 29 of gestation.

2.4.6 Food consumption

The quantity of food consumed by each animal was measured by weighing the feeders over days 0-7, 7-14 and 14-21 and 21-29 of gestation.

2.4.7 Water consumption

Water consumption was not measured.

2.4.8 Pathology

The animals were killed by an intravenous injection in the ear vena of Euthesate ? (sodium pentobarbital; ca. 1 ml/kg body weight, Apharmo, Arnhem, The Netherlands) on day 29 of pregnancy and examined for gross abnormalities. Any animal found dead or killed in extremis during the study was also examined macroscopically, and if present, the number of corpora lutea and implantations were recorded. Maternal tissues showing severe macroscopic abnormalities were removed and fixed in a neutral, aqueous phosphate buffered 4% solution of formaldehyde; these tissues were examined microscopically if necessary, after consultation with the Sponsor.

The uteri (including the fetuses), ovaries and placentas of all females killed on day 29 were examined for the following parameters:
- number of corpora lutea
- number of implantation sites[1]
- number of early and late resorptions
- number of live and dead fetuses
- sex of the fetuses
- number of grossly visible malformed fetuses and fetuses with external abnormalities
- weight of ovaries
- weight of uterus, containing placentas and fetuses

[1] If necessary the implantation sites were made visible following Salewski, E. (1964), Arch. Exp. Path. Pharmacol. 247, 367

- weight of uterus, empty
- weight of fetuses
- weight of the placentas
- gross evaluation of the placentas

2.4.9 Fetopathological examination

First all fetuses of each litter were examined by careful dissection for visceral anomalies. The sex of the fetuses was determined. To examine the head for visceral anomalies, half of the number of fetuses in each litter were decapitated and their heads were fixed in Bouin's fixative and subsequently free-hand sectioned according to Van Julsinga and Bennet (1977). The sections obtained were examined for visceral anomalies. After the visceral examinations the intact and decapitated bodies were eviscerated, fixed and 70% ethanol, cleared in potassium hydroxide and stained with Alizarin Red S after Dawson (1926). The skeletons of the control and high-dose group were examined for skeletal abnormalities and then archived. At Caesarian section 10 females of the high-dose group were pregnant. Since OECD guidelines recommend the examination of fetuses from 12 pregnant animals, the examination of the fetal skeletons were, after consultation with the sponsor, extended to the mid-dose group.

2.5 Reproductive performance

For each treatment or control group the following data were recorded:
- female fecundity index = (number of pregnant females/number of females mated) x 100
- pre-implantation loss = [(number of corpora lutea - number of implantation sites)/ number of corpora lutea] x 100
- post-implantation loss = [(number of implantation sites- number of live fetuses)/number of implantation sites] x 100
- gestation index = (number of females with live fetuses/number of females pregnant) x 100
- sex ratio = (number of live male fetuses/number of live fetuses) x 100

2.6 Statistical analysis of the results

The resulting data were analyzed using the methods mentioned below. $P < 0.05$ was considered as the level of significance.
Clinical findings were evaluated by Fisher's exact probability test.
Abnormal fetal findings were evaluated by Fisher's exact probability test; both litter and fetus were used as unit for statistical analysis. Statistical difference was considered to be relevant if an effect was detected on litter basis.
Body weight, body weight gain, organ weights and food consumption data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.
Fisher's exact probability test was used to evaluate the number of pregnant females and females with live fetuses. Number of corpora lutea, implantations, live and dead fetuses and early and late resorptions were evaluated by Kruskal-Wallis

nonparametric analysis of variance followed by the Mann-Whitney U-test .

2.7 Retention of records, samples and specimens

A reference sample of the test substance, raw data, the master copy of the final report and all other information relevant to the quality and integrity of the study, including tissue specimens, were retained in the archives of the TNO Nutrition and Food Research Institute for a period of at least five years (tissue specimens), 10 years (reference sample of the test substance) or at least 15 years (raw data) after reporting of the study. At the end of the five year storage period, the sponsor will be asked whether the tissue specimens can be discarded, should be stored for an additional period, or transferred to the archives of the sponsor.

2.8 Deviations from the protocol

- From 1 January 1999 onwards Reproduction toxicology is part of the Department Target Organ Toxicology headed by Drs H.H. Emmen.
- Due to re-scheduling of activities body weights for randomisation purposes were not recorded on day -3 as was mentioned in the protocol but on day -1.
- The female rabbits were inseminated with 1 ml of a pooled semen sample as provided by Broekman Institute B.V., Someren, The Netherlands. The amount of sperm cells/ml in the samples was not determined by the supplier.
- Due to technical problems, preparation of the diets took up several days (1 April 1998 - 9 April 1998). Furthermore, two different moulds were used giving pellets of different size (diets of group A, B and C larger pellets, diets of group C and D smaller pellets. From 9 April 1998 until the end of the study the animals of group C were fed the smaller pellets).
- Since the preparation of the diets took up more days, the samples for analysis, taken just after diet preparation, were stored in a freezer at <-18 ^{0}C pending analysis. The stability of the test substance in the diets was demonstrated in samples just after thawing, after storage of thawed samples at ambient temperature in the animal room in an open container for 7 days and after storage of samples for about another 7 weeks in a freezer at < -18^{0}C.
- The diets were not stored in a refrigerator as was mentioned in the protocol but in a freezer at <-18 ^{0}C to ensure stability of the test diets.
- The homogeneity and stability of the test substance in the diets was not only conducted in the diets of the low- and high-dose groups as was mentioned in the protocol but also in the diet of the mid-dose group at request of the sponsor.
- Due to the large amount of food which had to be made, the components of the food were mixed in three batches (See 2.4.3). In one of the three batches of the control group too much of the SDS standard diet was used. As a result of this, in this batch the concentration of the potato starch was 8.9% (instead of 10%) and of D-L-methionine, vitamin B6 and vitamin B12 the concentrations were 0.09% (instead of 1%), 35.65 mg/kg (instead of 40 mg/kg) and 44.44 μg/kg (instead of 50 μg/kg), respectively.
- Several times from 22-29 April 1998 and from 4 May - 10 May 1998 temperatures exceeded 22 ^{0}C; a maximum of 24 ^{0}C was reached on 10 May 1998.

- Due to technical failure from 29-30 April 1998 light in the animal room was on all night.

The above deviations are not considered to have influenced the validity of the study.

3 Results

3.1 Analyses of trehalose in the diet (Annex 3)

The analyses of trehalose in the diet are presented in Annex 3.
At all concentrations, the test substance was homogeneously distributed in the diets and stable when stored for 7 days at room temperature and for at least 52 days in the freezer (<-18 ^{0}C). Due to increased day-to-day background fluctuations in the concentration of a substance that co-resolves with trehalose during analysis, low-dose levels (2.5% trehalose) in pelleted diets could not be determined precisely (compared to the validation data). Therefore, it is not possible to determine if (considering the performance of the method for pelleted diets) the low-dose deviated less than 10% from the intended level of 2.5%. Considering the increased background fluctuations it was concluded that the mid-dose level (5%) is close-to-intended, although the relative difference with the expected value is -14%. The content of the test substance in the high-dose level (10%) was close to intended.

3.2 Clinical signs and mortality (Table 1 and Appendix 2)

Clinical signs and mortalities of the females during the gestation period are presented in Table 1.
Animal D113 did not eat and drank small amounts for several days. On gestation day 9, this animal was anaesthetized with ca. 1.5 ml Nembutal for an extensive clinical examination at which time no problems were observed. Animal D113 was sacrificed on gestation day 16. Animal D117 was found dead on gestation day 28, the perineum of this animal was soiled with faeces. Furthermore, animal A19 had a wounded ear for several days during pregnancy and female D101 had a pale appearance and showed decreased activity on the last day of gestation.
Further, daily clinical observations during the gestation period did not reveal any remarkable findings in the animals appearance, general condition or behaviour between the dosing and control groups.

3.3 Macroscopic findings at necropsy (Table 2 and Appendix 3)

The findings at necropsy of the sacrificed and dead animals and the findings at Caesarian section are presented in Table 2.
At necropsy of animal D113 and D117 it was observed that both animals had a hairball in the stomach. The cause of death of female D117 could not be determined. At necropsy, both animals were pregnant. At scheduled sacrifice on gestation day 29, it was observed that the liver of animals A31, B43, B57, D103 and D121 had a dark appearance. None of these animals were pregnant, no relation with trehalose treatment was supposed as no dose relationship was observed. Animal D103 also had haemorrhagic ovaries.
Gross examination did not reveal any significant differences of the maternal organs and tissues amongst the groups. The few macroscopic findings observed, are common in rabbits.

3.4 Body weight and body weight change (Table 3 and 4 and Appendix 4)

The mean body weights and body weight changes of the pregnant animals during gestation are presented in Table 3 and 4.
No statistically significant differences in mean body weights and body weight changes were observed between the control and the groups fed trehalose in the diet.

3.5 Food and water consumption (Table 5 and Appendix 5)

Food consumption expressed as g/kg body weight/day is presented in Table 5.
No statistically significant difference in food consumption was observed between the control and the groups fed trehalose in the diet. Some animals ate little for several days (see Appendix 5 for individual data). Furthermore, although water consumption was not measured in this study it was noticed that animal D113 drank little between gestation day 4 and 7 and on gestation day 15. Animal D117 drank little at gestation day 23.

3.6 Test substance intake

The test substance intake was calculated from the food consumption expressed in g/kg body weight/day Table 5.

	Test substance intake (g trehalose/kg body weight/day) (mean ± standard error of the mean)		
	2.5% trehalose low-dose group	5% trehalose mid-dose group	10% trehalose high-dose group
Gestation period: days 0-7	0.77 ± 0.03	1.34 ± 0.11	2.82 ± 0.23
days 7-14	0.71 ± 0.04	1.52 ± 0.10	2.58 ± 0.25
days 14 -21	0.45 ± 0.08	0.97 ± 0.26	1.50 ± 0.30
days 21-29	0.21 ± 0.05	0.48 ± 0.11	1.04 ± 0.14

3.7 Reproduction data and litter data (Table 6 and Appendices 6 and 7)

The pregnancy status of the reproduction and litter data are given in Table 6.
The two animals in the high-dose group that were sacrificed and found dead were pregnant. Furthermore, one early delivery was observed in the high-dose group (D101)
At Caesarian section, 12 of 16 females in the control group, 12 of 16 females in the low-dose group, 14 of 16 females in the mid-dose group and 10 of 13 females in the high-dose group were pregnant. The female fecundity index was 75, 75, 87 and 81% for the control, low-, mid- and high-dose, respectively. The gestation index was 100% for all groups.
There were no statistically significant differences between the control group and the groups fed trehalose in the diet in the number of corpora lutea, implantations, live and dead fetuses, early and late resorptions, pre- and post implantation loss, or

in the sex ratio of the fetuses.

3.8 Organ weights (Table 7 and Appendices 8)

Mean reproductive organ weights and net maternal body weight change during gestation are given in Table 7. Except for a statistically significant increase in mean weight of the empty uterus of the animals of the high-dose group, no remarkable differences in gravid and empty uterus weight (low- and mid-dose groups), ovary weight, carcass weight and net weight change (body weight gain from day 0 to day 29 of gestation minus gravid uterine weight) were observed between the control group and the groups fed trehalose in the diet.
The increase in mean weight of the empty uterus was considered to be related to the increased number of fetuses in the high-dose group (8.4 in the high-dose group versus 6.5 in the control group).

3.9 Fetal external observations (Table 8 and Appendix 9)

Fetal external observations are summarized in Table 8.
In the control group one fetus (fetus 9 of female A13) was found which showed proboscis and ectopic eyes. No other external findings were observed.

3.10 Findings of the placenta (Table 9 and Appendix 9)

Findings of the placenta are given in Table 9.
In 1 female (B47) of the low-dose group a bilobed placenta was observed. No statistically significant difference was observed for the individual findings.

3.11 Fetal weight and placental weight (Table 10 and Appendices 10 and 11)

The mean fetal weights and the placental weights are given in Table 10. No significant differences in the mean fetal body weight and placenta weights were observed between the control group and the groups fed trehalose in the diet.

3.12 Fetopathological examination

3.12.1 Visceral examination (Tables 11-13 and Appendix 12)

Visceral malformations
No statistically significant differences were observed in visceral malformations between the fetuses of the control group and the fetuses of the low-, mid-dose and high-dose groups (Table 11). Fetus 9 of female A13 showed proboscis and ectopic eyes (see also 3.9) and, furthermore, ventricles of the brain were missing. From fetus 6 of female A13 the subclavian artery was absent. From fetus 3 of female C69 the right kidney and ureter were missing.

Visceral anomalies

The number and type of visceral anomalies observed were normal for rabbits (Table 12). No statistically significant differences were observed in individual findings amongst the control and the trehalose-dosed groups.

Visceral variations
The number and type of visceral variations observed were normal for rabbits (Table 13). No statistically significant differences were observed amongst the control and the trehalose-treated groups.

In summary, there were no treatment-related changes in fetal soft tissues examination.

3.12.2 Skeletal examination (Tables 14-17 and Appendix 13)

Skeletal malformations
Except for two fused ribs observed in one fetus in the control group, no skeletal malformations were observed in the fetuses of the control and mid- and high-dose group (Table 14).

Skeletal anomalies
The number and type of skeletal anomalies were normal for rabbits (Table 15). No statistically significant differences were observed between the control and trehalose-treated groups.

Skeletal variations
The observed fetal skeletal variations are normal for rabbits (Table 16).
Except for a statistically significant increase in the incidence of accessory ribs in the mid-dose groups no statistically significant effects in fetal skeletal variations were observed between the control group and the trehalose-treated groups. Since the effect on accessory ribs was only observed in the mid-dose group and not in the high-dose groups and because the effect was only statistically significant when expressed on fetus-basis and not when expressed on litter-basis this effect was considered to be not related to treatment.

Variations in the ossification of the skeletons
The variations in the ossification of the skeletons are presented in Table 17. The incidence of one or two incomplete ossified thoracal bodies was statistically significantly increased in the mid- and high-dose groups when expressed on fetus-basis. On litter-basis the effect on ossification of the thoracal bodies was only statistically significant in the mid-dose group and not in the high-dose group. The incidence of three or more incomplete ossified thoracal bodies was decreased, although non-significantly, in the mid- and high-dose groups. The incidence of total incomplete ossified thoracal bodies was statistically significantly increased in the mid-dose group when expressed on fetus-basis. No statistically significant effect was observed when the incidence was expressed on litter-basis.
Furthermore, in the high-dose group no effects of trehalose treatment were observed on the incidence of total incomplete ossified thoracal bodies. For these reasons, the effect of trehalose on ossification of the thoracal bodies was not

considered to be treatment related.
In the mid-dose group, the incidence of fetuses with unossified distal epiphysis of the humerus was statistically significantly increased when expressed on fetus-basis. No effect was observed when the incidence was expressed on litter-basis. Furthermore, in the high-dose group no effect of trehalose treatment on the incidence of unossified distal epiphysis of humerus was observed. The incidental effect of trehalose on ossification of the distal epiphysis of the humerus was considered to be not related to treatment.
Furthermore, no other effects of trehalose on the ossification of fetal skeletons were observed.

4 Discussion and conclusion

Trehalose was fed in the diet to pregnant New Zealand White rabbits (16 animals per dose group) from days 0-29 of gestation. The concentrations in the diet were 0, 2.5, 5 and 10%. On gestation day 29 the dams were killed and macroscopically examined. Reproductive organs were weighed and fetuses were examined after Caesarian section. The viscera of the fetuses of all groups and the skeletons of the fetuses of the control, mid- and high-dose groups were examined.

One animal of the high-dose hardly ate and drank sparingly for several days and for that reason the animal was sacrificed on gestation day 16. Another animal of the high-dose group was found dead on gestation day 28. At necropsy, it was observed that both animals had a hairball in the stomach. Daily clinical observations during the gestation period did not reveal any other remarkable differences in the animals appearance, general condition or behaviour amongst the dosing and control groups.
Gross examination, at scheduled sacrifice on gestation day 29, did not reveal any significant differences of the maternal organs and tissues amongst the groups. The few macroscopic findings observed are common in rabbits.

No statistically significant differences in mean body weights, body weight changes and food consumption were observed amongst the control and the groups fed trehalose in the diet. It was noticed that some animals did not eat or drink for several days. The test substance intake during the gestation period ranged from 0.21 ± 0.05 - 0.77 ± 0.03 , 0.48 ± 0.11 - 1.34 ± 0.11 and 1.04 ± 0.14 - 2.82 ± 0.23 (mean $\pm$ standard error of the mean) g trehalose/kg body weight/day for the low-, mid- and high-dose group, respectively.

One early delivery was observed in 1 female (D101) of the high-dose group. At Caesarian section, 12 of 16 females in the control group, 12 of 16 females in the low-dose group, 14 of 16 females in the mid-dose group and 10 of 13 females in the high-dose group were pregnant.
The female fecundity index was 75, 75, 87 and 81% for the control, low-, mid- and high-dose groups respectively. The gestation index was 100% for all the groups. There were no statistically significant differences between the control group and the groups fed trehalose in the diet in the number of corpora lutea, implantations, live and dead fetuses and early and late resorptions nor in the pre- and post implantation loss or in the sex ratio of the fetuses.
Except for a statistically significant increase in the mean weight of the empty uterus of the animals of the high-dose group, no remarkable differences in gravid and empty uterus weight, ovary weight, carcass weight and net weight change (body weight gain from day 0 to day 29 of gestation minus gravid uterine weight) were observed between the control group and the groups fed trehalose in the diet. The increase in mean weight of the empty uterus was related to the increased number of fetuses in the high-dose group (8.4 in the high-dose group versus 6.5 in the control group) and was not considered to be treatment related.

Fetal external observations of the fetuses and placentas at Caesarian section did not reveal any remarkable findings which could be related to treatment. One fetus

in the control group showing proboscis and ectopic eyes
Furthermore, no significant differences in the mean fetal body weight and placenta weights were observed between the control group and the groups fed trehalose in the diet.

At fetal examination no treatment-related changes in fetal soft tissues were observed.

Since the observed skeletal variations in the mid-dose group and the effects on ossification in the mid- and high-dose group were only statistically significant when expressed on fetus-basis and not on litter-basis, these effects were considered to be not related to treatment.

At the mid- and high-dose concentrations, the test substance was homogeneously distributed in the diets and stable when stored for 7 days at room temperature and for at least 52 days in the freezer (< -18 °C). Due to increased day-to day background fluctuations in the concentration of a substance that co-resolves with trehalose during analysis, low-dose levels (2.5% trehalose) in pelleted diets could not be determined precisely (compared to the validation data). Therefore, it is not possible to determine if (considering the performance of the method for pelleted diets) the low-dose deviated less than 10% from the intended level of 2.5%. Considering the increased background fluctuations it was concluded that the mid-dose level (5%) is close-to-intended, although the relative difference with the expected value is -14%. The content of the test substance in the high-dose level (10%) was 'close to intended'.

On the basis of the results obtained in this study it was concluded that:
trehalose, when administered in the diet did not induce maternal nor developmental toxicity at concentration of 2.5, 5 and 10% for the low-, mid- and high-dose group, respectively.

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE: 1 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING GESTATION FEMALES

		DAY OF GESTATION																															
	GROUP#	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	TOTAL
# OF FEMALES EXAMINED	A	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	
	B	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	
	C	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	
	D	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	15	15	15	15	15	15	15	15	15	15	15	15	14	
NO REMARKABLE CLINICAL OBSERVATIONS	A	16	16	16	16	16	16	16	16	16	16	16	16	15	15	15	15	15	15	15	15	15	15	15	15	15	16	16	16	16	16	
	B	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	15	
	C	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	
	D	16	16	16	16	16	16	16	16	16	16	16	16	16	16	15	15	15	15	15	15	15	15	15	15	15	15	15	15	14	12	
DEAD: pregnant, interim kill	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
DEAD: pregnant, died	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
GENERAL CONDITION: pale	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
BEHAVIOUR: decreased activity	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
SKIN: sparsely haired	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2

Statistical key: Fishers exact test * p< 0.05 ** p< 0.01 # p< 0.001

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE: 1 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING GESTATION FEMALES

		DAY OF GESTATION																																
	GROUP#	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	TOTAL
# OF FEMALES EXAMINED	A	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	
	B	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	
	C	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	
	D	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	15	15	15	15	15	15	15	15	15	15	15	15	14	
SKIN: encrustation(s)	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
EARS: wound(s)	A	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERINEUM: soiled with faeces	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

Statistical key: Fishers exact test * p< 0.05 ** p< 0.01 # p< 0.001

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:2 SUMMARY OF PARENTAL NECROPSY OBSERVATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
DAMS	N	16	16	16	16
SKIN: alopecia/sparsely haired	N	0 f	1	1	2
	%	0.0	6.3	6.3	13
SKIN: encrustation(s)	N	0 f	0	0	1
	%	0.0	0.0	0.0	6.3
LIVER: dark appearance	N	1 f	2	0	2
	%	6.3	13	0.0	13
STOMACH: hairball	N	0 f	0	0	2
	%	0.0	0.0	0.0	13
OVARIES: haemorrhage(s)	N	0 f	0	0	1
	%	0.0	0.0	0.0	6.3

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:3 MEAN MATERNAL BODY WEIGHT DURING GESTATION (g)

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
DAY 0	MEAN	4336.5 d	4314.3	4353.8	4395.5
	S.E.	84.86	121.11	76.65	76.36
	N	12	12	14	13
DAY 7	MEAN	4460.6 d	4419.6	4381.5	4465.9
	S.E.	83.60	112.67	89.46	82.96
	N	12	12	14	13
DAY 14	MEAN	4540.6 d	4490.4	4480.1	4520.5
	S.E.	88.65	105.34	75.91	102.66
	N	12	12	14	13
DAY 21	MEAN	4556.3 d	4501.3	4411.1	4524.6
	S.E.	86.20	112.87	72.39	82.09
	N	12	12	14	12
DAY 29	MEAN	4467.9 d	4334.6	4275.4	4500.5
	S.E.	93.71	116.58	80.28	100.02
	N	12	12	14	10

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:4 MEAN MATERNAL BODY WEIGHT CHANGE DURING GESTATION (g)

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
DAYS 0 TO 7	MEAN	124.1 d	105.3	27.7	70.5
	S.E.	13.04	18.54	41.22	28.11
	N	12	12	14	13
DAYS 7 TO 14	MEAN	80.0 d	70.8	98.6	54.6
	S.E.	18.21	22.16	27.74	30.39
	N	12	12	14	13
DAYS 14 TO 21	MEAN	15.8 d	10.9	-69.1	-73.9
	S.E.	35.21	35.69	51.15	50.03
	N	12	12	14	12
DAYS 21 TO 29	MEAN	-88.4 d	-166.8	-135.6	-60.6
	S.E.	56.84	47.43	41.31	50.80
	N	12	12	14	10

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:5 MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- g/kg/day

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
DAYS 0 TO 7	MEAN	33.1 d	30.6	26.8	28.2
	S.E.	0.78	1.08	2.27	2.33
	N	12	12	14	13
DAYS 7 TO 14	MEAN	33.6 d	28.6	30.4	25.8
	S.E.	2.55	1.59	1.96	2.54
	N	11	12	14	13
DAYS 14 TO 21	MEAN	23.1 d	18.2	19.5	15.0
	S.E.	3.23	3.16	5.18	2.96
	N	10	12	13	12
DAYS 21 TO 29	MEAN	15.1 d	8.2	9.7	10.4
	S.E.	2.73	1.85	2.27	1.37
	N	10	12	12	10

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:6 SUMMARY OF REPRODUCTION DATA

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Females inseminated	N	16	16	16	16
Pregnant at C-section	N	12	12	14	10
Dams with no Viable Fetuses	N	0	0	0	0
Dams with Live Fetuses	N	12	12	14	10
Female fecundity index[1]	%	75	75	87	81
Gestation index[2]	%	100	100	100	100
Corpora Lutea	N	111	119	130	100
No. per animal	MEAN	9.25 u	9.92	9.29	10.00
	S.E.	0.808	0.679	0.559	0.683
Implantation Sites	N	88	108	107	87
No. per animal	MEAN	7.33 u	9.00	7.64	8.70
	S.E.	0.987	0.674	0.676	0.716
Preimplantation Loss	N	23	11	23	13
% per animal	MEAN	22.26 u	8.98	17.50	13.21
	S.E.	7.536	2.871	5.437	4.281
Live Fetuses	N	78	97	95	84
No. per animal	MEAN	6.50 u	8.08	6.79	8.40
	S.E.	0.848	0.570	0.482	0.733
% per animal	MEAN	90.59 u	90.47	91.20	96.41
	S.E.	3.484	2.299	2.752	1.869
Postimplantation Loss	N	10	11	12	3
% impl. loss per animal	MEAN	9.41 u	9.53	8.80	3.59
	S.E.	3.484	2.299	2.752	1.869
Dead Fetuses	N	2	4	4	0
No. per animal	MEAN	0.17 u	0.33	0.29	0.00
	S.E.	0.112	0.188	0.163	0.000

Statistical key: u= Kruskal-Wallis & Mann-Whitney U

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:6 SUMMARY OF REPRODUCTION DATA

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Pregnant at C-section	N	12	12	14	10
Resorptions: Total	N	8	7	8	3
No. per animal	MEAN	0.67 u	0.58	0.57	0.30
	S.E.	0.355	0.229	0.251	0.153
Resorptions: Early	N	4	4	7	3
No. per animal	MEAN	0.33 u	0.33	0.50	0.30
	S.E.	0.188	0.188	0.251	0.153
Resorptions: Late	N	4	3	1	0
No. per animal	MEAN	0.33 u	0.25	0.07	0.00
	S.E.	0.333	0.179	0.071	0.000
Live Male Fetuses	N	38 f	47	45	36
	%	49	48	47	43
Live Female Fetuses	N	40 f	50	50	48
	%	51	52	53	57

Statistical key: f= Fishers exact test u= Kruskal-Wallis & Mann-Whitney U

[1]) Female fecundity index: (number of females pregnant / number of females inseminated) x 100
[2]) Gestation index: (number of females with live pups / number of females pregnant) x 100

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:7 MEAN REPRODUCTIVE ORGAN WEIGHTS AND NET MATERNAL BODY WEIGHT CHANGE DURING GESTATION (g)

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
GRAVID UTERUS	MEAN	357.62	d	380.31	349.75	453.01
	S.E.	35.680		19.967	20.111	38.939
	N	12		11	14	10
CARCASS	MEAN	4110.3	d	3905.3	3925.7	4047.5
	S.E.	105.34		111.03	72.39	97.53
	N	12		11	14	10
NET WEIGHT CHANGE FROM DAY 0	MEAN	-226.2	d	-367.9	-428.1	-337.3
	S.E.	100.96		88.59	106.40	82.17
	N	12		11	14	10
EMPTY UTERUS	MEAN	55.513	d	58.656	56.383	70.944*
	S.E.	4.7931		2.5452	3.4097	4.2677
	N	12		12	14	10
OVARIES	MEAN	0.980	d	0.967	0.945	1.034
	S.E.	0.0322		0.0309	0.0406	0.0710
	N	12		12	14	10

Statistical key: d= ANOVA & Dunnett test * = p<0.05
N=Number of pregnant females

Carcass Weight : Terminal Body Weight minus Gravid Uterus weight
Net Weight Change from Day 0 : Carcass Weight minus Day 0 Body Weight

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:8 SUMMARY OF FETAL EXTERNAL OBSERVATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	12	14	10
Fetuses Evaluated	N	78	97	95	84
M NOSE: proboscis					
Fetal Incidence	N	1 f	0	0	0
	%	1.3	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	8.3	0.0	0.0	0.0
M EYES: ectopic					
Fetal Incidence	N	1 f	0	0	0
	%	1.3	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	8.3	0.0	0.0	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:9 SUMMARY OF FETAL EXTERNAL UNCLASSIFIED FINDINGS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	12	14	10
Fetuses Evaluated	N	78	97	95	84
PLACENTA: bilobed					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	1.0	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	8.3	0.0	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:10 SUMMARY OF FETAL MEANS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
PLACENTA WEIGHT UNITS: GRAMS					
of all Viable Fetuses	MEAN	3.7972 d	2.9591	3.2368	3.3042
	S.E.	.40973	.18912	.19753	.17021
	N	12	12	14	10
of Male Fetuses	MEAN	3.9062 d	3.0606	3.2984	3.4936
	S.E.	.41824	.20941	.22090	.18549
	N	12	12	14	10
of Female Fetuses	MEAN	3.3190 d	2.8225	3.1984	3.0945
	S.E.	.29725	.19186	.21973	.17870
	N	11	12	14	10
FETAL WEIGHT UNITS: GRAMS					
of all Viable Fetuses	MEAN	38.614 d	32.640	34.805	36.748
	S.E.	2.4125	2.0373	1.9162	1.3073
	N	12	12	14	10
of Male Fetuses	MEAN	39.435 d	33.398	34.483	37.411
	S.E.	2.4917	2.0302	1.8694	1.5649
	N	12	12	14	10
of Female Fetuses	MEAN	35.823 d	31.605	35.129	35.875
	S.E.	1.8860	2.2143	2.0775	1.4736
	N	11	12	14	10

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:11 SUMMARY OF FETAL VISCERAL MALFORMATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	12	14	10
Fetuses Evaluated	N	78	97	95	84
Heads Evaluated	N	36	45	44	41
NOSE: proboscis					
Fetal Incidence	N	1 f	0	0	0
	%	2.8	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	8.3	0.0	0.0	0.0
EYES: ectopic					
Fetal Incidence	N	1 f	0	0	0
	%	2.8	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	8.3	0.0	0.0	0.0
BRAIN: ventricles missing					
Fetal Incidence	N	1 f	0	0	0
	%	2.8	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	8.3	0.0	0.0	0.0
KIDNEYS: missing					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	1.1	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	7.1	0.0
URETERS: missing					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	1.1	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	7.1	0.0
CIRCULATORY SYST: absent subclavian					
Fetal Incidence	N	1 f	0	0	0
	%	1.3	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	8.3	0.0	0.0	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:12 SUMMARY OF FETAL VISCERAL ANOMALIES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	12	14	10
Fetuses Evaluated	N	78	97	95	84
Heads Evaluated	N	36	45	44	41
EYES: folded retina					
Fetal Incidence	N	3 f	4	3	6
	%	8.3	8.9	6.8	14.6
Litter Incidence	N	2 f	4	2	3
	%	17	33	14	30
EYES: small hole in vitreous body					
Fetal Incidence	N	1 f	0	0	0
	%	1.3	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	8.3	0.0	0.0	0.0
HEART: pericard filled with haemorrhagic fluid					
Fetal Incidence	N	6 f	9	10	12
	%	7.7	9.3	11	14
Litter Incidence	N	4 f	6	7	7
	%	33	50	50	70
GALLBLADDER: small					
Fetal Incidence	N	1 f	4	3	0
	%	1.3	4.1	3.2	0.0
Litter Incidence	N	1 f	3	3	0
	%	8.3	25	21	0.0
THORACIC CAVITY: containing haemorrhagic fluid					
Fetal Incidence	N	0 f	0	1	0
	%	0.0	0.0	1.1	0.0
Litter Incidence	N	0 f	0	1	0
	%	0.0	0.0	7.1	0.0
ABDOMEN: containing haemorrhagic fluid					
Fetal Incidence	N	3 f	4	12	6
	%	3.8	4.1	13	7.1
Litter Incidence	N	2 f	3	4	5
	%	17	25	29	50

Statistical key: f= Fishers exact test * = p<0.05

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:13 SUMMARY OF FETAL VISCERAL VARIATIONS

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12		12	14	10
Fetuses Evaluated	N	78		97	95	84
Heads Evaluated	N	36		45	44	41
EYES: not well-defined soft lens						
Fetal Incidence	N	0	f	1	1	2
	%	0.0		2.2	2.3	4.8
Litter Incidence	N	0	f	1	1	2
	%	0.0		8.3	7.1	20
SPLEEN: pale appearance						
Fetal Incidence	N	3	f	2	5	1
	%	3.8		2.1	5.3	1.2
Litter Incidence	N	1	f	2	2	1
	%	8.3		17	14	10
CIRCULATORY SYST: minor variation aortic-arch area						
Fetal Incidence	N	77	f	97	95	84
	%	99		100	100	100
Litter Incidence	N	12	f	12	14	10
	%	100		100	100	100

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:14 SUMMARY OF FETAL SKELETAL MALFORMATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
RIBS: two ribs fused					
Fetal Incidence	N	1 f		0	0
	%	1.3		0.0	0.0
Litter Incidence	N	1 f		0	0
	%	8.3		0.0	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:15 SUMMARY OF FETAL SKELETAL ANOMALIES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
STERNEBRAE: two sternebrae fused					
Fetal Incidence	N	3 f		1	0
	%	3.8		1.1	0.0
Litter Incidence	N	2 f		1	0
	%	17		7.1	0.0
STERNEBRAE: three or more sternebrae fused					
Fetal Incidence	N	1 f		0	0
	%	1.3		0.0	0.0
Litter Incidence	N	1 f		0	0
	%	8.3		0.0	0.0
THORACAL BODIES: one or two supernumerary bodies					
Fetal Incidence	N	0 f		1	0
	%	0.0		1.1	0.0
Litter Incidence	N	0 f		1	0
	%	0.0		7.1	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:16 SUMMARY OF FETAL SKELETAL VARIATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
RIBS: accessory lumbar rib(s)					
Fetal Incidence	N	41 f		69*	53
	%	53		73	63
Litter Incidence	N	12 f		14	10
	%	100		100	100
STERNEBRAE: irregular shape of one sternebra					
Fetal Incidence	N	3 f		1	0
	%	3.8		1.1	0.0
Litter Incidence	N	3 f		1	0
	%	25		7.1	0.0
STERNEBRAE: irregular shape of two or more sternebrae					
Fetal Incidence	N	0 f		1	0
	%	0.0		1.1	0.0
Litter Incidence	N	0 f		1	0
	%	0.0		7.1	0.0

Statistical key: f= Fishers exact test * = p<0.05

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:17 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
FRONTAL: incompletely ossified					
Fetal Incidence	N	1 f		4	0
	%	2.4		7.8	0.0
Litter Incidence	N	1 f		4	0
	%	8.3		29	0.0
PARIETAL: incompletely ossified					
Fetal Incidence	N	0 f		3	0
	%	0.0		5.9	0.0
Litter Incidence	N	0 f		3	0
	%	0.0		21	0.0
HYOID: incompletely ossified					
Fetal Incidence	N	7 f		8	6
	%	16.7		15.7	13.9
Litter Incidence	N	5 f		4	5
	%	42		29	50
HYOID: unossified					
Fetal Incidence	N	1 f		0	1
	%	2.4		0.0	2.3
Litter Incidence	N	1 f		0	1
	%	8.3		0.0	10
RIBS: one rib incompletely ossified					
Fetal Incidence	N	1 f		0	0
	%	1.3		0.0	0.0
Litter Incidence	N	1 f		0	0
	%	8.3		0.0	0.0
STERNEBRAE: one sternebra incompletely ossified					
Fetal Incidence	N	30 f		31	25
	%	38		33	30
Litter Incidence	N	10 f		12	10
	%	83		86	100

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:17 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
STERNEBRAE: two sternebrae incompletely ossified					
Fetal Incidence	N	12 f		14	16
	%	15		15	19
Litter Incidence	N	8 f		8	8
	%	67		57	80
STERNEBRAE: three or more sternebrae incomp. ossified					
Fetal Incidence	N	3 f		3	1
	%	3.8		3.2	1.2
Litter Incidence	N	3 f		2	1
	%	25		14	10
STERNEBRAE: one sternebra unossified					
Fetal Incidence	N	21 f		26	28
	%	27		27	33
Litter Incidence	N	10 f		11	10
	%	83		79	100
STERNEBRAE: two sternebrae unossified					
Fetal Incidence	N	10 f		5	7
	%	13		5.3	8.3
Litter Incidence	N	5 f		4	3
	%	42		29	30
THORACAL BODIES: one or two incomplete ossified bodies					
Fetal Incidence	N	1 f		17#	9*
	%	1.3		18	11
Litter Incidence	N	1 f		10**	4
	%	8.3		71	40

Statistical key: f= Fishers exact test * = p<0.05 ** = p<0.01 # = p<0.001

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:17 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
THORACAL BODIES: three or more incomplete ossified bodies					
Fetal Incidence	N	5 f		5	2
	%	6.4		5.3	2.4
Litter Incidence	N	3 f		1	1
	%	25		7.1	10
THORACAL BODIES: incomplete ossified bodies (total)					
Fetal Incidence	N	6 f		22**	11
	%	7.7		23.2	13.1
Litter Incidence	N	4 f		10	4
	%	33.3		71	40
CORACOID: incompletely ossified					
Fetal Incidence	N	12 f		7	15
	%	15		7.4	18
Litter Incidence	N	8 f		7	6
	%	67		50	60
CORACOID: unossified					
Fetal Incidence	N	50 f		69	50
	%	64		73	60
Litter Incidence	N	12 f		14	9
	%	100		100	90
METACARPALS: 1-2 metacarpals incompletely ossified					
Fetal Incidence	N	24 f		26	35
	%	31		27	42
Litter Incidence	N	8 f		10	10
	%	67		71	100
METACARPALS: 3-5 metacarpals incompletely ossified					
Fetal Incidence	N	0 f		1	0
	%	0.0		1.1	0.0
Litter Incidence	N	0 f		1	0
	%	0.0		7.1	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:17 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
METACARPALS: 1-2 metacarpals unossified					
Fetal Incidence	N	18 f		30	12
	%	23		32	14
Litter Incidence	N	6 f		7	7
	%	50		50	70
PHALANGES FRONT PROX: 1-4 digits incompletely ossified					
Fetal Incidence	N	1 f		5	1
	%	1.3		5.3	1.2
Litter Incidence	N	1 f		5	1
	%	8.3		36	10
PHALANGES FRONT MED: 1-4 digits incompletely ossified					
Fetal Incidence	N	41 f		51	51
	%	53		54	61
Litter Incidence	N	12 f		13	10
	%	100		93	100
PHALANGES FRONT MED: 3-6 digits unossified					
Fetal Incidence	N	15 f		25	21
	%	19		26	25
Litter Incidence	N	7 f		11	6
	%	58		79	60
PHALANGES FRONT PROX: 1-5 digits unossified					
Fetal Incidence	N	3 f		1	0
	%	3.8		1.1	0.0
Litter Incidence	N	1 f		1	0
	%	8.3		7.1	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:17 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
DIST EP OF FEMUR: incompletely ossified					
Fetal Incidence	N	20 f		13	23
	%	26		14	27
Litter Incidence	N	8 f		7	9
	%	67		50	90
DIST EP OF FEMUR: unossified					
Fetal Incidence	N	44 f		66	52
	%	56		69	62
Litter Incidence	N	11 f		13	10
	%	92		93	100
PROX EP OF TIBIA: incompletely ossified					
Fetal Incidence	N	3 f		4	10
	%	3.8		4.2	12
Litter Incidence	N	3 f		4	5
	%	25		29	50
PROX EP OF TIBIA: unossified					
Fetal Incidence	N	63 f		84	71
	%	81		88	85
Litter Incidence	N	11 f		14	10
	%	92		100	100
TALUS: unossified					
Fetal Incidence	N	4 f		6	0
	%	5.1		6.3	0.0
Litter Incidence	N	3 f		4	0
	%	25		29	0.0

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:17 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
TALUS: incompletely ossified					
Fetal Incidence	N	4 f		7	1
	%	5.1		7.4	1.2
Litter Incidence	N	2 f		4	1
	%	17		29	10
PHALANGES HIND PROX: 1-4 digits incompletely ossified					
Fetal Incidence	N	1 f		0	0
	%	1.3		0.0	0.0
Litter Incidence	N	1 f		0	0
	%	8.3		0.0	0.0
PHALANGES HIND PROX: 5-8 digits incompletely ossified					
Fetal Incidence	N	1 f		1	0
	%	1.3		1.1	0.0
Litter Incidence	N	1 f		1	0
	%	8.3		7.1	0.0
PHALANGES HIND PROX: 3-6 digits unossified					
Fetal Incidence	N	1 f		0	0
	%	1.3		0.0	0.0
Litter Incidence	N	1 f		0	0
	%	8.3		0.0	0.0
PHALANGES HIND MED: 1-4 digits incompletely ossified					
Fetal Incidence	N	43 f		56	42
	%	55		59	50
Litter Incidence	N	11 f		13	8
	%	92		93	80

Statistical key: f= Fishers exact test

STUDY NO.1993 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN NEW ZEALAND WHITE RABBITS

TABLE:17 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	12	0	14	10
Fetuses Evaluated	N	78	0	95	84
Heads Evaluated	N	42	0	51	43
PHALANGES HIND MED: 5-8 digits incompletely ossified					
Fetal Incidence	N	1 f		3	0
	%	1.3		3.2	0.0
Litter Incidence	N	1 f		2	0
	%	8.3		14	0.0
PHALANGES HIND MED: 1-4 digits unossified					
Fetal Incidence	N	2 f		5	0
	%	2.6		5.3	0.0
Litter Incidence	N	1 f		3	0
	%	8.3		21	0.0
PROX EP OF HUMERUS: incompletely ossified					
Fetal Incidence	N	13 f		11	10
	%	17		12	12
Litter Incidence	N	8 f		9	6
	%	67		64	60
PROX EP OF HUMERUS: unossified					
Fetal Incidence	N	52 f		74	60
	%	67		78	71
Litter Incidence	N	10 f		14	10
	%	83		100	100
DIST EP OF HUMERUS: incompletely ossified					
Fetal Incidence	N	13 f		10	11
	%	17		11	13
Litter Incidence	N	9 f		7	7
	%	75		50	70
DIST EP OF HUMERUS: unossified					
Fetal Incidence	N	18 f		37*	26
	%	23		39	31
Litter Incidence	N	5 f		11	7
	%	42		79	70

Statistical key: f= Fishers exact test