TNO report

V98.551 final

Oral embryotoxicity/teratogenicity study with trehalose in rats

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Datum:
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Project number:
471003

At the request of:
Hayashibara Company Ltd.
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Okyama 700, Japan

Study director:
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Number of pages:
216

Summary

1. Trehalose was fed in the diet to mated female Wistar rats (28 animals per dose group) from days 0-21 of gestation. The concentrations in the diet were 0, 2.5, 5 and 10%. On gestation day 21 the dams were killed and macroscopically examined. Reproductive organs were weighed and fetuses were examined after Cesarian section. The viscera and skeletons of the fetuses of the control and high-dose group were examined.

2. The test substance was homogeneously distributed in the diets. The diets were stable at room temperature and for 42 days in the refrigerator (2-10°C). The content of the test substance measured in the batch diets prepared for this study was close to intended at all dose levels.

3. No mortalities were observed.
   One animal of the control and high-dose group showed haemorrhagic discharge on gestation day 14 and 21, respectively.
   Further, daily clinical observations during the gestation period did not reveal any remarkable findings in the animals' appearance, general condition or behaviour amongst the dosing and control groups.

4. 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. The test substance intake during the gestation period ranged from 1.4-2.0, 2.8-3.9 and 5.5-7.8 g trehalose/kg body weight/day for the low-, mid and high-dose group, respectively.

5. For all dose groups 28 females were mated; early delivery was observed in 1 female (A51) of the control group and 2 females (B93 and B111) of the low-dose group. At Cesarian section, 21, 23, 24 and 24 females in the control group, low-dose, mid-dose and high-dose group were pregnant.
   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.
   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 21 of gestation minus gravid uterine weight) were observed between the control group and the groups fed trehalose in the diet.

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

7. Upon fetal examination there were no treatment-related changes in fetal soft tissues or fetal skeletal examination.

8. 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% (1.4-2.0, 2.8-3.9 and 5.5-7.8 g trehalose/kg body weight/day) for the low-, mid and high-dose group, respectively.

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] 09-07-1998

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

[signed] 09-07-1998

Dr B.M. Kulig
(Management) Date

Quality Assurance Statement

On:	Oral embryotoxicity/teratogenicity study with trehalose in rats
Report Number:	V98.551
Date :	9 July 1998

The protocol and amendment 1 and 2 were inspected on 13 January 1998, 15 January 1998 and 14 April 1998, respectively
The experimental phase of this study was inspected by the Quality Assurance Unit of TNO Nutrition and Food Research Institute as follows:

Date of inspection:	Date of report:
22 January 1998	22 January 1998
22 January 1998 (Analysis)	23 January 1998
28 January 1998	28 January 1998
19 February 1998	19 February 1998

This report was audited as follows:

Dates of audit:	Date of report:
29 and 30 June and 1 and 2 July 1998	2 July 1998
1, 6 and 7 July 1998 (Analysis)	7 July 1998
9 July 1998	9 July 1998

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]	09-07-1998
M.W. van Marwijk (Quality Assurance Officer)	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.M.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., 1-2-3-Shimoishii, 1-Chome, Okyama 700, Japan, the possible effects of the test substance on the pregnant female rat and the development of the embryo and fetus consequent to exposure of the female from fertilization (gestation day 0) to Cesarian section shortly before term (gestation day 21) were investigated.

2 Experimental

The study was conducted according to the protocol approved by the Sponsor, entitled "Protocol for an oral embryotoxicity study with trehalose in rats (P 471003)" approved by the Study Director on 16 December 1997 and Amendments 1 and 2 dated 13 January 1998 and 25 March 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	: 14 January 1998
b. experimental start date	: 25 January 1998
c. termination in-life part	: 24 February 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 rats. The rat was used because this species is recommended as a suitable species for this type of study.
Hundred and twenty four female rats of ca. 11 weeks and 62 male rats of ca. 12 weeks old, Wistar outbred (Crl:(WI)WU BR), were obtained from a colony maintained under SPF-conditions at Charles River Wiga, Germany. At the commencement of the study the female rats were at least 12 weeks old.

2.2.2 Animal allocation

Upon arrival, the rats were quarantined and checked for overt signs of ill health and anomalies. During the quarantine period serological examinations were conducted in a random sample. After the results of serology indicated an acceptable microbiological status, the animals were acclimatized for a period of 9 days.
After mating the mated females were distributed over the 4 experimental groups in such a way that the animals from the same day of pregnancy were equally distributed over all groups.
In the mid-dose group 2 females (C151 and C167) were mated by the same male (48); all other females mated by the same male were placed in different groups (Appendix 1).

2.2.3 Identification of the test system

During the acclimatization period, the animals were identified by a temporary tailmark. During the mating period, males were identified by their cage number. After allocation to the groups, the individual female rats were identified by a unique odd animal identification number which is tattooed and clipped in the ears. During the study, each group of rats was coded by a letter and a colour. Each cage was provided with a card showing the colour code, animal identification number(s), group letter and study number.

2.3 Experimental conditions

2.3.1 Animal maintenance

2.3.1.1 Environment

From arrival the rats were housed under conventional conditions in animal room 25.04.
The room was ventilated with about 10 air changes per hour and maintained at a temperature of 22 $\pm$ 3 °C and a relative humidity of 30 - 70 %, except for short periods during cleaning activities when the upper limit for humidity was exceeded (maximum of 93%). The number of air changes was about 10 per hour. Lighting was artificial by fluorescent tubes, time switch controlled at a sequence of 12 hours light (07:30-19:30), 12 hours dark.

2.3.1.2 Caging

During the quarantine and acclimatization period, the males and females were housed in groups of 4 per sex, in suspended stainless steel group cages (45x32x18 cm) with wire mesh floor and front. For mating one male and two females were housed together in smaller suspended wire mesh cages (18x32x18 cm) with wire mesh floor and front. Mated females were housed individually in wire mesh cages (18x32x18 cm), which were placed in another cage rack. The location of the mated females in the new cage racks was determined by the date of mating (females found sperm-positive on the same date were considered a "lot") and by the animal number (within each lot the mated females were housed in order of animal number).
After the mating period, surplus females and males were sacrificed.

2.3.2 Feed and drinking water

The rats were provided with feed and water *ad libitum* from arrival until the end of the study.
The feed was provided as a powder, in stainless steel cans, covered by a perforated steel plate that serves to prevent spillage. The feed in the feeders was refreshed once per week and topped up when necessary.
During the quarantine and acclimatization period, the rats were fed a closed formula diet obtained from SDS (Special Diets Services, Witham, England). Each batch of the diet (batch no. 4177) was analysed by the supplier for nutrients and contaminants (see Annex 1.1 for the certificate of analysis).
From day 0 of gestation, the female rats were fed a somewhat modified diet (batch no. 4070) supplemented with different concentrations of the test substance. The modification consisted of the omission of 20% barley from the diet (see Annex 1.2 for the certificate of analysis). The barley was replaced by the test substance and/or pregelatinized potato starch (Paselli WA 4, AVEBE, Foxhol, the Netherlands; see Annex 1.3 for certificate of analysis). For concentrations in the diet see section 2.4.3.
Diets were prepared by mixing the various ingredients in a mechanical blender and stored in a refrigerator.
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

Initially, 2 females were caged with each male for mating until pregnancy occurs. Every consecutive morning vaginal examinations were made to ascertain copulation by detection of sperm cells in a vaginal smear. Upon evidence of copulation, positive

females were housed individually. The day a sperm-positive smear or a vaginal plug was detected was considered to be gestation day 0.

2.4.1 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 from fertilization (gestation day 0) up to Cesarian section (gestation day 21). The test substance was incorporated in the diet as indicated in sections 2.3.2 and 2.4.3.

2.4.2 Dose levels and groups

The study comprised four groups of 28 mated female rats: 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 (%)		
			trehalose	Pregelatinized potato starch	
A	control	white	0	20	28
B	low-dose	blue	2.5	17.5	28
C	mid-dose	green	5	15	28
D	high-dose	red	10	10	28

[1]: SDS rodent diet from which 20% cereals has been left out.

2.4.3 Observations, analyses and measurements

2.4.3.1 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 analysis of samples from 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.

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 of diet preparation, after storage at room temperature in an open container (for 7 days) and after storage in a refrigerator in a closed container (for about 5 weeks).
To determine the homogeneity of the test substance in the diet, 5 samples per dose level (low-, mid- and high-dose) taken once at different locations in the feed container and 1 control sample were analyzed.
Diet samples will be taken immediately after preparation of the diets and stored at < -18 °C pending analysis.

2.4.3.2 Clinical signs

Each female was observed daily from the start of the study and, if necessary, handled to appraise its 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 animals from the study. During weekends and holidays only one check per day was carried out.

2.4.4 Body weight

Body weights of the rats were recorded on days 0, 7, 14 and 21 of gestation.

2.4.5 Food consumption

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

2.4.6 Water consumption

Water consumption was not measured.

2.4.7 Pathology

The females were killed by decapitation after ether anaesthesia on gestation day 21 and examined for gross abnormalities. Maternal tissue showing severe macroscopic abnormalities was removed and fixed in a neutral, aqueous phosphate buffered 4% solution of formaldehyde.

The uteri (including the fetuses), ovaries and placentas of all females killed on day 21 were examined for the following parameters:
- number of corpora lutea
- number of implantation sites[1]

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

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

2.4.8 Fetopathological examination

First all fetuses of each litter were examined carefully for anomalies. The sex of the fetuses was determined. Half of the number of fetuses in each litter was fixed in Bouin's fixative and subsequently examined for soft tissue anomalies according to a method modified after Barrow and Taylor (1969) and then discarded.
The other half of the foetuses were fixed in 70% ethanol, subsequently partly eviscerated and then cleared in potassium hydroxide and stained with Alizarin Red S modified after Dawson (1926). These foetuses were examined for skeletal abnormalities and then retained.
The fetopathological examinations were initially restricted to all foetuses of the animals of the control group and the high dose group. Only when alterations were observed in the high-dose group, the examinations were, after consultation with the sponsor, extended to the intermediate-dose groups.

2.5 Reproductive performance

For each 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 a level of significance.
Clinical findings were evaluated by Fisher's exact probability test.
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

- Female C151 and C167 mated with the same male (no. 48) were placed in the same group (group C).
- Initially 112 females were placed with 56 males (2 females per male); after another week the remaining 12 females were placed with 6 males (2 females per male).
- The homogeneity and stability at the day of diet preparation, after 7 days animal room and after ca. 5 weeks refrigerator of the test substance in the diets was not determined only in the low-dose and high-dose diets but also in the mid-dose diet.

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.
The test substance was homogeneously distributed in the diets. The diets were stable at room temperature and for 42 days in the refrigerator (2-10 °C). The content of the test substance measured in the batch diets prepared for this study on 22 January 1998 was close to intended at all dose levels.

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.
No mortalities were observed.
Animal A53 and D 219 of the control and high-dose group showed haemorrhagic discharge on gestation day 21 and 14, respectively.
Further, daily clinical observations during the gestation period did not reveal any remarkable findings in the animals' appearance, general condition or behaviour amongst the dosing and control groups.

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

The findings at Caesarian section are presented in Table 2.
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 rats.

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

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

3.5 Food consumption (Table 4 and Appendix 5)

Food consumption expressed as g/kg body weight/day is presented in Table 4.
No statistically significant difference in food consumption was observed amongst the control and the groups fed trehalose in the diet.

3.6 Test substance intake

The test substance intake was calculated from the food consumption expressed in g/kg body weight/day Table 4.

	Test substance intake (g trehalose/kg body weight/day)		
	2.5% trehalose low-dose group	5% trehalose mid-dose group	10% trehalose high-dose group
Gestation period: days 1-7	2.0	3.9	7.8
days 7-14	1.8	3.7	7.3
days 14 -21	1.4	2.8	5.5

3.7 Reproduction data and litter data (Table 5 and Appendices 6 and 7)

The pregnancy status of the reproduction and litter data are given in Table 5.
For all dose groups 28 females were mated; early delivery was observed in 1 female (A51) of the control group and 2 females (B93 and B111) of the low-dose group.
At Cesarian section, 21, 23, 24 and 24 females in the control group, low-dose, mid-dose and high-dose group were pregnant.
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.

3.8 Organ weights (Table 6 and Appendices 8)

Mean reproductive organ weights and net maternal body weight change during gestation are given in Table 6. 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 21 of gestation minus gravid uterine weight) were observed between the control group and the groups fed trehalose in the diet.

3.9 Fetal external observations (Table 7 and Appendix 9)

Fetal external observations are summarized in Table 7.
In the low- and high-dose group a statistically significantly decreased number of large fetuses (i.e. fetus weight > 125% of the mean fetal body weight) were observed.
Furthermore, in the low-dose group a statistically significantly decreased number of small fetuses (i.e. fetus weight < 75% of the mean fetal body weight) was observed.
The differences in the number of large and small fetuses were not considered to be an effect of the test substance for reason of their incidental nature and lack of dose response.
In the low-dose group a fetus with a flexed hindlimb and in the control and high-dose groups a fetus with a filiformed tail were observed. No other external findings were observed.

3.10 Findings of the placenta (Table 8 and Appendix 9)

Findings of the placenta are given in Table 8.
In 2 females (A31 and A39) of the control and in 1 female of the high-dose group (D207) 2 placentas were fused. In 1 female of the mid-dose group (C145) 3 placentas were fused. No statistically significant difference was observed for the individual findings.

3.11 Fetal weight and placental weight (Table 9 and Appendices 10 and 11)

The mean fetal weights and the placental weights are given in Table 9. 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 10-12 and Appendix 12)

Visceral malformations
No visceral malformations were observed in the fetuses of the control and high-dose group (Table 10).

Visceral anomalies
The number and type of visceral anomalies observed were normal for rats (Table 11). No statistically significant differences were observed amongst the control and the high-dose group fed trehalose in the diet.

Visceral variations
The number and type of visceral variations observed were normal for rats (Table 12). No statistically significant differences were observed amongst the control and the high-dose group fed trehalose in the diet.

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

3.12.2 Skeletal examination (Tables 13-16 and Appendix 13)

Skeletal malformations
No skeletal malformations were observed in the fetuses of the control and high-dose group (Table 13).

Skeletal anomalies
In the high-dose group 2 fetuses with dislocated metatarsals were observed (Table 14).

Skeletal variations
No differences were observed in the number of fetuses with skeletal variations (Table 15). The skeletal variations observed were normal for rats.

Variations in the ossification of the skeletons
Apart from the statistically significant difference in ossification of the proximal phalanges of the hindlimbs no effects were observed (Table 16). This incidental finding was considered not to be related to treatment as no other difference in ossification was observed.

4 Discussion and conclusion

Trehalose was fed in the diet to mated female Wistar rats (28 animals per dose group) from days 0-21 of gestation. The concentrations in the diet were 0, 2.5, 5 and 10%. On gestation day 21 the dams were killed and macroscopically examined. Reproductive organs were weighed and fetuses were examined after Cesarian section. The viscera and skeletons of the fetuses of the control and high-dose group were examined.

The test substance was homogeneously distributed in the diets. The diets were stable at room temperature and for 42 days in the refrigerator (2-10 °C). The content of the test substance measured in the batch diets prepared for this study was close to intended at all dose levels.

No mortalities were observed.
One animal of the control and high-dose group showed haemorrhagic discharge on gestation day 14 and 21, respectively.
Further, daily clinical observations during the gestation period did not reveal any remarkable findings in the animals' appearance, general condition or behaviour amongst the dosing and control groups.

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. The test substance intake during the gestation period ranged from 1.4-2.0, 2.8-3.9 and 5.5-7.8 g trehalose/kg body weight/day for the low-, mid and high-dose group, respectively.

For all dose groups 28 females were mated; early delivery was observed in 1 female (A51) of the control group and 2 females (B93 and B111) of the low-dose group. At Cesarian section, 21, 23, 24 and 24 females in the control group, low-dose, mid-dose and high-dose group were pregnant.
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.
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 21 of gestation minus gravid uterine weight) were observed between the control group and the groups fed trehalose in the diet.

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

Upon fetal examination there were no treatment-related changes in fetal soft tissues or fetal skeletal examination.

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% (1.4-2.0, 2.8-3.9 and 5.5-7.8 g trehalose/kg body weight/day) for the low-, mid and high-dose group, respectively.

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

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	TOTAL
# OF FEMALES EXAMINED	A	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	
	B	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	
NO REMARKABLE CLINICAL OBSERVATIONS	A	28	28	28	28	28	28	28	25	25	25	25	25	25	25	23	23	23	23	23	23	23	18	
	B	28	28	28	28	28	28	28	27	27	27	27	27	27	27	26	26	26	26	26	27	27	21	
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	20	
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	26	27	27	27	27	27	27	20	
DEAD: scheduled sacrifice	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	28
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	28
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	28
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	28
SKIN: encrustation(s)	A	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	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
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKIN: sparsely haired	A	0	0	0	0	0	0	0	2	2	2	2	2	2	2	4	4	4	4	4	4	4	9	9
	B	0	0	0	0	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	2	1	7	8
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	8
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	8	8
VAGINA: haemorrhagic discharge	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	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
	C	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	1	0	0	0	0	0	0	0	1

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:2 SUMMARY OF PARENTAL NECROPSY OBSERVATIONS

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
DAMS	N	28		28	28	28
SKIN: alopecia/sparsely haired	N	9	f	8	6	8
	%	32		29	21	29
SKIN: encrustation(s)	N	1	f	0	1	1
	%	3.6		0.0	3.6	3.6
VAGINA: haemorrhagic discharge	N	2	f	0	0	0
	%	7.1		0.0	0.0	0.0
UTERUS: swollen	N	1	f	1	0	0
	%	3.6		3.6	0.0	0.0
UTERUS: hydrometra	N	1	f	0	0	0
	%	3.6		0.0	0.0	0.0
UTERUS: haematometra	N	0	f	1	0	0
	%	0.0		3.6	0.0	0.0
EARS: partly missing	N	1	f	0	0	0
	%	3.6		0.0	0.0	0.0

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:3.1 MEAN MATERNAL BODY WEIGHT DURING GESTATION (g)

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
DAY 0	MEAN	212.72 d	211.40	212.86	215.76
	S.E.	4.152	2.683	2.889	2.949
	N	22	25	24	24
DAY 7	MEAN	233.22 d	233.82	238.12	237.49
	S.E.	4.445	2.725	2.245	3.163
	N	22	25	24	24
DAY 14	MEAN	258.72 d	257.45	260.07	258.96
	S.E.	4.529	3.332	2.499	2.964
	N	22	25	24	24
DAY 21	MEAN	295.98 d	289.51	293.39	296.65
	S.E.	4.901	3.739	3.452	3.173
	N	21	23	24	24

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:3.2 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	20.50 d	22.42	25.26	21.73
	S.E.	1.451	1.141	2.858	1.183
	N	22	25	24	24
DAYS 7 TO 14	MEAN	25.50 d	23.63	21.95	21.48
	S.E.	1.125	1.051	1.306	1.783
	N	22	25	24	24
DAYS 14 TO 21	MEAN	35.46 d	32.79	33.32	37.69
	S.E.	2.917	1.802	1.900	2.248
	N	21	23	24	24

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:4 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	77.78 d	79.11	78.10	77.58
	S.E.	1.858	1.180	1.288	1.075
	N	22	25	24	24
DAYS 7 TO 14	MEAN	73.81 d	73.19	74.27	72.52
	S.E.	1.130	1.444	1.514	0.818
	N	22	25	24	24
DAYS 14 TO 21	MEAN	53.42 d	54.24	55.93	54.62
	S.E.	1.670	1.461	1.530	1.676
	N	21	23	24	24

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:5 SUMMARY OF REPRODUCTION DATA

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Pregnant at C-section	N	21	23	24	24
Dams with no Viable Fetuses	N	0	0	0	0
Dams with Live Fetuses	N	21	23	24	24
Female fecundity	%	79	89	86	86
Corpora Lutea	N	288	315	320	333
No. per animal	MEAN	13.71 u	13.70	13.33	13.88
	S.E.	0.379	0.291	0.627	0.501
Implantation Sites	N	268	289	282	300
No. per animal	MEAN	12.76 u	12.57	11.75	12.50
	S.E.	0.441	0.326	0.755	0.507
Pre-implantation Loss	N	20	26	38	33
% per animal	MEAN	7.03 u	7.95	13.17	9.26
	S.E.	1.735	1.964	5.032	3.548
Live Fetuses	N	264	271	275	281
No. per animal	MEAN	12.57 u	11.78	11.46	11.71
	S.E.	0.461	0.397	0.754	0.476
% per animal	MEAN	98.38 u	94.35	97.56	94.21
	S.E.	0.761	2.686	0.978	1.768
Post-implantation Loss	N	4	18	7	19
% impl. loss per animal	MEAN	1.62 u	5.65	2.44	5.79
	S.E.	0.761	2.686	0.978	1.768
Dead Fetuses	N	0	1	0	1
No. per animal	MEAN	0.00 u	0.04	0.00	0.04
	S.E.	0.000	0.043	0.000	0.042

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

Female fecundity index : number of females pregnant * 100 / number of females mated
Pre-implantation loss : number of corpora lutea - number of implantation sites * 100 / number of corpora lutea
Post-implantation loss : number of implantation sites - number of live fetuses * 100 / number of implantation sites

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:5 SUMMARY OF REPRODUCTION DATA

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Pregnant at C-section	N	21		23	24	24
Resorptions: Total	N	4		17	7	18
No. per animal	MEAN	0.19	u	0.74	0.29	0.75
	S.E.	0.088		0.357	0.112	0.243
Resorptions: Early	N	4		12	6	16
No. per animal	MEAN	0.19	u	0.52	0.25	0.67
	S.E.	0.088		0.176	0.109	0.197
Resorptions: Late	N	0		5	1	2
No. per animal	MEAN	0.00	u	0.22	0.04	0.08
	S.E.	0.000		0.217	0.042	0.058
Live Male Fetuses	N	140	f	142	145	152
Sex ratio	%	53		52	53	54
Live Female Fetuses	N	124	f	129	130	129
	%	47		48	47	46

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

Sex ratio : number of male fetuses date n * 100 / total number of fetuses

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:6 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	72.90 d	67.29	64.85	68.70
	S.E.	2.224	1.963	3.891	2.695
	N	21	23	24	24
CARCASS	MEAN	223.08 d	222.22	228.54	227.95
	S.E.	4.449	3.854	4.534	4.534
	N	21	23	24	24
NET WEIGHT CHANGE FROM DAY 0	MEAN	8.64 d	10.71	15.68	12.19
	S.E.	3.691	3.693	4.752	3.536
	N	21	23	24	24
EMPTY UTERUS	MEAN	4.660 d	4.370	4.022	4.466
	S.E.	0.1543	0.1439	0.2117	0.1775
	N	21	23	24	24
OVARIES	MEAN	0.119 d	0.116	0.115	0.110
	S.E.	0.0053	0.0052	0.0055	0.0054
	N	21	23	24	24

Statistical key: d= ANOVA & Dunnett test

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

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:7 SUMMARY OF FETAL EXTERNAL OBSERVATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	23	24	24
Fetuses Evaluated	N	264	272	275	282
Live	N	264	271	275	281
Dead	N	0	1	0	1
V GROSS EXAM: large fetus					
Fetal Incidence	N	9 f	1*	7	0**
	%	3.4	0.4	2.5	0.0
Litter Incidence	N	3 f	1	4	0
	%	14	4.3	17	0.0
V GROSS EXAM: small fetus					
Fetal Incidence	N	7 f	0*	7	1
	%	2.7	0.0	2.5	0.4
Litter Incidence	N	3 f	0	5	1
	%	14	0.0	21	4.2
A LIMBS: flexed					
Fetal Incidence	N	0 f	1	0	0
	%	0.0	0.4	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	4.3	0.0	0.0
M TAIL: filiformed					
Fetal Incidence	N	1 f	0	0	1
	%	0.4	0.0	0.0	0.4
Litter Incidence	N	1 f	0	0	1
	%	4.8	0.0	0.0	4.2

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

Large fetus : > 125 % of the mean fetal body weight of the control group (viz. 5.555 g for males and 5.228 g for females).
Small fetus : < 75 % of the mean fetal body weight of the control group (viz. 3.333 g for males and 3.137 g for females).

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:8 SUMMARY OF FETAL EXTERNAL UNCLASSIFIED FINDINGS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	23	24	24
Fetuses Evaluated	N	264	272	275	282
Live	N	264	271	275	281
Dead	N	0	1	0	1
PLACENTA: fused					
Fetal Incidence	N	4 f	0	3	2
	%	1.5	0.0	1.1	0.7
Litter Incidence	N	2 f	0	1	1
	%	9.5	0.0	4.2	4.2

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:9 SUMMARY OF FETAL MEANS (g)

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
PLACENTA WEIGHT					
of all Viable Fetuses	MEAN	0.5026 d	0.4547	0.5100	0.4818
	S.E.	.02125	.00950	.02638	.02018
	N	21	23	24	24
of Male Fetuses	MEAN	0.5183 d	0.4682	0.5012	0.4979
	S.E.	.02311	.01070	.01641	.02479
	N	21	23	23	24
of Female Fetuses	MEAN	0.4870 d	0.4402	0.4900	0.4645
	S.E.	.02133	.00859	.02729	.01724
	N	21	23	23	24
FETAL WEIGHT					
of all Viable Fetuses	MEAN	4.3257 d	4.2935	4.3475	4.4152
	S.E.	.10201	.03784	.12131	.06778
	N	21	23	24	24
of Male Fetuses	MEAN	4.4441 d	4.4088	4.3863	4.5344
	S.E.	.11386	.04323	.10509	.07160
	N	21	23	23	24
of Female Fetuses	MEAN	4.1825 d	4.1607	4.1657	4.2895
	S.E.	.09567	.03746	.11800	.06811
	N	21	23	23	24

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:10 SUMMARY OF FETAL VISCERAL MALFORMATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	126	0	0	135
TOTAL FETAL VISCERAL MALFORMATIONS					
Fetal Incidence	N	0 f			0
	%	0.0			0.0
Litter Incidence	N	0 f			0
	%	0.0			0.0

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:11 SUMMARY OF FETAL VISCERAL ANOMALIES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	126	0	0	135
URINARY BLADDER: dilated					
Fetal Incidence	N	3 f			6
	%	2.4			4.4
Litter Incidence	N	2 f			3
	%	9.5			13
URETERS: hydroureter					
Fetal Incidence	N	5 f			9
	%	4.0			6.7
Litter Incidence	N	3 f			4
	%	14			17
URETERS: kinked					
Fetal Incidence	N	0 f			3
	%	0.0			2.2
Litter Incidence	N	0 f			3
	%	0.0			13

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:12 SUMMARY OF FETAL VISCERAL VARIATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	126	0	0	135
KIDNEYS: increased renal pelvic cavitation					
Fetal Incidence	N	4 f			9
	%	3.2			6.7
Litter Incidence	N	2 f			7
	%	9.5			29
URETERS: bent					
Fetal Incidence	N	4 f			5
	%	3.2			3.7
Litter Incidence	N	3 f			2
	%	14			8.3

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:13 SUMMARY OF FETAL SKELETAL MALFORMATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	138	0	0	146
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	0 f			0
	%	0.0			0.0
Litter Incidence	N	0 f			0
	%	0.0			0.0

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:14 SUMMARY OF FETAL SKELETAL ANOMALIES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	138	0	0	146
METATARSALS: dislocated					
Fetal Incidence	N	0 f			2
	%	0.0			1.4
Litter Incidence	N	0 f			1
	%	0.0			4.2

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:15 SUMMARY OF FETAL SKELETAL VARIATIONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	138	0	0	146
STERNEBRAE: irregular ossification of one sternebra					
Fetal Incidence	N	1 f			0
	%	0.7			0.0
Litter Incidence	N	1 f			0
	%	4.8			0.0
STERNEBRAE: irregular ossification of two sternebrae					
Fetal Incidence	N	1 f			1
	%	0.7			0.7
Litter Incidence	N	1 f			1
	%	4.8			4.2
STERNEBRAE: irregular shape of one sternebra					
Fetal Incidence	N	4 f			4
	%	2.9			2.7
Litter Incidence	N	3 f			3
	%	14			13
STERNEBRAE: irregular shape of two or more sternebrae					
Fetal Incidence	N	6 f			9
	%	4.3			6.2
Litter Incidence	N	5 f			8
	%	24			33
THORACAL BODIES: one or two bodies irregularly ossified					
Fetal Incidence	N	0 f			1
	%	0.0			0.7
Litter Incidence	N	0 f			1
	%	0.0			4.2

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:16 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21		0	0	24
Fetuses Evaluated	N	138		0	0	146
FRONTAL: incompletely ossified						
Fetal Incidence	N	94	f			104
	%	68				71
Litter Incidence	N	18	f			20
	%	86				83
PARIETAL: incompletely ossified						
Fetal Incidence	N	83	f			97
	%	60				66
Litter Incidence	N	15	f			18
	%	71				75
INTER PARIETAL: incompletely ossified						
Fetal Incidence	N	2	f			6
	%	1.4				4.1
Litter Incidence	N	2	f			3
	%	9.5				13
HYOID: incompletely ossified						
Fetal Incidence	N	0	f			2
	%	0.0				1.4
Litter Incidence	N	0	f			1
	%	0.0				4.2
STERNEBRAE: one sternebra incompletely ossified						
Fetal Incidence	N	21	f			22
	%	15				15
Litter Incidence	N	13	f			14
	%	62				58
STERNEBRAE: two sternebrae incompletely ossified						
Fetal Incidence	N	8	f			10
	%	5.8				6.8
Litter Incidence	N	7	f			7
	%	33				29

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:16 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	138	0	0	146
STERNEBRAE: three or more sternebrae incomp. ossified					
Fetal Incidence	N	2 f			2
	%	1.4			1.4
Litter Incidence	N	2 f			2
	%	9.5			8.3
STERNEBRAE: one sternebra unossified					
Fetal Incidence	N	0 f			1
	%	0.0			0.7
Litter Incidence	N	0 f			1
	%	0.0			4.2
CERVICAL BODIES: one or two incomplete ossified bodies					
Fetal Incidence	N	68 f			57
	%	49			39
Litter Incidence	N	21 f			24
	%	100			100
CERVICAL BODIES: three or more incomplete ossified bodies					
Fetal Incidence	N	10 f			6
	%	7.2			4.1
Litter Incidence	N	8 f			6
	%	38			25
CERVICAL BODIES: one or two unossified bodies					
Fetal Incidence	N	35 f			44
	%	25			30
Litter Incidence	N	15 f			18
	%	71			75

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:16 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21		0	0	24
Fetuses Evaluated	N	138		0	0	146
CERVICAL BODIES: three or more unossified bodies						
Fetal Incidence	N	96	f			96
	%	70				66
Litter Incidence	N	20	f			23
	%	95				96
THORACAL BODIES: one or two incomplete ossified bodies						
Fetal Incidence	N	5	f			1
	%	3.6				0.7
Litter Incidence	N	2	f			1
	%	9.5				4.2
METACARPALS: 1-2 metacarpals incompletely ossified						
Fetal Incidence	N	1	f			1
	%	0.7				0.7
Litter Incidence	N	1	f			1
	%	4.8				4.2
METACARPALS: 3-6 unossified						
Fetal Incidence	N	0	f			1
	%	0.0				0.7
Litter Incidence	N	0	f			1
	%	0.0				4.2
PHALANGES FRONT PROX: 1-4 digits incompletely ossified						
Fetal Incidence	N	103	f			106
	%	75				73
Litter Incidence	N	21	f			24
	%	100				100

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:16 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	138	0	0	146
PHALANGES FRONT PROX: 5-8 digits incompletely ossified					
Fetal Incidence	N	2 f			1
	%	1.4			0.7
Litter Incidence	N	2 f			1
	%	9.5			4.2
PHALANGES FRONT DIST: 1-5 digits incompletely ossified					
Fetal Incidence	N	15 f			19
	%	11			13
Litter Incidence	N	8 f			10
	%	38			42
PHALANGES FRONT DIST: 6-10 digits incompletely ossified					
Fetal Incidence	N	0 f			4
	%	0.0			2.7
Litter Incidence	N	0 f			2
	%	0.0			8.3
PHALANGES FRONT PROX: 3-6 digits unossified					
Fetal Incidence	N	26 f			29
	%	19			20
Litter Incidence	N	13 f			13
	%	62			54
PHALANGES FRONT PROX: 7-10 digits unossified					
Fetal Incidence	N	4 f			2
	%	2.9			1.4
Litter Incidence	N	4 f			2
	%	19			8.3

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:16 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21		0	0	24
Fetuses Evaluated	N	138		0	0	146
PHALANGES FRONT DIST: 1-5 digits unossified						
Fetal Incidence	N	1	f			3
	%	0.7				2.1
Litter Incidence	N	1	f			2
	%	4.8				8.3
METATARSALS: 0-2 metatarsals incompletely ossified						
Fetal Incidence	N	38	f			30
	%	28				21
Litter Incidence	N	15	f			12
	%	71				50
METATARSALS: 0-2 metatarsals unossified						
Fetal Incidence	N	13	f			15
	%	9.4				10
Litter Incidence	N	8	f			10
	%	38				42
METATARSALS: 3-5 metatarsals unossified						
Fetal Incidence	N	0	f			1
	%	0.0				0.7
Litter Incidence	N	0	f			1
	%	0.0				4.2
PHALANGES HIND PROX: 1-4 digits incompletely ossified						
Fetal Incidence	N	45	f			57
	%	33				39
Litter Incidence	N	18	f			23
	%	86				96

Statistical key: f= Fishers exact test

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:16 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL		B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21		0	0	24
Fetuses Evaluated	N	138		0	0	146
PHALANGES HIND PROX: 5-8 digits incompletely ossified						
Fetal Incidence	N	38	f			42
	%	28				29
Litter Incidence	N	15	f			15
	%	71				63
PHALANGES HIND DIST: 1-5 digits incompletely ossified						
Fetal Incidence	N	1	f			4
	%	0.7				2.7
Litter Incidence	N	1	f			3
	%	4.8				13
PHALANGES HIND DIST: 6-10 digits incompletely ossified						
Fetal Incidence	N	1	f			1
	%	0.7				0.7
Litter Incidence	N	1	f			1
	%	4.8				4.2
PHALANGES HIND PROX: 3-6 digits unossified						
Fetal Incidence	N	42	f			64*
	%	30				44
Litter Incidence	N	17	f			22
	%	81				92
PHALANGES HIND PROX: 7-10 digits unossified						
Fetal Incidence	N	63	f			66
	%	46				45
Litter Incidence	N	19	f			19
	%	90				79

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

STUDY NO.1991 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH TREHALOSE IN RATS

TABLE:16 SUMMARY OF VARIATION IN OSSIFICATION OF FETAL SKELETONS

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Litters Evaluated	N	21	0	0	24
Fetuses Evaluated	N	138	0	0	146
PHALANGES HIND DIST: 1-5 digits unossified					
Fetal Incidence	N	2 f			0
	%	1.4			0.0
Litter Incidence	N	1 f			0
	%	4.8			0.0

Statistical key: f= Fishers exact test