

TNO report
V99.280 final

Oral two-generation reproduction study with trehalose in rats

Volume 1 of 3

TNO Nutrition and Food Research Institute

Utrechtseweg 48
P.O. Box 360
3700 AJ Zeist
The Netherlands

Phone +31 30 694 41 44
Fax +31 30 695 72 24

Date:
27 September 1999

Authors:
Dr Ir A.P.M. Wolterbeek
Ir D.H. Waalkens-Berendsen

Project number:
471004
TNO Study number:
1992

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

Study director:
Ir D.H. Waalkens-Berendsen

All rights reserved
No part of this publication may be reproduced and/or published by print, photoprint, microfilm or any other means without the previous written consent of TNO.

In case this report was drafted on instructions, the rights and obligations of contracting parties are subject to either the Standard Conditions for Research Instructions given to TNO or the relevant agreement concluded between the contracting parties
Submitting the report for inspection to parties who have a direct interest is permitted

Number of pages:

(Volume 1: pages 1-....., Text, Tables 1-50, Annexes 1-4)

© TNO



1. Trehalose was given for two successive generations to male and female Wistar rats in the diet at concentrations of 0, 2.5, 5 and 10%. In each generation one litter was raised.
2. From the data of this study it can be concluded that dietary administration of trehalose to rats at concentrations up to 10% (w/w) for two consecutive generations had no maternal toxic effects and had no effects on reproduction of the parental F0- and F1-generation or the development of the pups of the F0- and F1-generation. The no observed adverse effect level (NOAEL) for reproductive effects after dietary administration of trehalose to rats is at least 7.09 (males pre-mating), 7.61 (females pre-mating), 6.16 (gestation) and 14.09 (lactation) g/kg body weight/day.
3. The test substance was homogeneously distributed in the diets and was stable when stored for 7 days at room temperature or stored for 6 weeks in the refrigerator (2-10°C). The concentrations of the test substance measured in each batch of the diets prepared were close to the intended concentration (2.5, 5 and 10%).
4. Daily clinical observations of the F0- and F1-animals during the pre-mating, gestation and lactation period did not reveal remarkable findings in the animals' appearance, general condition or behaviour which could be related to trehalose treatment.
5. Statistically significant intergroup differences in maternal body weights and body weight changes were not-consistent by reproductive period, dose or generation and were considered not related to treatment.
6. The statistically significant differences in food consumption between the control and treatment groups of the F0- and F1-generation were not considered to be treatment related, because of the inconsistent nature of the affected groups.
7. The test substance intake for the F0- and F1 males during the pre-mating period ranged from 1.24-2.90 (mean \pm standard error = 1.73 \pm 0.10), 2.38-5.65 (3.45 \pm 0.20) and 4.89-12.43 (7.09 \pm 0.45) g/kg body weight/day for the low-, mid- and high-dose group, respectively. The test substance intake for the F0- and F1 females during the pre-mating period ranged from 1.47-2.85 (1.86 \pm 0.09), 2.88-5.48 (3.74 \pm 0.16) and 5.94-11.73 (7.61 \pm 0.34)g/kg body weight/day for the low-, mid- and high-dose group, respectively. During the gestation period the test substance ranged from 1.06-1.74 (1.49 \pm 0.13), 2.22-3.51 (3.06 \pm 0.23) and 4.40-7.21(6.16 \pm 0.48) g/kg body weight/day for the low-, mid- and high-dose group, respectively. During the lactation period the test substance ranged from 2.30-4.34 (3.33 \pm 0.54), 4.98-8.94 (6.88 \pm 1.09), 10.10-17.94 (14.09 \pm 2.19) g/kg body weight/day for the low-, mid- and high-dose group, respectively.
8. From the results of this study it was concluded that in both generations no effects of trehalose treatment were observed on any reproduction variables

determined: precoital time, mating index, male and female fertility, female fecundity index, gestation index, duration of gestation, and number of females with (all) stillborn pups and post-implantation loss.

9. For both generations, no adverse effects of trehalose were observed on the number of pups delivered, the number of liveborn and stillborn pups, pup mortality, sex ratio, pup observations, pup body weights and pup body weight changes.
10. No effect of the test substance was observed on absolute and relative spleen weights.
11. At autopsy of both generations, no treatment-related macroscopic changes were observed.
Microscopical examination did not reveal treatment-related histopathological changes in either generation. The histopathological changes observed are common findings in rats of this age and strain. Furthermore, they were about equally distributed amongst the groups or they occurred in only one or a few animals.

Contents

Summary	2
Statement of GLP compliance	5
Authentication of co-operating scientists	6
Quality Assurance Statement	7
Testing facility	10
Contributors	10
1 Introduction	11
2 Experimental	11
2.1 Test substance	11
2.2 Test system	12
2.3 Experimental conditions	12
2.4 Experimental procedures	14
2.5 Fertility and reproductive performance	18
2.6 Statistical analysis of the results	19
2.7 Retention of records, samples and specimens	19
2.8 Deviations from the protocol	20
3 Results	21
3.1 Analyses of trehalose in the diets (Annex 4)	21
3.2 Clinical signs (Tables 1-8, Appendices 3-10)	21
3.3 Body weights and body weight changes (Tables 9-24, Appendices 11-18)	21
3.4 Food consumption (Tables 25-32, Appendices 19-26)	22
3.5 Test substance intake (Tables 33 and 34)	22
3.6 Fertility and reproductive performance (Tables 35 and 36, Appendices 27-32)	23
3.7 Litter data (Tables 35-42, Appendices 31-40)	23
3.8 Parental necropsy observations (Tables 43-50, Appendices 41-46)	25
4 Discussion and conclusion	26

Revised final report

This revised final report has been issued as the result of comments by the sponsor on figures in paragraph 7 of the summary, in section 3.5 and in the paragraph about test substance intake of the discussion and conclusion of the final report, dated 27 September 1999.

The comments have been documented and retained with the other documentation in the archives of the TNO Nutrition and Food Research Institute.

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 (1997). Organization for Economic Co-operation and Development (OECD), Paris, ENV/MC/CHEM(98)17.

[signed]

27-09-1999

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

Date

[signed]

27-09-1999

Dr H.H. Emmen
(Management)

Date

Authentication of co-operating scientists

I, the undersigned, hereby declare that the pathology data presented in this report were compiled by me or under my supervision, and accurately reflect the data obtained.

[signed]

27-09-1999

Drs M.V.W. Wijnands
(Pathologist)

Date

Quality Assurance Statement

On: Oral two-generation reproduction study with trehalose in rats
Report Number: V99.280
Date : 12 October 1999

The protocol of this study was inspected on 13 January 1998 and Amendment 1 and 2 were inspected on 14 January 1998 and on 14 April 1998, respectively.

The experimental phase of the study was inspected by the Quality Assurance Unit of TNO Nutrition and Food Research Institute as follows:

Date of inspection:	Date of report:
15 January 1998	15 January 1998
22 January 1998 (analysis)	23 January 1998
26 January 1998	26 January 1998
1 April 1998	1 April 1998
27 April 1998	27 April 1998
8 May 1998	8 May 1998
29 June 1998	29 June 1998
12 August 1998	12 August 1998
8 September 1998	8 September 1998
1 October 1998	2 October 1998

This report was audited as follows:

Dates of audit:	Date of report:
13, 14 April 1999	14 April 1999
6-9, 12-14 April 1999	15 April 1999
27 September 1999	27 September 1999
12 October 1999	12 October 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]

27-09-1999

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



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
Utrechtsweg 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.



Rijsdijk, 24 April 1998

A. Jaspers, PhD

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

Testing facility

The study was conducted by:
TNO Nutrition and Food Research Institute
Toxicology Division and Analytical Sciences Division
P.O. Box 360, 3700 AJ Zeist, the Netherlands
Telephone +31 30 69 44 144
Telefax +31 30 69 57 224
Visitors address: Utrechtseweg 48, Zeist, the Netherlands

Contributors

Study director	: Ir D.H. Waalkens-Berendsen ¹
Deputy study director	: Ir D. Jonker ¹
Trainee study director	: Dr Ir A.P.M. Wolterbeek ¹
Study assistant	: A. Dijkstra ¹
Biotechnicians	: M.R. van Kooten-van Someren, C. M. Schlosser, C. D. van den Berg-Zwan ¹
Senior biotechnician	: G. van Beek ¹
Pathologist	: Drs M.V.W. Wijnands ¹
Diet preparation	: J.Y.C. de Smit ¹
Senior histotechnician	: E.C.M. van Oostrum ¹
Test substance analyses in the diet, Principal investigator	: Drs E.Ch.Th. Gevers ²

¹ Toxicology Division, TNO Nutrition and Food Research Institute

² Analytical Sciences Division, TNO Nutrition and Food Research Institute

1 Introduction

At the request of Hayashibara Company Ltd., 2-3-Shimoishii, 1-chome, Okayama 700, Japan the possible effects of trehalose on male and female reproductive performance and on the growth and development of the offspring were examined in a two-generation reproduction study with rats.

The test substance was administered in the diet at concentrations of 0, 2.5, 5 and 10% over two successive generations. In each generation one litter was raised.

2 Experimental

The study was conducted according to the protocol approved by the Sponsor, entitled "Protocol for an oral two-generation reproduction study with trehalose in rats (P471004)" approved by the Study Director on 16 December 1997 and 2 amendments to the protocol approved by the Study Director on 12 January 1998 and 25 March 1998, respectively. The TNO study number was 1992.

The study was carried out in accordance with the OECD Principles Practice and OECD Test guideline 416 adopted 26 May 1983.

The following time schedule was used:

- a. arrival of the animals : 7 January 1998
- b. experimental start date : 19 January 1998
- c. termination date in-life part : 2 October 1998
(F1-generation)

2.1 Test substance

The test substance was characterized as follows:

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	: $C_{12}H_{22}O_{11} \cdot 2H_2O$
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

For certificate of analysis see Annex 1.

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 albino rats. The rat was used because this species is considered a suitable species for this type of study.

One hundred and sixteen males and 116 females rats, Wistar outbred (CrI:(WI)WU BR) of 4-5 weeks of age were obtained from a colony maintained under SPF-conditions at Charles River Deutschland, Sulzfeld, Germany.

2.2.2 Animal allocation

Upon arrival, the rats were taken to their animal room (animal room 15.18) and checked for overt signs of ill health and anomalies. During the quarantine period (2 days) serological investigation of the microbiological status was conducted. After the results of serology indicated an acceptable microbiological status, the animals were acclimatized for a period of 10 days. Shortly before the start of the study, the animals were proportionately allocated by body weight to the various groups by computer randomization (Appendices 1 and 2).

Surplus animals were kept in the animal room for monitoring during the study.

2.2.3 Identification of the test system

During the acclimatization period, the animals were identified by a temporary tailmark.

F0 (parent)-animals:

Following allocation, 3 days before the start of the study, the individual male and female rats were identified by a unique animal identification number which was tattooed and clipped in the ears.

F1-animals:

Selected pups were identified by a unique animal identification number which was tattooed and clipped in the ears.

Males were identified by even numbers and females by odd numbers.

Each dosing group was coded by a letter and a colour (see section 2.4.3).

Each cage was provided with a card showing the colour code, animal identification number(s), cage number, group letter and study number.

2.3 Experimental conditions

2.3.1 Animal maintenance

After the quarantine period the animals were housed under conventional conditions in animal room 15.18 and from 12 March 1998 onwards in animal room 15.20. No other animals were housed in the same room during the study. The room was ventilated with about 10 air changes per hour and was maintained at a temperature of 19-25°C. On 25 January 1998 and 2 February 1998 temperature was lower than 19°C for several hours; a minimum of 17°C was reached. From 10 - 11 May 1998

temperature was higher than 25°C for several hours; the highest temperature reached was 27°C. Relative humidity was 40-70% except during room cleaning.

On 2, 6, 7, 9, 19-21 and 25 June 1998, on 12, 20 and 21 July 1998, on 11 August 1998 and on 2, 3 and 9 September 1998 humidity was higher than 70% for several hours reaching a maximum of 85%. Lighting was artificial with a sequence of 12 hours light and 12 hours dark.

During the acclimatization and pre-mating periods, 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 one female were housed together in smaller suspended cages (18x32x18 cm) with wire mesh floor and front. Mated F0-females were housed individually in wire mesh suspended cages (18x32x18 cm). After the mating period, non-mated females were housed individually until sacrifice and males were returned to their group cage. On day 17 of the gestation period, the females were housed in macrolon cages (45x32x18 cm) with sterilized dust-free saw dust and wood shavings as nesting material; during the lactation period the females and their litters remained in these cages. At or shortly after postnatal (PN) day 21, F1-animals were housed in groups of about 4 per sex, in suspended stainless steel group cages (45x32x18 cm) with wire mesh floor and front.

2.3.2 Feed and drinking water

Feed and water were provided *ad libitum* from the arrival of the rats 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 reduce 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 was analysed by the supplier for nutrients and contaminants. The analytical certificates pertaining to the batches used in this study (batch no. 4070, 4177 and 4670) are presented in Annex 2.

From the start of the study the rats were fed a modified diet (see section 2.4.3). The modification consisted of the omission of 20% barley from the diet which was replaced by the test substance and/or pregelatinized potato starch (Paselli WA 4, AVEBE, Foxhol, the Netherlands; see Annex 2.4 for certificate of analysis). The various ingredients were homogeneously distributed in the diets by mixing them in a mechanical blender. The diets were stored in a refrigerator (2-10°C) for maximally 39 days. The diets were prepared ten times.

Drinking water (tap-water) was supplied in polypropylene bottles with rubber stoppers and stainless steel nipples, that were cleaned approximately weekly and refilled when necessary. Tap water suitable for human consumption (quality guidelines according to Dutch legislation based on EEC Council Directive 80/778/EEC) was supplied by N.V. Waterleidingbedrijf Midden-Nederland. Results of the routine physical, chemical and microbiological examination of drinking water as conducted by the supplier are supplied to TNO Nutrition and Food Research Institute. In addition, the supplier periodically (twice per year) analyses 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 3.

2.4 Experimental procedures

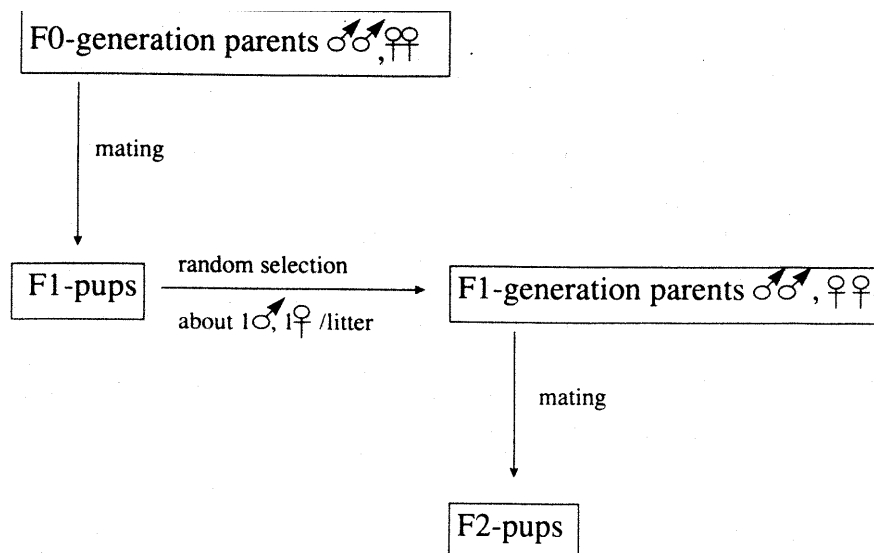
2.4.1 Experimental schedules and mating procedure

After allocation to the treatment groups, the F0-generation animals were kept on their respective diets until sacrifice.

After 10 weeks of treatment (pre-mating period), each female was caged with one male from the same treatment group until pregnancy occurred or 3 weeks had elapsed. Every consecutive morning during the mating period, vaginal smears were made to ascertain copulation by detection of sperm cells in the smear. Upon evidence of copulation, females were housed individually for the birth and rearing their young (F1-generation, see schematic presentation of two-generation study). The day a sperm-positive smear was detected was considered as gestation day 0. Mating pairs were clearly identified. After weaning of the litters F0-males were sacrificed and necropsied. The morning after birth was considered PN day 1. Consequently, for litters that were born during the day, but after the morning observation, that day was considered PN day 0.

On PN day 4, litters of more than 8 pups were adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 males and 4 females per litter. Before culling, all pups were examined externally for abnormalities after which the culled pups were preserved in a neutral aqueous phosphate buffered solution containing 4% formaldehyde.

Schematic presentation of a two-generation study:



given in Annex 3.

2.4 Experimental procedures

2.4.1 Experimental schedules and mating procedure

After allocation to the treatment groups, the F0-generation animals were kept on their respective diets until sacrifice.

After 10 weeks of treatment (pre-mating period), each female was caged with one male from the same treatment group until pregnancy occurred or 3 weeks had elapsed. Every consecutive morning during the mating period, vaginal smears were made to ascertain copulation by detection of sperm cells in the smear. Upon evidence of copulation, females were housed individually for the birth and rearing their young (F1-generation, see schematic presentation of two-generation study). The day a sperm-positive smear was detected was considered as gestation day 0. Mating pairs were clearly identified. After weaning of the litters F0-males were sacrificed and necropsied. The morning after birth was considered PN day 1. Consequently, for litters that were born during the day, but after the morning observation, that day was considered PN day 0.

On PN day 4, litters of more than 8 pups were adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 males and 4 females per litter. Before culling, all pups were examined externally for abnormalities after which the culled pups were preserved in a neutral aqueous phosphate buffered solution containing 4% formaldehyde.

Schematic presentation of a two-generation study:

On PN day 21, the F1-pups were weaned and shortly thereafter 28 males and 28 females were selected at random from as many litters as possible in each group to rear the next (F2) generation. Mating of siblings was avoided and no identical breeding pairs were formed. The other pups were discarded after an external examination. After weaning the F0-females were sacrificed and necropsied.

The animals selected from the F1-litters to rear the F2-generation were treated at the same dose levels as their parents from the day of weaning until sacrifice.

The F1-animals were mated at the end of a pre-mating period of about 10 weeks. The procedures followed to rear the F2-generation litters were identical to those described for the F0-generation to rear the F1-generation.

2.4.2 Administration of the test substance

2.4.2.1 Route and duration of administration

The oral route was used because this is an anticipated route of exposure in humans. F0-animals were exposed to constant concentrations of the test substance in the diet for 10 weeks prior to mating, throughout the mating and gestation periods and during the lactation period until sacrifice. F1- and F2-animals received comparable amounts of the test substance in the diets from weaning until sacrifice.

2.4.2.2 Preparation of the test diets

The diets were prepared as described in section 2.3.2 at concentrations described in section 2.4.3.

2.4.3 Experimental groups and dose levels

The study was comprised of four groups, viz. three test groups receiving different concentrations of trehalose and one control group. The doses were selected in consultation with the sponsor. Each group of F0- and F1-generation parents consisted of 28 males and 28 females.

The various treatment groups are presented in the scheme below.

Group	Treatment	colour code	dietary supplement		Number of males	Number of females
			trehalose	pregelatinized potato starch		
A	control	white	0	20	28	28
B	low-dose	blue	2.5	17.5	28	28
C	mid-dose	green	5	15	28	28
D	high-dose	red	10	10	28	28

¹ SDS RM3 modified rodent diet (RM3(E)MOD SQC FG-TNO) from which 20% cereals were left out.

2.4.4 Observations, analyses and measurements

Unless otherwise mentioned all observations, analyses and measurements were conducted for both generations.

2.4.4.1 Test substance analysis in the diet

Analyses to determine the homogeneity, content and stability of the test substance in the diets were conducted using HPLC. The trehalose concentrations measured in the test diets were corrected for the amount of disaccharides measured in blank RM3 diet. The stability of the test substance under (simulated) experimental conditions was demonstrated by analysing samples of diets on the day of diet preparation (day 0), after storage in an open container at (animal) room temperature for 7 days and after storage in a closed container in a refrigerator (2-10°C) for 6 weeks.

The homogeneity of the test substance was determined by analysing samples of diets of the low-, mid- and high-dose groups, taken once at 5 different locations in the feed containers.

The content of the test substance in the diets was determined in the diets of all batches prepared. Diet samples were taken immediately after preparation of the diets and stored at ca. -18°C pending analysis.

All analysis were performed at the Analytical Sciences Division of TNO Nutrition and Food Research Institute. The analytical method, its validation and details of the samples analysed are presented in Annex 4 of this report.

2.4.4.2 Clinical signs

Each animal was carefully observed daily in the morning hours. On working days, all cages were checked again in the afternoon. On Saturdays, Sundays and public holidays only one check per day was performed. All abnormalities, signs of ill health or reaction were recorded.

2.4.4.3 Body weight

Body weights of all F0-parents were recorded once during the acclimatization period at allocation to the various treatment groups. For both generations, body weights of male animals were recorded weekly until sacrifice. Body weights of female rats were recorded during the pre-mating and mating periods, on days 0, 7, 14 and 21 of gestation, and during lactation on PN days 1, 7, 14 and 21. Body weights of mated females which produced no litter were recorded up to day 21 of the presumed gestation period.

Non-mated females were weighed weekly after the mating period. All animals were weighed at sacrifice.

2.4.4.4 Food consumption

The quantity of food consumed by the animals was measured on a cage basis, by weighing the feeders. In the report the food consumption is expressed in g/kg body weight/day and g/animal/day.

Food consumption of the male animals was measured weekly, except during the mating period when food consumption was not measured.

Food consumption of female animals was measured weekly during the pre-mating

period. Food consumption of females was not recorded during the mating period. Food consumption of mated females was recorded weekly during pregnancy (days 0-7, 7-14 and 14-21) and lactation (days 1-7, 7-14 and 14-21). Food consumption of mated females which produced no litter was recorded up to day 21 of the presumed gestation period.

2.4.4.5 Test substance intake

The test substance intake was assessed on the basis of food intake, body weight and nominal dietary levels of the test substance.

2.4.4.6 Water consumption

Water consumption was not measured.

2.4.4.7 Parturition and litter evaluation

At the end of the gestation period, females were examined twice daily for signs of parturition. Any difficulties that occurred during parturition were recorded.

To keep nest disturbance to a minimum, the litters were examined only once daily for dead pups.

The total litter size, number of each sex, the number of stillbirths, livebirths, grossly malformed pups, and pups showing abnormalities were recorded on PN day 1.

Furthermore, the number of live and dead pups, pups showing malformations or abnormalities were recorded on PN days 4, 7, 14, and 21.

2.4.4.8 Pup weight

The litters were weighed on PN days 1, 4 (before and after culling), 7 and 14. At weaning (PN day 21), all pups were weighed individually.

2.4.4.9 Gross necropsy of pups and weanlings

All stillborn pups, pups found dead and pups that were killed because they were moribund during the study were examined macroscopically for structural abnormalities or pathological changes.

2.4.4.10 Gross necropsy and histology of parental animals

All surviving male and female parents of the F0- and F1-generation were killed by decapitation after ether anaesthesia, after weaning of their litters, and/or if they were no longer necessary for assessment of reproductive effects. A complete gross examination of each animal and collection of tissue samples for microscopic observations was performed.

At necropsy the spleen was weighed.

Samples of the following tissues and organs of all parents of the F0- and F1-generation were preserved in a neutral, aqueous phosphate buffered solution containing 4% formaldehyde, except for the testes which was preserved in Bouin's fixative:

- ovaries
- uterus
- vagina

- testes
- epididymides
- seminal vesicles (with coagulating glands and their fluids)
- prostate
- pituitary
- spleen
- organs or tissues showing macroscopic abnormalities

Tissues for microscopic examination were processed, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin, except for the sections of the testes which were stained with PAS haematoxylin. Microscopic examination was done on the collected organs of all rats of the high-dose group and of the control group and on the macroscopic abnormalities of all groups.

In addition, the reproductive organs of the males that fail to sire and non-pregnant females of the low- and mid-dose group were examined.

2.5 Fertility and reproductive performance

The following data are presented for each group:

- number of females placed with males
- number of males mated with females
- number of successful copulations (= number of females mated)
- number of males that became sires
- number of pregnant females
- number of females surviving delivery
- number of females with liveborn and (all) stillborn pups
- number of pups delivered (live- and stillborn)
- number of live pups at day n
- number of pups lost (= number of pups dying after live birth)
- number of pups culled and alive after culling
- number of litters lost entirely (= number of litters in which all pups died or were stillborn)
- number of male pups at day n
- number of implantations
- number of lost implantations (= number of implantations that did not result in live or stillborn births)

The following factors were calculated:

- pre-coital time = time between the start of mating and successful copulation
- duration of gestation = time between gestation day 0 and day of delivery
- mating index = (number of females mated/number of females placed with males) x 100
- male fertility index = (number of males that became sire/number of males placed with females) x 100
- female fertility index = (number of pregnant females/number of females placed with males) x 100
- female fecundity index = (number of pregnant females/number of females mated)x 100

- gestation index = (number of females with live pups/number of females pregnant) x 100
- live birth index = (number of pups born alive/number of pups born) x 100
- viability index (days 4-21) = (number of live weanlings/number of pups alive on day 4 post partum) x 100
- pup mortality day n = (number of dead pups on day n/total number of pups on day n) x 100
- sex ratio day n = (number of live male pups on day n/ number of live pups on day n) x 100
- number of lost implantations = number of implantations sites - number of pups born alive
- post-implantation loss = [(number of implantation sites - number of pups born alive)/number of implantation sites] x 100

2.6 Statistical analysis of the results

Statistical procedures used in the evaluation of data were as follows:

- for (pup) body weights and food consumption: one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests
- for clinical signs and developmental markers: Fisher's exact probability test
- for pre-coital time and duration of gestation: Kruskal-Wallis followed by Mann-Whitney U-tests
- for number of females pregnant, females with liveborn, females surviving delivery, females with (all) stillborn pups, number of live- and stillborn pups, number of pups/litters lost, number of male pups and number of implantation sites: Fisher's exact probability test
- for mean number of pups delivered, mean number of pups alive, mean number of implantations and post-implantation loss: Kruskal-Wallis followed by Mann-Whitney U-tests
- for pathological changes: Fisher's exact probability test

All tests were two-sided and a value of $P < 0.05$ was considered statistically significant (significant).

Statistical evaluations on variables associated with the pups were considered on a litter basis in accordance with standard procedures. Additional evaluations on a per pup basis were performed to attempt to identify any specific dose-related effect that may have occurred.

2.7 Retention of records, samples and specimens

The software package used to capture data was the "Grosse system".

A reference sample of the test substance will be retained for ten years. Unless otherwise stipulated, remaining test substance will be retained for at least six months after submission of the final report.

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, paraffin blocks and microscopic slides, will be retained in the archives of TNO Nutrition and Food

Research Institute for a period of at least five years (tissue specimens, paraffin blocks) or at least 15 years (slides, 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 and paraffin blocks can be discarded, should be stored for an additional period, or transferred to the archives of the Sponsor.

2.8 Deviations from the protocol

- ◆ In addition there were other contributors mentioned in the list of contributors who were not included in the study protocol. Drs M.V.W. Wijnands was responsible for pathology instead of Dr R.A. Woutersen as was mentioned in the protocol.
- ◆ From 1 January 1999 onwards Reproduction toxicology is part of the Department Target Organ toxicology headed by Drs H.H. Emmen.
- ◆ At arrival the animals were about 4-5 weeks of age, instead of 3-4 weeks as mentioned in the protocol.
- ◆ Due to fluctuations in outside temperature, on 25 January 1998 and 2 February 1998 temperature was lower than 19°C for several hours; a minimum of 17°C was reached. From 10 - 11 May 1998 temperature was higher than 25°C for several hours; the highest temperature reached was 27°C.
- ◆ Due to climatic circumstances, on 2, 6, 7, 9, 19-21 and 25 June 1998, on 12, 20 and 21 July 1998, on 11 August 1998 and on 2, 3 and 9 September 1998 humidity was higher than 70% for several hours reaching a maximum of 85%.
- ◆ Stability and homogeneity were tested in all diets (low-, mid- and high-dose).
- ◆ Diet samples for analysis were stored at ca. -18°C.

These deviations were not considered to have influenced the validity of the study.

3 Results

3.1 Analyses of trehalose in the diets (Annex 4)

The results of the analyses of trehalose in the diet are presented in Annex 4. Summarising, the test substance was homogeneously distributed in the diets and stable when stored for 7 days at ambient temperature in the animal room or stored for 6 weeks in a refrigerator (2-10°C).

The measured concentrations of the test substance in the diets were within the acceptable range ($\pm 10\%$ of the intended concentration).

3.2 Clinical signs (Tables 1-8, Appendices 3-10)

Clinical signs during the pre-mating, gestation and lactation periods are given in Tables 1-4 and 5-8 for the F0- and F1-generation, respectively.

The number of F1-females with a sparsely haired skin was statistically significantly increased in all dose groups during the pre-mating period, in the mid- and high-dose groups during the gestation period and in the high-dose group during the lactation period. This finding was considered not to be related to treatment since sparsely haired skin is a normal finding in this strain of rats.

Daily clinical observations of the F0- and F1-animals during the pre-mating, gestation and lactation period did not reveal other remarkable findings in the animals' appearance, general condition or behaviour amongst the dosing and control groups.

3.3 Body weights and body weight changes (Tables 9-24, Appendices 11-18)

Body weights and body weight changes during the pre-mating, gestation and lactation periods are presented in Tables 9-16 and 17-24 for the F0- and F1-generation, respectively.

Mean body weight changes of the F0-males of the mid- and high-dose groups were significantly increased in week 8-9 of the pre-mating period. On day 21 of the gestation period of the F0-generation, body weights of the females of the mid-dose group were significantly increased. Mean body weight changes of the F1-females of the low-dose group were significantly decreased in weeks 7-8 and 8-9 of the pre-mating period. Mean body weight changes of the F1-females of the high-dose group were significantly decreased between days 14-21 of the lactation period.

The significant differences in body weights and body weight changes between the control and treatment groups were not dose dependent and inconsistent over time and reproductive period and generation. Therefore, trehalose was considered to have no effect on maternal body weights and body weight changes.

3.4 Food consumption (Tables 25-32, Appendices 19-26)

The mean food consumption during the pre-mating, gestation and lactation period expressed in g/kg body weight/day and g/animal/day is given in Tables 25-28 and Tables 29-32 for the F0- and F1-generation, respectively.

Food consumption (g/animal/day) of the F0-males of the mid-dose group was significantly decreased in week 1-2 of the pre-mating period. Food consumption (g/animal/day) of the F0-females of the mid-dose group was significantly increased in week 5-6 of the pre-mating period and between days 7-14 of the lactation period. Food consumption (g/animal/day) of the F0-females of the high-dose group was significantly increased between days 7-14 and 14-21 of the lactation period. Furthermore, food consumption (g/kg/day) of the F0-females of the mid- and high-dose groups was significantly increased in week 5-6 of the pre-mating period. In the F1-generation, food consumption (g/animal/day) of the males of the mid-dose group was significantly increased in week 8-9 of the pre-mating period. In the pre-mating period of the F1-generation, food consumption (g/kg/day) of the males of the high-dose group was significantly increased in weeks 0-1, 1-2, 3-4 and 8-9 and of the females of the high-dose group in week 9-10.

In conclusion, the significant differences in food consumption between the control and treatment groups were not dose dependent and inconsistent over time, reproduction period and generation. Therefore, trehalose was considered to have no effect on food consumption.

3.5 Test substance intake (Tables 33 and 34)

Test substance intake, expressed as g/kg body weight/day, is presented in Tables 33 (F0-generation) and 34 (F1-generation). The test substance intake was calculated from the food consumption expressed in g/kg body weight/day (Tables 25-28 and 29-32 for the F0- and F1-generation, respectively).

Since pups started eating around PN day 14, test substance intake in lactation week 3 is an unrealistic figure and excluded from determining the test substance intake during the lactation period.

The intake of trehalose by male- and female rats of low-, mid- and high-dose groups of the F0- and F1-generation was dose-dependent and clearly represented the concentration of trehalose in the diets (the intake of trehalose by the animals of the low-, mid- and high-dose groups correlates as 1:2:4, respectively).

The test substance intake for the F0- and F1 males during the pre-mating period ranged from 1.24-2.90 (mean \pm standard error = 1.73 ± 0.10), 2.38-5.65 (3.45 ± 0.20) and 4.89-12.43 (7.09 ± 0.45) g/kg body weight/day for the low-, mid- and high-dose group, respectively. The test substance intake for the F0- and F1 females during the pre-mating period ranged from 1.47-2.85 (1.86 ± 0.09), 2.88-5.48 (3.74 ± 0.16) and 5.94-11.73 (7.61 ± 0.34) g/kg body weight/day for the low-, mid- and high-dose group, respectively. During the gestation period the test substance ranged from 1.06-1.74 (1.49 ± 0.13), 2.22-3.51 (3.06 ± 0.23) and 4.40-7.21 (6.16 ± 0.48) g/kg body weight/day for the low-, mid- and high-dose group, respectively. During the lactation period the test substance ranged from 2.30-4.34 (3.33 ± 0.54), 4.98-8.94 (6.88 ± 1.09), 10.10-17.94

(14.09±2.19) g/kg body weight/day for the low-, mid- and high-dose group, respectively.

3.6 Fertility and reproductive performance (Tables 35 and 36, Appendices 27-32)

Fertility and reproductive performances are presented in Tables 35 (F0-generation) and 36 (F1-generation).

All females of all groups were mated; all mating indices were 100%.

Pre-coital time was comparable amongst all groups for both generations.

The number of pregnant females was 24, 26, 24, 27 and 23, 25, 25, 25 for the control, low-, mid- and high-dose group of the F0- and F1-generation, respectively. The female fecundity index and male- and female fertility index ranged from 86-96% in the F0-generation and from 82-89% in the F1-generation. Duration of gestation was comparable in all groups for both generations.

The number of females with liveborn pups was 24, 26, 24, 27 and 23, 25, 25, 25 for the control, low-, mid- and high-dose group of the F0- and F1-generation, respectively. In both generations, there were no females which delivered only dead pups. Stillborn pups were observed in 7, 6, 4, 4 and 3, 3, 3, 2 litters of the control, low-, mid- and high-dose group of the F0- and F1-generation, respectively. The gestation index was 100% for all groups of both generations. Post-implantation loss was 16.05, 14.84, 12.87, 13.91% and 13.81, 15.78, 11.32, 11.69% for the control, low-, mid- and high-dose group of the F0- and F1-generation, respectively.

3.7 Litter data (Tables 35-42, Appendices 31-40)

3.7.1 Litter size and sex (Tables 35 and 36, Appendices 31-34)

Litter data are presented in Tables 35 and 36 for the F0- and F1-generation, respectively.

The number of pups delivered was 259, 271, 266, 296 and 217, 244, 245, 237 for the control, low-, mid- and high-dose group of the F0- and F1-generation, respectively.

The numbers of liveborn and stillborn pups were significantly increased and decreased, respectively, in the high-dose group of the F0-generation and in the mid- and high-dose groups of the F1-generation. In the other groups no effect of trehalose was observed on the number of liveborn and stillborn pups of either generation. The number of liveborn pups on PN day 1 was 237, 255, 248, 288 and 197, 223, 236, 233 for the control, low-, mid- and high-dose group of the F0- and F1-generation, respectively.

The pup mortality (days 1-4) was significantly decreased in the low- and high-dose groups of the F0-generation.

After culling, at PN day 4, the number of pups was 158, 196, 171, 203 and 164, 172, 181, 185 for the control, low-, mid- and high dose group of the F0- and F1-generation, respectively. Between PN day 4-21 3, 0, 3, 1 pups of the control, low-, mid- and high-dose groups of the F0-generation died or were missing, respectively. Of the F1-generation, 1 pup of the low-dose group died between PN day 4 and 21. The number of litters in which all pups died or were missing between PN days 0-21 was 3, 0, 2, 1 and 1, 1, 1, 0 for the control, low-, mid- and high-dose group of the F0-

and F1-generation, respectively.

The sex ratio was comparable in all dose groups on PN days 1 and 21 for both generations.

3.7.2 Pup observations (Tables 37 and 38, Appendices 35 and 36)

Pup observation on PN days 1, 4, 7, 14 and 21 are presented in Tables 37 and 38 for the F0- and F1-generation, respectively. No significant findings in any variables were obtained when evaluated using the standard litter basis.

When a per pup basis was used statistical significances were obtained.

On PN day 1 of the F0-generation, the number of pale pups, small pups and pups having no milk in the stomach in the low- and high-dose groups was significantly decreased. In the mid-dose group the number of cold pups was significantly increased compared to the control group.

On PN day 4 of the F0-generation, the number of small pups was significantly lower in the low- and high-dose groups than in the control group. Furthermore, on PN day 7 of the F0-generation, the number of large pups was significantly higher in the mid- and high-dose groups than in the control group.

On PN day 1 of the F1-generation, the number of small pups in the low-dose group was significantly increased and the number of large pups was significantly decreased.

On PN day 4 the number of small pups was significantly increased in the low-dose group and significantly decreased in the high-dose group. In the low-dose group, the number of small pups was significantly increased on PN day 7 and the number of sparsely haired pups was significantly increased on PN day 14. On PN day 21, the number of large pups was significantly increased in the high-dose group.

All these findings were only significant on a pup basis and not on a litter basis. Furthermore, no dose or generational (F0, F1) relationships were observed. The findings were normal for pups of this age. For these reasons the findings are considered to be not related to treatment.

3.7.3 Pathology of pups (Tables 39 and 40, Appendices 37 and 38)

No grossly malformed pups were observed.

The results of the examinations of stillborn pups and pups that died or that were killed because they were moribund during the study are presented in Tables 39 and 40 for the F0- and F1-generation, respectively.

At macroscopic examinations no findings were observed which indicated an abnormal development of the pups for either generation.

3.7.4 Litter weight (Tables 41 and 42, Appendices 39 and 40)

The mean pup weight and pup body weight changes as calculated from the litter weights on PN days 1, 4, 7, 14 and the mean pup weight on PN day 21 by weighing the individual male and female pups are presented in Tables 41 and 42 for the F0- and F1-generation, respectively.

Mean pup weights of all groups were comparable on PN days 1, 4, 7, 14 and 21 for both generations except for the pup body weights of the mid-dose group of the F0-generation measured on PN day 7 and 21 which was significantly increased (Table 41).

Body weight changes of the pups of the mid-dose group of the F0-generation were significantly increased between PN days 4 and 7 compared to the control group (Table 41). In the F1-generation, pup body weight changes of the low-dose group was statistically significantly decreased between PN days 1-4 (Table 42).

Since the statistically significant differences in body weights and body weight changes between the control and treatment groups were not consistent for treatment group, time period or generation, the differences were considered incidental. Therefore, trehalose was considered to have no effect on body weights and body weight changes of the pups of the F0- and F1-generation.

3.8 Parental necropsy observations (Tables 43-50, Appendices 41-46)

3.8.1 Spleen weights of parental animals (Tables 43-46, Appendices 41-44)

Absolute (g) and relative weights (g/kg body weight) of the spleen of the males and females are presented in Tables 43-44 and 45-46 for the F0- and F1-generation, respectively.

No intergroup differences were observed on absolute and relative spleen weights of the males and females of the F0- and F1-generation.

3.8.2 Macroscopic and microscopic observation of parental animals (Tables 47-50, Appendices 45 and 46)

At necropsy of both generations, no treatment-related macroscopic changes were observed.

Microscopic examination did not reveal treatment-related histopathologic changes in either generation. The histopathologic changes observed are common findings in rats of this age and strain. Furthermore, they were about equally distributed amongst the groups or they occurred in only one or a few animals.

4 Discussion and conclusion

In this paragraph the results of the study are restated:

Trehalose was given to male and female Wistar rats in the diet at concentrations of 0, 2.5, 5 and 10% over two successive generations. In each generation one litter was raised.

The test substance was homogeneously distributed in the diets and stable when stored for 7 days at room temperature or stored for 6 weeks in the refrigerator (2-10°C). The concentrations of the test substance measured in each batch of the diets prepared were within the acceptable range ($\pm 10\%$ of the intended concentration)

Daily clinical observations of the F0- and F1-animals during the pre-mating, gestation and lactation period did not reveal remarkable findings in the animals' appearance, general condition or behaviour which could be related to trehalose treatment.

The test substance had no consistent adverse effect on maternal or paternal body weights and body weight changes.

Trehalose had no effect on food-consumption of males and females of both generations.

The test substance intake for the F0- and F1 males during the pre-mating period ranged from 1.24-2.90 (mean \pm standard error = 1.73 ± 0.10), 2.38-5.65 (3.45 ± 0.20) and 4.89-12.43 (7.09 ± 0.45) g/kg body weight/day for the low-, mid- and high-dose group, respectively. The test substance intake for the F0- and F1 females during the pre-mating period ranged from 1.47-2.85 (1.86 ± 0.09), 2.88-5.48 (3.74 ± 0.16) and 5.94-11.73 (7.61 ± 0.34) g/kg body weight/day for the low-, mid- and high-dose group, respectively. During the gestation period the test substance ranged from 1.06-1.74 (1.49 ± 0.13), 2.22-3.51 (3.06 ± 0.23) and 4.40-7.21 (6.16 ± 0.48) g/kg body weight/day for the low-, mid- and high-dose group, respectively. During the lactation period the test substance ranged from 2.30-4.34 (3.33 ± 0.54), 4.98-8.94 (6.88 ± 1.09), 10.10-17.94 (14.09 ± 2.19) g/kg body weight/day for the low-, mid- and high-dose group, respectively.

From the results of this study it was concluded that in both generations, trehalose had no effect on any reproduction parameters determined: pre-coital time, mating index, male and female fertility, female fecundity index, gestation index, duration of gestation, and number of females with (all) stillborn pups and post-implantation loss.

Trehalose had no adverse effects on the number of pups delivered, the number of liveborn and stillborn pups, pup mortality, sex ratio, pup observations, pup body weights and pup body weight changes.

No effect of the test substance was observed on absolute and relative spleen weights.

At necropsy of both generations, no treatment-related macroscopic changes were observed.

Microscopic examination did not reveal treatment-related histopathologic changes in either generation. The histopathologic changes observed are common findings in rats of this age and strain. Furthermore, they were about equally distributed amongst the groups or they occurred in only one or a few animals.

From the data of this study it can be concluded that dietary administration of trehalose to rats at concentrations up to 10% (w/w) for two consecutive generations had no maternal toxic effects and had no effects on reproduction of the parental F0- and F1-generation or the development of the pups of the F0- and F1-generation. The no observed adverse effect level for reproductive effects after dietary administration of trehalose to rats is at least 7.09 (males pre mating), 7.61 (females pre mating), 6.16 (gestation) and 14.09 (lactation) g/kg body weight/day.

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 1 SUMMARY OF CLINICAL OBSERVATIONS MALES

	GROUP#	WEEK OF STUDY															TOTAL		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		15	16
# OF ANIMALS EXAMINED	A	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0
	B	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0
NORMAL																			
NO REMARKABLE CLINICAL OBSERVATIONS	A	28	28	28	25	23	22	23	27	25	25	26	26	26	27	27	27	13	
	B	28	28	28	28	27	25	24	24	23	23	23	24	23	24	23	23	12	
	C	27	24	25	27	27	27	27	27	25	26	25	26	24	24	24	24	11	
	D	28	28	28	28	27	26	26	25	25	25	22	22	23	22	21	21	12	
DEAD																			
DEAD: scheduled sacrifice	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
SKIN/FUR																			
SKIN: sparsely haired	A	0	0	0	3	5	6	5	1	3	3	2	2	2	1	1	1	1	7
	B	0	0	0	0	0	2	2	2	3	3	3	2	3	3	3	3	0	5
	C	2	4	2	0	0	0	0	0	1	0	1	0	2	2	2	2	1	8
	D	0	0	0	0	1	1	1	2	2	2	5	4	4	5	6	6	1	6
MOUTH/NOSE																			
MOUTH: malocclusion of incisors	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	1	1	2	2	2	2	2	2	2	2	2	2	2	2
	C	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
	D	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	2	1	2
MOUTH: ENCRUSTATIONS	A	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	1	0	0	0	0	0	1
	C	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
NOSE: encrustation(s)	A	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
	C	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	1	0	0	0	0	0	1
EYES																			
EYES: encrustations	A	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
	C	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	1	1	1	1	1	1	1	1	1	1	1

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

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 1 SUMMARY OF CLINICAL OBSERVATIONS

MALES

	GROUP#	WEEK OF STUDY															TOTAL			
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		15	16	
# OF ANIMALS EXAMINED	A	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0	
	B	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	0
EYES:blepharospasm	A	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
	C	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	1	0	0	0	0	0	0	0	0	0	0	1
GENITALS																				
TESTES: small	A	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
	C	0	0	0	0	0	0	0	0	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	0	0	0	0	0

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

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 2 SUMMARY OF CLINICAL OBSERVATIONS FEMALES

	GROUP#	WEEK OF STUDY																				TOTAL	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20
# OF ANIMALS EXAMINED	A	28	28	28	28	28	28	28	28	28	28	28	28	1	1	1	1	1	1	1	1	0	
	B	28	28	28	28	28	28	28	28	28	28	28	28	1	1	0	0	0	0	0	0	0	
	C	28	28	28	28	28	28	28	28	28	28	28	28	0	0	0	0	0	0	0	0	0	
	D	28	28	28	28	28	28	28	28	28	28	28	28	2	0	0	0	0	0	0	0	0	
NORMAL																							
NO REMARKABLE CLINICAL OBSERVATIONS	A	28	28	28	25	24	24	24	24	22	21	21	1	1	1	1	1	1	1	1	1	-	
	B	28	28	25	24	24	24	23	23	24	24	24	0	0	-	-	-	-	-	-	-	-	
	C	28	28	24	24	25	24	23	21	20	21	21	-	-	-	-	-	-	-	-	-	-	
	D	28	28	27	23	23	23	23	23	22	21	21	2	-	-	-	-	-	-	-	-	-	
DEAD																							
DEAD: scheduled sacrifice	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14	28
SKIN/FUR																							
SKIN: sparsely haired	A	0	0	2	3	4	4	4	4	5	6	6	0	0	0	0	1	1	0	0	0	-	8
	B	0	0	3	4	4	4	5	6	4	4	4	1	1	-	-	-	-	-	-	-	-	6
	C	0	0	4	4	3	4	5	7	10	7	7	-	-	-	-	-	-	-	-	-	-	11
	D	0	0	3	5	5	5	5	6	6	7	7	0	-	-	-	-	-	-	-	-	-	10
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
	B	0	0	0	0	0	0	1	0	0	0	0	0	0	-	-	-	-	-	-	-	-	1
	C	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	0
	D	0	0	0	0	0	0	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-	1
EYES																							
EYES: encrustations	A	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	-	1
	B	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
	D	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	0
EYES: ptosis																							
	A	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	-	1
	B	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
	D	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	0

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

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 3 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING GESTATION FEMALES

	GROUP#	DAY OF GESTATION																														TOTAL		
		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		30	
# OF FEMALES EXAMINED	A	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	26	14	4	4	4	4	4	4	4	4	4	4
	B	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	26	10	2	2	2	2	2	2	2	2	2	2
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	27	16	4	4	4	4	4	4	4	4	4	4
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	27	14	1	1	1	1	1	1	1	1	1	1
NO REMARKABLE CLINICAL OBSERVATIONS	A	20	20	20	20	20	20	20	20	20	20	20	20	20	22	22	22	22	22	22	22	21	10	4	4	4	4	4	4	4	4	4	4	
	B	24	24	24	24	24	24	24	24	24	24	24	24	24	25	25	25	25	25	25	25	23	7	1	1	1	1	1	1	1	1	1	1	
	C	21	21	21	21	21	21	22	22	22	22	22	23	23	23	23	23	23	23	23	23	22	13	3	3	3	3	3	3	4	4	4	4	
	D	22	22	22	22	22	22	22	22	22	23	23	23	23	24	25	25	25	25	25	25	24	12	1	1	1	1	1	1	1	1	1	1	
SKIN: sparsely haired	A	6	6	6	6	6	6	6	6	6	6	6	6	6	4	4	4	4	4	4	4	4	3	0	0	0	0	0	0	0	0	0	8	
	B	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	3	3	3	3	1	1	1	1	1	1	1	1	1	5	
	C	7	7	7	7	7	7	5	5	5	5	5	4	4	4	4	4	4	4	4	4	2	1	1	1	1	1	1	0	0	0	8		
	D	6	6	6	6	6	6	5	5	4	4	4	4	3	3	3	3	3	3	3	3	2	0	0	0	0	0	0	0	0	0	6		
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	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	2	
MOUTH: malocclusion of incisors	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	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	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		
NOSE: 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	1	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	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
EYES: encrustations	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	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	0	0		
EYES: ptosis	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	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	0	0		

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

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 4 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING LACTATION FEMALES

	GROUP#	DAY OF LACTATION																				TOTAL																																																																								
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20	21																																																																						
# OF FEMALES EXAMINED	A	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	B	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	C	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	D	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
NO REMARKABLE CLINICAL OBSERVATIONS	A	15	15	15	15	15	14	15	15	15	15	15	15	15	13	13	13	13	13	13	13	13	15	B	22	22	22	22	22	22	22	22	22	22	22	22	22	23	23	23	24	24	24	24	21	C	20	19	19	19	19	19	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	17	D	22	21	21	21	21	21	17	17	17	17	17	17	17	16	16	16	16	16	16	16	15	16			
SKIN: sparsely haired	A	8	8	8	8	8	9	8	8	8	8	8	8	8	10	10	10	10	10	10	10	8	12	B	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	2	2	2	2	5	7	C	3	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	7	D	5	6	6	6	6	6	10	10	10	10	10	10	10	11	11	11	11	11	11	11	12	11	13	
SKIN: encrustation(s)	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	
MOUTH: malocclusion of incisors	A	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	C	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	0	0	0	0	0	0	0	0		
EYES: encrustations	A	1	1	1	1	1	1	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	C	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		
EYES: ptosis	A	1	1	1	1	1	1	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	C	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		

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 5 SUMMARY OF CLINICAL OBSERVATIONS

MALES

	GROUP#	WEEK OF STUDY													TOTAL		
		0	1	2	3	4	5	6	7	8	9	10	11	12		13	
# OF ANIMALS EXAMINED	A	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
	C	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
NORMAL																	
NO REMARKABLE CLINICAL OBSERVATIONS	A	27	27	27	26	24	24	21	21	22	23	21	21	21	21	21	21
	B	27	27	26	26	26	26	25	24	23	23	24	25	25	25	25	25
	C	27	27	22	23	20	20	18	17	16	15	15	14	15	16	16	16
	D	28	28	27	27	26	26	24	24	24	23	23	24	24	24	23	23
DEAD																	
DEAD: scheduled sacrifice	A	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	28	28
	C	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	28	28
SKIN/FUR																	
SKIN: sparsely haired	A	1	1	1	2	4	4	7	7	6	5	7	7	7	7	10	10
	B	0	0	0	0	0	0	1	1	2	2	1	1	2	4	6	6
	C	0	0	5	4	6	6	8	9	10	10	11	11	10	9	12	12
	D	0	0	0	0	1	1	3	3	3	3	3	3	1	2	3	3
MOUTH/NOSE																	
MOUTH: malocclusion of incisors	A	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
	B	0	0	1	1	1	1	1	2	2	1	1	1	1	1	2	2
	C	0	0	0	0	1	1	1	1	1	2	2	2	2	2	2	2
	D	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
NOSE: haemorrhagic discharge	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	C	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

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 5 SUMMARY OF CLINICAL OBSERVATIONS

MALES

	GROUP#	WEEK OF STUDY													TOTAL		
		0	1	2	3	4	5	6	7	8	9	10	11	12		13	
# OF ANIMALS EXAMINED	A	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	
	C	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	
NOSE: encrustation(s)																	
	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	C	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	1	0	0	0	1
EYES																	
EYES: encrustations																	
	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	1	1	2	2	1	1	1	1	2
	C	0	0	0	0	1	1	0	0	0	2	2	2	2	2	2	2
	D	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
ABDOMEN/FAECES/URINE																	
ABDOMEN: umbilical hernia																	
	A	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
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
GENITALS																	
TESTES: cryptorchidism																	
	A	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
	C	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EXTREMITIES																	
TAIL: missing/dead																	
	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
tailtip	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	C	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

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 6 SUMMARY OF CLINICAL OBSERVATIONS

FEMALES

	GROUP#	WEEK OF STUDY																	TOTAL									
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		17								

# OF ANIMALS EXAMINED	A	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	0	0	0	0	0	0	0	0	0
	B	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	0	0	0	0	0	0	0	0	0
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	0	0	0	0	0	0	0	0	0
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	0	0	0	0	0	0	0	0	0
NORMAL																												
NO REMARKABLE CLINICAL OBSERVATIONS	A	28	28	28	25	25	26	25	24	24	24	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	28	28	25	25	24	24	23	22	19	16	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	28	28	25	22	22	22	22	23	16	16	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D	27	27	24	24	24	24	22	21	20	19	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DEAD																												
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	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	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	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	0	0	0	0	28	28
SKIN/FUR																												
SKIN: sparsely haired	A	0	0	0	3	3	2	3	4	4	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
	B	0	0	3	3	4	4	5	6	9	12	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15*
	C	0	0	3	6	6	6	6	5	12	12	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15*
	D	1	1	3	3	3	3	5	6	7	9	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15*
MOUTH/NOSE																												
MOUTH: malocclusion of incisors	A	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	B	0	0	0	0	0	0	0	0	0	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	C	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
MOUTH: tooth missing	A	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
	C	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	D	0	0	1	1	1	1	1	1	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
EYES																												
EYES: encrustations	A	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	B	0	0	0	0	0	0	0	0	0	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	C	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

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 7 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING GESTATION FEMALES

	GROUP#	DAY OF GESTATION																													TOTAL			
		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	30	
# 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	11	5	5	5	5	5	5	5	5	5	5
	B	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	27	7	3	3	3	3	3	3	3	3	3	3
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	13	3	3	3	3	3	3	3	3	3	3
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	3	3	3	3	3	3	3	3	3	3
NO REMARKABLE CLINICAL OBSERVATIONS	A	24	23	23	23	23	23	24	24	24	24	24	24	24	23	23	23	23	23	23	23	23	9	5	5	5	5	5	5	5	5	5	5	
	B	17	17	17	17	17	17	21	21	21	21	21	21	21	17	17	17	17	17	17	17	17	5	2	2	2	2	2	2	2	2	2	2	
	C	16	16	16	16	16	16	16	16	16	16	16	16	16	10	10	10	10	10	10	10	10	7	1	1	1	1	1	1	1	1	1	1	
	D	15	15	15	15	15	15	18	18	18	18	18	18	18	16	16	16	16	16	16	16	16	6	3	3	3	3	3	3	3	3	3	3	
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	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	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	0	
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	
SKIN: sparsely haired	A	4	4	4	4	4	4	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	2	0	0	0	0	0	0	0	0	0	7	
	B	10	10	10	10	10	10	6	6	6	6	6	6	6	11	11	11	11	11	11	11	10	2	1	1	1	1	1	1	1	1	13		
	C	12	12	12	12	12	12	12	12	12	12	12	12	12	17	17	17	17	17	17	17	17	6	2	2	2	2	2	2	2	2	2	20**	
	D	13	13	13	13	13	13	10	10	10	10	10	10	10	12	12	12	12	12	12	12	12	8	0	0	0	0	0	0	0	0	0	18**	
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	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	
	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	0	
	D	0	0	0	0	0	0	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	0	0	0	0	0	0	0	0	0	3	
SKIN: nodule	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	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	
	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	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	0	
SKIN: wound(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	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	0	
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 7 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING GESTATION FEMALES

	GROUP#	DAY OF GESTATION																														TOTAL			
		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		30		
# 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	11	5	5	5	5	5	5	5	5	5	5		
	B	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	27	7	3	3	3	3	3	3	3	3	3		
	C	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	13	3	3	3	3	3	3	3	3	3	3		
	D	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	14	3	3	3	3	3	3	3	3	3	3		
MOUTH: malocclusion of incisors	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	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1		
	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	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	0		
EYES: encrustations	A	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1		
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1		
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0		
TAIL: missing/dead tailtip	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	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	0	0	
	C	0	0	0	0	0	0	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	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	0	0	0	0	0	0	0	0	0	0	
TAIL: scaliness	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	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	0	0	
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0
TAIL: wound(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	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	0	0	0
	C	0	0	0	0	0	0	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	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	0	0	0	0	0	0	0	0	0	0	0

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 8 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING LACTATION FEMALES

	GROUP#	DAY OF LACTATION																				TOTAL	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20
# OF FEMALES EXAMINED	A	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	C	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	D	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
NO REMARKABLE CLINICAL OBSERVATIONS	A	18	18	18	18	17	17	17	18	18	18	18	18	18	18	19	19	19	19	19	19	19	21
	B	17	17	18	18	18	18	18	14	14	14	14	14	14	14	14	14	14	15	15	15	15	15
	C	13	12	12	12	13	13	13	14	14	14	14	14	14	14	14	14	15	15	15	15	15	15
	D	11	11	11	11	11	11	11	11	11	11	11	12	12	12	11	11	11	11	11	11	11	10
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
	B	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
	D	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
GENERAL CONDITION: poor health	A	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
	C	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	D	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	5	5	5	5	6	6	6	5	5	5	5	5	5	4	4	4	4	4	4	4	2	6
	B	7	7	6	6	6	6	10	10	10	10	10	10	10	9	10	10	9	9	9	9	8	11
	C	12	12	12	12	12	12	11	11	11	11	11	11	11	11	11	10	10	10	10	10	10	12
	D	14	14	14	14	14	14	14	14	14	14	13	13	13	14	14	14	14	14	14	14	15	17**
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
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	2
SKIN: nodule	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	C	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

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 8 SUMMARY OF MATERNAL CLINICAL OBSERVATIONS DURING LACTATION FEMALES

	GROUP#	DAY OF LACTATION																				TOTAL		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20	21
# OF FEMALES EXAMINED	A	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	C	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
	D	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
SKIN: wound(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
	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	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
FUR: piloerection	A	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
	C	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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
MOUTH: malocclusion of incisors	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	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	0	0	0	0	0	0	0	1	1
EYES: encrustations	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	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	0	0	0	0	0	0	0	0	0
TAIL: missing/dead tailtip	A	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
	C	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	0	0	0	0	0	0	0	0	0

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

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 9 MEAN BODY WEIGHTS (g)

		MALES				
		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE	
WEEK	0	MEAN S.E. N	169.99 d 2.365 28	168.15 1.798 28	167.80 1.888 28	171.12 1.779 28
WEEK	1	MEAN S.E. N	215.84 d 3.003 28	213.55 2.006 28	210.62 2.454 28	214.75 2.191 28
WEEK	2	MEAN S.E. N	252.68 d 3.550 28	248.86 2.313 28	243.14 3.063 28	250.79 3.204 28
WEEK	3	MEAN S.E. N	282.37 d 4.055 28	276.48 2.779 28	273.17 3.390 28	280.54 3.907 28
WEEK	4	MEAN S.E. N	303.79 d 4.526 28	296.57 3.552 28	294.90 4.112 28	302.29 4.363 28
WEEK	5	MEAN S.E. N	325.02 d 4.832 28	314.68 4.478 28	313.27 4.666 28	320.16 4.352 28
WEEK	6	MEAN S.E. N	342.91 d 4.961 28	330.14 4.795 28	330.49 5.029 28	339.11 4.833 28
WEEK	7	MEAN S.E. N	358.02 d 5.272 28	344.18 4.780 28	347.99 5.460 28	354.96 5.109 28
WEEK	8	MEAN S.E. N	372.49 d 5.364 28	360.81 4.447 28	364.45 5.648 28	369.40 5.329 28
WEEK	9	MEAN S.E. N	380.45 d 5.694 28	370.69 4.512 28	375.93 5.968 28	381.53 5.658 28
WEEK	10	MEAN S.E. N	390.84 d 5.789 28	381.03 4.653 28	385.13 6.597 28	391.41 5.921 28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 9 MEAN BODY WEIGHTS (g)

		MALES			
		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
WEEK	11	MEAN 402.12 d S.E. 5.833 N 28	393.79 4.631 28	398.77 6.326 28	405.48 5.706 28
WEEK	12	MEAN 409.18 d S.E. 6.094 N 28	401.47 4.983 28	406.23 6.284 28	416.22 6.355 28
WEEK	13	MEAN 419.54 d S.E. 6.126 N 28	413.16 5.191 28	415.67 6.602 28	426.98 6.347 28
WEEK	14	MEAN 430.26 d S.E. 6.338 N 28	422.83 5.199 28	424.98 7.194 28	435.57 6.461 28
WEEK	15	MEAN 436.32 d S.E. 6.451 N 28	431.40 5.772 28	434.22 7.398 28	442.56 6.791 28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 10 MEAN BODY WEIGHTS (g)

FEMALES

		A CONTROL			B 2.5% TREHALOSE		C 5% TREHALOSE		D 10% TREHALOSE	
WEEK	0	MEAN	146.68 d	145.03	146.51	145.97				
		S.E.	1.924	1.212	1.334	1.451				
		N	28	28	28	28				
WEEK	1	MEAN	162.25 d	160.78	162.21	162.24				
		S.E.	2.191	1.330	1.660	1.533				
		N	28	28	28	28				
WEEK	2	MEAN	172.69 d	170.97	174.44	174.38				
		S.E.	2.400	1.488	1.758	1.934				
		N	28	28	28	28				
WEEK	3	MEAN	182.13 d	180.08	184.46	183.76				
		S.E.	2.697	1.741	2.016	2.324				
		N	28	28	28	28				
WEEK	4	MEAN	189.77 d	188.20	192.80	190.43				
		S.E.	2.832	1.890	2.176	2.377				
		N	28	28	28	28				
WEEK	5	MEAN	197.46 d	194.76	199.35	197.98				
		S.E.	2.996	2.031	2.155	2.415				
		N	28	28	28	28				
WEEK	6	MEAN	203.87 d	200.09	206.72	204.73				
		S.E.	3.062	1.928	2.279	2.590				
		N	28	28	28	28				
WEEK	7	MEAN	208.73 d	206.05	211.99	209.57				
		S.E.	3.227	2.259	2.236	2.650				
		N	28	28	28	28				
WEEK	8	MEAN	212.74 d	210.23	216.47	214.45				
		S.E.	3.362	2.541	2.609	2.826				
		N	28	28	28	28				
WEEK	9	MEAN	214.64 d	212.81	220.09	218.12				
		S.E.	3.406	2.423	2.328	2.777				
		N	28	28	28	28				
WEEK	10	MEAN	217.73 d	215.96	222.46	221.15				
		S.E.	3.434	2.343	2.319	3.002				
		N	28	28	28	28				

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 11 MEAN BODY WEIGHT CHANGE (g)

			MALES				
			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	45.85 d	45.40	42.82	43.63
			S.E.	1.168	0.849	0.945	1.149
			N	28	28	28	28
WEEK	1 TO	2	MEAN	36.84 d	35.31	32.52	36.04
			S.E.	1.365	1.146	1.852	1.656
			N	28	28	28	28
WEEK	2 TO	3	MEAN	29.69 d	27.62	30.03	29.75
			S.E.	1.096	0.961	1.106	1.234
			N	28	28	28	28
WEEK	3 TO	4	MEAN	21.42 d	20.09	21.73	21.74
			S.E.	0.964	1.775	0.937	0.950
			N	28	28	28	28
WEEK	4 TO	5	MEAN	21.24 d	18.11	18.37	17.87
			S.E.	0.771	1.425	0.858	1.965
			N	28	28	28	28
WEEK	5 TO	6	MEAN	17.89 d	15.45	17.22	18.96
			S.E.	0.669	0.921	0.749	0.920
			N	28	28	28	28
WEEK	6 TO	7	MEAN	15.11 d	14.04	17.50	15.85
			S.E.	0.660	0.778	0.717	1.421
			N	28	28	28	28
WEEK	7 TO	8	MEAN	14.46 d	16.64	16.45	14.44
			S.E.	0.652	1.540	0.723	0.607
			N	28	28	28	28
WEEK	8 TO	9	MEAN	7.97 d	9.88	11.48**	12.13#
			S.E.	0.753	0.738	0.718	0.673
			N	28	28	28	28
WEEK	9 TO	10	MEAN	10.38 d	10.34	9.20	9.89
			S.E.	0.623	0.741	1.163	0.746
			N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test ** = p<0.01 # = p<0.001

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 11 MEAN BODY WEIGHT CHANGE (g)

			MALES				
			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE				
WEEK	10 TO	11	MEAN	11.28 d	12.76	13.65	14.07
			S.E.	0.892	0.856	1.104	0.751
			N	28	28	28	28
WEEK	11 TO	12	MEAN	7.06 d	7.69	7.45	10.74
			S.E.	1.215	1.090	2.723	1.196
			N	28	28	28	28
WEEK	12 TO	13	MEAN	10.36 d	11.68	9.45	10.76
			S.E.	1.097	0.786	1.030	0.649
			N	28	28	28	28
WEEK	13 TO	14	MEAN	10.73 d	9.68	9.31	8.59
			S.E.	0.934	0.719	1.050	0.634
			N	28	28	28	28
WEEK	14 TO	15	MEAN	6.06 d	8.57	9.24	7.00
			S.E.	0.886	0.989	0.874	0.841
			N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 12 MEAN BODY WEIGHT CHANGE (g)

FEMALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	15.57 d	15.74	15.70	16.27
			S.E.	0.860	0.720	0.811	0.863
			N	28	28	28	28
WEEK	1 TO	2	MEAN	10.44 d	10.20	12.23	12.15
			S.E.	0.682	0.689	0.708	1.024
			N	28	28	28	28
WEEK	2 TO	3	MEAN	9.44 d	9.11	10.02	9.38
			S.E.	0.622	0.606	0.666	0.728
			N	28	28	28	28
WEEK	3 TO	4	MEAN	7.64 d	8.13	8.35	6.67
			S.E.	0.641	0.679	0.568	0.627
			N	28	28	28	28
WEEK	4 TO	5	MEAN	7.69 d	6.55	6.54	7.55
			S.E.	0.657	0.592	0.436	0.587
			N	28	28	28	28
WEEK	5 TO	6	MEAN	6.41 d	5.33	7.38	6.76
			S.E.	0.526	0.505	0.724	0.731
			N	28	28	28	28
WEEK	6 TO	7	MEAN	4.85 d	5.96	5.26	4.84
			S.E.	0.696	0.614	0.656	0.675
			N	28	28	28	28
WEEK	7 TO	8	MEAN	4.01 d	4.17	4.48	4.88
			S.E.	0.684	0.729	0.699	0.614
			N	28	28	28	28
WEEK	8 TO	9	MEAN	1.90 d	2.58	3.62	3.66
			S.E.	0.824	0.744	0.654	0.537
			N	28	28	28	28
WEEK	9 TO	10	MEAN	3.09 d	3.16	2.37	3.03
			S.E.	0.689	0.664	0.468	0.643
			N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:13 MEAN MATERNAL BODY WEIGHT DURING GESTATION (g)

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAY 0	MEAN	216.40 d	216.80	223.40	224.61
	S.E.	3.828	2.448	2.776	3.033
	N	23	25	24	27
DAY 7	MEAN	233.68 d	233.71	240.40	242.55
	S.E.	4.247	2.712	2.983	3.741
	N	23	25	24	27
DAY 14	MEAN	255.42 d	256.68	264.50	265.89
	S.E.	4.661	2.847	2.749	3.637
	N	23	25	24	27
DAY 21	MEAN	277.77 d	280.92	294.63*	288.96
	S.E.	4.881	3.354	3.564	4.238
	N	22	24	23	26

Statistical key: d= ANOVA & Dunnett test * = p<0.05

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:14 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	17.28 d	16.90	17.00	17.94
	S.E.	0.863	1.598	2.847	1.294
	N	23	25	24	27
DAYS 7 TO 14	MEAN	21.74 d	22.97	24.10	23.34
	S.E.	0.820	1.197	1.480	1.032
	N	23	25	24	27
DAYS 14 TO 21	MEAN	23.81 d	25.70	29.70	23.36
	S.E.	1.539	1.910	1.873	1.605
	N	22	24	23	26

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:15 MEAN MATERNAL BODY WEIGHTS DURING LACTATION (g)

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAY 1	MEAN	205.83 d	209.77	215.42	213.90
	S.E.	4.095	3.327	2.669	3.645
	N	24	26	24	27
DAY 7	MEAN	230.52 d	235.73	239.16	241.00
	S.E.	4.132	3.406	3.131	3.848
	N	21	26	22	26
DAY 14	MEAN	248.82 d	255.80	258.06	259.90
	S.E.	4.053	2.897	3.215	3.736
	N	21	26	22	26
DAY 21	MEAN	246.45 d	251.93	254.00	255.43
	S.E.	3.951	2.514	3.484	3.117
	N	21	26	22	26

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:16 MEAN MATERNAL BODY WEIGHT CHANGE DURING LACTATION (g)

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
DAYS 1 TO 7	MEAN	23.89 d	25.96	23.16	26.29
	S.E.	2.853	1.937	2.561	2.249
	N	21	26	22	26
DAYS 7 TO 14	MEAN	18.30 d	20.07	18.90	18.90
	S.E.	2.600	1.809	2.449	1.878
	N	21	26	22	26
DAYS 14 TO 21	MEAN	-2.37 d	-3.87	-4.06	-4.47
	S.E.	2.424	1.484	1.713	1.462
	N	21	26	22	26

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 17 MEAN BODY WEIGHTS (g)

MALES

		MALES				
		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE	
WEEK	0	MEAN S.E. N	138.04 d 2.194 28	138.32 2.481 28	142.33 2.285 28	131.90 3.945 28
WEEK	1	MEAN S.E. N	173.58 d 2.701 28	174.50 3.412 28	180.96 2.770 28	168.73 4.026 28
WEEK	2	MEAN S.E. N	212.95 d 3.491 28	214.24 4.091 28	221.43 3.270 28	206.12 4.212 28
WEEK	3	MEAN S.E. N	244.89 d 3.958 28	246.83 4.778 28	253.56 3.574 28	238.08 4.201 28
WEEK	4	MEAN S.E. N	270.65 d 4.307 28	274.40 5.412 28	280.53 3.961 28	264.41 4.068 28
WEEK	5	MEAN S.E. N	292.12 d 4.909 28	295.01 5.868 28	298.39 4.756 28	285.81 4.131 28
WEEK	6	MEAN S.E. N	307.35 d 5.168 28	312.94 6.375 28	313.76 5.535 28	301.81 4.253 28
WEEK	7	MEAN S.E. N	320.74 d 5.668 28	326.11 6.987 28	328.12 5.249 28	316.48 4.286 28
WEEK	8	MEAN S.E. N	335.22 d 5.829 28	337.65 7.466 28	343.90 4.992 28	329.59 4.438 28
WEEK	9	MEAN S.E. N	344.13 d 6.185 28	350.08 7.663 28	356.26 5.287 28	340.58 4.343 28
WEEK	10	MEAN S.E. N	354.11 d 6.439 28	361.90 8.047 28	368.26 5.622 28	351.71 4.528 28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 17 MEAN BODY WEIGHTS (g)

		MALES				
		A	B	C	D	
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE	
WEEK	11	MEAN	358.42 d	368.35	375.59	357.90
		S.E.	7.088	8.054	5.954	4.639
		N	28	28	28	28
WEEK	12	MEAN	361.36 d	373.30	381.39	362.54
		S.E.	7.147	8.107	6.533	5.345
		N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 18 MEAN BODY WEIGHT CHANGE (g)

			MALES				
			A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE	
WEEK	0 TO	1	MEAN	35.55 d	36.19	38.63	36.82
			S.E.	0.929	1.160	0.931	0.815
			N	28	28	28	28
WEEK	1 TO	2	MEAN	39.36 d	39.74	40.47	37.39
			S.E.	1.009	0.998	0.884	0.724
			N	28	28	28	28
WEEK	2 TO	3	MEAN	31.94 d	32.59	32.13	31.96
			S.E.	0.789	0.997	0.794	0.863
			N	28	28	28	28
WEEK	3 TO	4	MEAN	25.76 d	27.57	26.97	26.33
			S.E.	0.728	0.886	1.011	0.884
			N	28	28	28	28
WEEK	4 TO	5	MEAN	21.47 d	20.61	17.86	21.40
			S.E.	0.957	0.740	2.575	0.798
			N	28	28	28	28
WEEK	5 TO	6	MEAN	15.23 d	17.92	15.38	16.00
			S.E.	0.654	0.751	1.444	0.556
			N	28	28	28	28
WEEK	6 TO	7	MEAN	13.40 d	13.17	14.35	14.67
			S.E.	0.676	1.131	0.840	0.755
			N	28	28	28	28
WEEK	7 TO	8	MEAN	14.48 d	11.54	15.79	13.11
			S.E.	0.579	1.105	1.262	0.702
			N	28	28	28	28
WEEK	8 TO	9	MEAN	8.91 d	12.43	12.36	10.99
			S.E.	1.109	0.832	1.268	0.823
			N	28	28	28	28
WEEK	9 TO	10	MEAN	9.99 d	11.82	11.99	11.12
			S.E.	0.738	0.809	1.333	0.738
			N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 18 MEAN BODY WEIGHT CHANGE (g)

		MALES			
		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
WEEK 10 TO 11	MEAN	4.30 d	6.46	7.34	6.20
	S.E.	1.297	0.934	0.840	0.946
	N	28	28	28	28
WEEK 11 TO 12	MEAN	2.94 d	4.95	5.79	4.64
	S.E.	0.678	0.716	1.304	2.081
	N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 19 MEAN BODY WEIGHTS (g)

		FEMALES				
		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE	
WEEK	0	MEAN S.E. N	115.45 d 1.922 28	115.15 1.733 28	113.98 2.472 28	110.26 2.811 28
WEEK	1	MEAN S.E. N	133.45 d 2.092 28	133.73 2.099 28	131.94 3.024 28	129.82 2.510 28
WEEK	2	MEAN S.E. N	149.14 d 2.338 28	151.32 2.051 28	147.71 3.504 28	145.19 2.410 28
WEEK	3	MEAN S.E. N	163.68 d 2.626 28	165.50 2.468 28	162.30 3.758 28	158.35 2.439 28
WEEK	4	MEAN S.E. N	174.97 d 2.722 28	176.93 2.562 28	173.25 3.874 28	168.85 2.632 28
WEEK	5	MEAN S.E. N	183.58 d 2.847 28	185.76 2.725 28	181.95 4.027 28	176.64 2.539 28
WEEK	6	MEAN S.E. N	188.59 d 2.800 28	190.32 2.486 28	186.63 4.003 28	182.20 2.455 28
WEEK	7	MEAN S.E. N	193.44 d 2.983 28	196.51 2.618 28	192.40 4.017 28	187.67 2.466 28
WEEK	8	MEAN S.E. N	199.54 d 3.083 28	200.78 2.675 28	197.08 3.828 28	193.48 2.512 28
WEEK	9	MEAN S.E. N	205.05 d 3.077 28	204.00 2.645 28	202.05 3.722 28	197.76 2.702 28
WEEK	10	MEAN S.E. N	209.37 d 3.125 28	210.28 2.798 28	206.54 3.838 28	203.38 2.600 28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 20 MEAN BODY WEIGHT CHANGE (g)

			FEMALES				
			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	18.00 d	18.58	17.96	19.56
			S.E.	0.672	0.735	0.833	0.916
			N	28	28	28	28
WEEK	1 TO	2	MEAN	15.70 d	17.59	15.77	15.37
			S.E.	0.850	0.626	0.728	0.650
			N	28	28	28	28
WEEK	2 TO	3	MEAN	14.54 d	14.18	14.59	13.16
			S.E.	0.925	0.780	0.601	0.650
			N	28	28	28	28
WEEK	3 TO	4	MEAN	11.29 d	11.43	10.95	10.50
			S.E.	1.180	0.509	0.558	0.591
			N	28	28	28	28
WEEK	4 TO	5	MEAN	8.61 d	8.83	8.70	7.79
			S.E.	0.604	0.599	0.779	0.561
			N	28	28	28	28
WEEK	5 TO	6	MEAN	5.01 d	4.57	4.67	5.55
			S.E.	0.457	0.636	0.529	0.588
			N	28	28	28	28
WEEK	6 TO	7	MEAN	4.85 d	6.19	5.78	5.48
			S.E.	0.486	0.451	0.600	0.510
			N	28	28	28	28
WEEK	7 TO	8	MEAN	6.11 d	4.27*	4.68	5.80
			S.E.	0.512	0.454	0.547	0.567
			N	28	28	28	28
WEEK	8 TO	9	MEAN	5.51 d	3.22*	4.96	4.29
			S.E.	0.576	0.547	0.507	0.715
			N	28	28	28	28
WEEK	9 TO	10	MEAN	4.32 d	6.28	4.50	5.62
			S.E.	0.712	0.627	0.560	0.624
			N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test * = p<0.05

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:21 MEAN MATERNAL BODY WEIGHT DURING GESTATION (g)

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAY 0	MEAN	207.34 d	209.36	204.66	202.85
	S.E.	3.525	2.840	3.768	2.651
	N	23	25	25	25
DAY 7	MEAN	223.18 d	226.06	223.14	219.22
	S.E.	3.839	3.186	3.686	3.186
	N	23	25	25	25
DAY 14	MEAN	243.91 d	244.63	243.20	238.22
	S.E.	3.783	3.327	4.117	3.550
	N	23	25	25	25
DAY 21	MEAN	270.30 d	268.17	271.58	264.45
	S.E.	4.357	3.961	4.602	3.776
	N	22	24	25	24

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:22 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	15.84 d	16.70	18.48	16.37
	S.E.	0.915	0.768	1.010	0.988
	N	23	25	25	25
DAYS 7 TO 14	MEAN	20.73 d	18.56	20.06	19.00
	S.E.	0.982	0.713	1.009	0.786
	N	23	25	25	25
DAYS 14 TO 21	MEAN	26.10 d	23.52	28.38	25.70
	S.E.	1.816	1.852	1.943	2.097
	N	22	24	25	24

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:23 MEAN MATERNAL BODY WEIGHTS DURING LACTATION (g)

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAY 1	MEAN	201.30 d	198.91	199.07	196.07
	S.E.	3.742	3.044	3.992	3.311
	N	23	25	25	25
DAY 7	MEAN	223.17 d	223.88	227.35	222.15
	S.E.	3.547	3.998	3.547	2.676
	N	22	24	24	25
DAY 14	MEAN	241.20 d	248.75	248.56	245.50
	S.E.	3.360	4.153	3.037	3.337
	N	22	24	24	25
DAY 21	MEAN	249.10 d	253.53	252.85	245.01
	S.E.	2.939	3.975	3.218	3.125
	N	22	24	24	25

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:24 MEAN MATERNAL BODY WEIGHT CHANGE DURING LACTATION (g)

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 1 TO 7	MEAN	21.73 d	24.12	26.02	26.08
	S.E.	2.345	2.058	2.260	2.510
	N	22	24	24	25
DAYS 7 TO 14	MEAN	18.03 d	24.87	21.21	23.35
	S.E.	1.911	2.005	2.422	2.363
	N	22	24	24	25
DAYS 14 TO 21	MEAN	7.90 d	4.78	4.29	-0.49**
	S.E.	1.555	1.338	1.234	2.105
	N	22	24	24	25

Statistical key: d= ANOVA & Dunnett test ** = p<0.01

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 25.1 MEAN FOOD CONSUMPTION -- (g/animal/day)

MALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	19.21 d	18.70	18.63	18.78
			S.E.	0.216	0.179	0.332	0.223
			N	7	7	7	7
WEEK	1 TO	2	MEAN	19.98 d	19.42	18.83**	19.63
			S.E.	0.247	0.119	0.240	0.311
			N	7	7	7	7
WEEK	2 TO	3	MEAN	19.93 d	19.38	19.39	19.71
			S.E.	0.204	0.082	0.207	0.416
			N	7	7	7	7
WEEK	3 TO	4	MEAN	19.64 d	19.07	19.17	19.48
			S.E.	0.203	0.259	0.259	0.257
			N	7	7	7	7
WEEK	4 TO	5	MEAN	19.63 d	18.69	18.70	18.76
			S.E.	0.266	0.499	0.194	0.254
			N	7	7	7	7
WEEK	5 TO	6	MEAN	19.09 d	18.30	18.69	18.63
			S.E.	0.251	0.339	0.225	0.243
			N	7	7	7	7
WEEK	6 TO	7	MEAN	19.11 d	18.32	18.81	19.13
			S.E.	0.226	0.279	0.274	0.335
			N	7	7	7	7
WEEK	7 TO	8	MEAN	19.70 d	19.19	19.67	19.79
			S.E.	0.255	0.132	0.359	0.337
			N	7	7	7	7
WEEK	8 TO	9	MEAN	19.18 d	18.66	18.86	19.52
			S.E.	0.276	0.178	0.296	0.347
			N	7	7	7	7
WEEK	9 TO	10	MEAN	19.05 d	18.66	18.07	18.91
			S.E.	0.352	0.298	0.217	0.376
			N	7	7	7	7
WEEK	13 TO	14	MEAN	20.50 d	20.36	19.82	20.46
			S.E.	0.315	0.265	0.565	0.227
			N	7	7	7	7
WEEK	14 TO	15	MEAN	19.44 d	19.45	19.18	19.86
			S.E.	0.266	0.349	0.381	0.208
			N	7	7	7	7

Statistical key: d= ANOVA & Dunnett test ** = p<0.01

N=Number of cages

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 25.2 MEAN FOOD CONSUMPTION -- (g/kg/day)

MALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	99.58 d	98.00	98.44	97.34
			S.E.	0.797	0.960	1.224	0.764
			N	7	7	7	7
WEEK	1 TO	2	MEAN	85.31 d	84.02	82.97	84.32
			S.E.	0.868	0.530	0.582	0.900
			N	7	7	7	7
WEEK	2 TO	3	MEAN	74.53 d	73.81	75.14	74.19
			S.E.	1.015	0.491	0.574	1.030
			N	7	7	7	7
WEEK	3 TO	4	MEAN	67.06 d	66.57	67.52	66.86
			S.E.	0.898	0.615	0.735	0.291
			N	7	7	7	7
WEEK	4 TO	5	MEAN	62.46 d	61.11	61.51	60.32
			S.E.	0.971	1.188	0.624	1.016
			N	7	7	7	7
WEEK	5 TO	6	MEAN	57.18 d	56.75	58.09	56.53
			S.E.	0.762	0.609	0.580	0.602
			N	7	7	7	7
WEEK	6 TO	7	MEAN	54.56 d	54.34	55.45	55.15
			S.E.	0.762	0.626	0.594	0.873
			N	7	7	7	7
WEEK	7 TO	8	MEAN	53.98 d	54.47	55.20	54.65
			S.E.	0.986	0.536	0.429	0.755
			N	7	7	7	7
WEEK	8 TO	9	MEAN	50.99 d	51.05	50.94	52.00
			S.E.	1.008	0.572	0.540	0.747
			N	7	7	7	7
WEEK	9 TO	10	MEAN	49.43 d	49.66	47.51	48.93
			S.E.	1.154	0.738	0.551	0.781
			N	7	7	7	7
WEEK	13 TO	14	MEAN	48.26 d	48.74	47.14	47.50
			S.E.	0.734	0.709	1.153	0.837
			N	7	7	7	7
WEEK	14 TO	15	MEAN	44.87 d	45.52	44.68	45.27
			S.E.	0.597	0.462	0.834	0.787
			N	7	7	7	7

Statistical key: d= ANOVA & Dunnett test N=Number of cages

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 26.1 MEAN FOOD CONSUMPTION -- (g/animal/day)

FEMALES

			A	B	C	D
			CONTROL	2.5%	5%	10%

WEEK	TO		MEAN	TREHALOSE	TREHALOSE	TREHALOSE
0	TO	1	14.09 d	13.54	13.98	14.16
			S.E.	0.096	0.186	0.193
			N	7	7	7
1	TO	2	13.98 d	13.67	14.28	14.16
			S.E.	0.112	0.367	0.190
			N	7	7	7
2	TO	3	13.65 d	13.61	14.17	14.04
			S.E.	0.217	0.303	0.258
			N	7	7	7
3	TO	4	13.25 d	13.40	14.02	13.41
			S.E.	0.273	0.257	0.366
			N	7	7	7
4	TO	5	13.15 d	13.01	13.63	13.84
			S.E.	0.211	0.257	0.341
			N	7	7	7
5	TO	6	12.37 d	12.42	13.57**	13.24
			S.E.	0.124	0.332	0.297
			N	7	7	7
6	TO	7	12.42 d	12.71	13.05	12.91
			S.E.	0.265	0.231	0.193
			N	7	7	7
7	TO	8	12.29 d	12.45	12.79	12.87
			S.E.	0.248	0.265	0.310
			N	7	7	7
8	TO	9	12.18 d	12.47	12.71	13.08
			S.E.	0.147	0.279	0.353
			N	7	7	7
9	TO	10	12.13 d	12.56	12.73	13.05
			S.E.	0.141	0.238	0.372
			N	7	7	7

Statistical key: d= ANOVA & Dunnett test ** = p<0.01

N=Number of cages

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 26.2 MEAN FOOD CONSUMPTION -- (g/kg/day)

FEMALES

			A	B	C	D
			CONTROL	2.5%	5%	10%

WEEK	TO		MEAN	TREHALOSE	TREHALOSE	TREHALOSE
0 TO	1		91.16 d	88.61	90.55	91.89
			S.E.	0.823	1.055	0.893
			N	7	7	7
1 TO	2		83.48 d	82.45	84.82	84.11
			S.E.	0.640	1.634	0.686
			N	7	7	7
2 TO	3		76.97 d	77.58	78.92	78.39
			S.E.	1.445	0.988	0.687
			N	7	7	7
3 TO	4		71.28 d	72.81	74.35	71.72
			S.E.	1.437	0.805	1.875
			N	7	7	7
4 TO	5		67.95 d	67.94	69.51	71.22
			S.E.	0.979	0.867	1.057
			N	7	7	7
5 TO	6		61.66 d	62.92	66.79#	65.71**
			S.E.	0.604	1.077	0.744
			N	7	7	7
6 TO	7		60.22 d	62.56	62.36	62.32
			S.E.	1.023	0.796	0.571
			N	7	7	7
7 TO	8		58.33 d	59.80	59.70	60.63
			S.E.	1.018	0.891	0.799
			N	7	7	7
8 TO	9		57.05 d	58.99	58.22	60.44
			S.E.	0.690	0.958	0.925
			N	7	7	7
9 TO	10		56.12 d	58.62	57.54	59.36
			S.E.	0.941	0.940	0.925
			N	7	7	7

Statistical key: d= ANOVA & Dunnett test ** = p<0.01 # = p<0.001 N=Number of cages

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:27.1 MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- g/animal/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 0 TO 7	MEAN	14.52 d	14.57	15.12	15.59
	S.E.	0.368	0.308	0.542	0.341
	N	23	25	24	27
DAYS 7 TO 14	MEAN	16.59 d	16.70	17.54	17.44
	S.E.	0.315	0.252	0.264	0.290
	N	23	25	24	27
DAYS 14 TO 21	MEAN	11.80 d	11.82	12.41	12.18
	S.E.	0.432	0.368	0.495	0.312
	N	22	24	23	26

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:27.2 MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- g/kg/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 0 TO 7	MEAN	64.50 d	64.68	65.18	66.66
	S.E.	1.159	1.104	2.205	0.963
	N	23	25	24	27
DAYS 7 TO 14	MEAN	67.91 d	68.12	69.52	68.67
	S.E.	0.837	0.795	0.835	0.837
	N	23	25	24	27
DAYS 14 TO 21	MEAN	44.58 d	44.09	44.43	44.02
	S.E.	1.734	1.256	1.824	1.105
	N	22	24	23	26

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:28.1 MEAN MATERNAL FOOD CONSUMPTION DURING LACTATION -- g/animal/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 1 TO 7	MEAN	21.06 d	22.31	22.74	22.93
	S.E.	1.038	0.853	1.157	0.636
	N	21	26	22	26
DAYS 7 TO 14	MEAN	39.94 d	42.66	44.49*	44.77*
	S.E.	1.349	1.149	1.225	1.006
	N	21	26	22	26
DAYS 14 TO 21	MEAN	49.24 d	50.83	53.26	53.97*
	S.E.	1.671	1.566	0.851	0.932
	N	21	26	22	26

Statistical key: d= ANOVA & Dunnett test * = p<0.05

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:28.2 MEAN MATERNAL FOOD CONSUMPTION DURING LACTATION -- g/kg/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 1 TO 7	MEAN	96.60 d	100.30	99.61	100.97
	S.E.	4.625	3.702	4.626	2.809
	N	21	26	22	26
DAYS 7 TO 14	MEAN	166.67 d	173.73	178.82	179.43
	S.E.	4.963	4.543	4.148	4.213
	N	21	26	22	26
DAYS 14 TO 21	MEAN	198.55 d	200.11	208.32	209.94
	S.E.	5.830	5.770	3.216	3.643
	N	21	26	22	26

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 29.1 MEAN FOOD CONSUMPTION -- (g/animal/day)

MALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	17.83 d	18.15	18.25	18.66
			S.E.	0.310	0.297	0.354	0.516
			N	7	7	7	7
WEEK	1 TO	2	MEAN	18.82 d	19.22	19.47	19.38
			S.E.	0.390	0.287	0.338	0.447
			N	7	7	7	7
WEEK	2 TO	3	MEAN	19.80 d	19.85	20.25	20.09
			S.E.	0.380	0.307	0.317	0.347
			N	7	7	7	7
WEEK	3 TO	4	MEAN	19.42 d	19.66	20.19	19.96
			S.E.	0.348	0.285	0.343	0.289
			N	7	7	7	7
WEEK	4 TO	5	MEAN	19.22 d	19.59	19.38	19.77
			S.E.	0.429	0.252	0.622	0.275
			N	7	7	7	7
WEEK	5 TO	6	MEAN	19.32 d	19.55	19.58	19.63
			S.E.	0.396	0.185	0.751	0.313
			N	7	7	7	7
WEEK	6 TO	7	MEAN	18.89 d	18.89	19.29	19.27
			S.E.	0.408	0.232	0.405	0.290
			N	7	7	7	7
WEEK	7 TO	8	MEAN	18.77 d	18.56	19.61	19.30
			S.E.	0.363	0.443	0.219	0.313
			N	7	7	7	7
WEEK	8 TO	9	MEAN	17.97 d	18.44	19.29*	18.78
			S.E.	0.400	0.249	0.322	0.280
			N	7	7	7	7
WEEK	9 TO	10	MEAN	17.91 d	18.47	18.72	18.47
			S.E.	0.375	0.166	0.527	0.254
			N	7	7	7	7

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 29.1 MEAN FOOD CONSUMPTION -- (g/animal/day)

MALES

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
WEEK	11 TO 12	MEAN	17.72 d	18.25	18.56
		S.E.	0.512	0.183	0.526
		N	7	7	7
WEEK	12 TO 13	MEAN	17.99 d	18.10	18.63
		S.E.	0.429	0.197	0.441
		N	7	7	7

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 29.2 MEAN FOOD CONSUMPTION -- (g/kg/day)

MALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	114.52 d	116.19	112.93	124.30**
			S.E.	1.899	2.406	1.893	2.243
			N	7	7	7	7
WEEK	1 TO	2	MEAN	97.39 d	98.98	96.79	103.50*
			S.E.	1.347	1.668	1.565	1.357
			N	7	7	7	7
WEEK	2 TO	3	MEAN	86.55 d	86.17	85.27	90.54
			S.E.	1.106	1.479	1.008	1.193
			N	7	7	7	7
WEEK	3 TO	4	MEAN	75.39 d	75.48	75.59	79.50*
			S.E.	0.946	1.242	0.935	0.837
			N	7	7	7	7
WEEK	4 TO	5	MEAN	68.32 d	68.86	66.90	71.90
			S.E.	1.012	1.005	1.738	0.940
			N	7	7	7	7
WEEK	5 TO	6	MEAN	64.51 d	64.38	63.88	66.83
			S.E.	1.044	0.921	1.833	0.739
			N	7	7	7	7
WEEK	6 TO	7	MEAN	60.20 d	59.15	60.09	62.34
			S.E.	0.922	0.831	0.668	0.770
			N	7	7	7	7
WEEK	7 TO	8	MEAN	57.29 d	55.93	58.40	59.75
			S.E.	0.905	1.262	0.909	0.790
			N	7	7	7	7
WEEK	8 TO	9	MEAN	52.93 d	53.65	55.09	56.08**
			S.E.	0.641	0.644	0.549	0.749
			N	7	7	7	7
WEEK	9 TO	10	MEAN	51.35 d	51.91	51.64	53.36
			S.E.	0.496	0.460	0.988	0.612
			N	7	7	7	7

Statistical key: d= ANOVA & Dunnett test * = p<0.05 ** = p<0.01
N=Number of cages

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 29.2 MEAN FOOD CONSUMPTION -- (g/kg/day)

MALES

		A	B	C	D
		CONTROL	2.5%	5%	10%
			TREHALOSE	TREHALOSE	TREHALOSE
WEEK 11 TO 12	MEAN	49.26 d	49.23	49.00	50.90
	S.E.	0.588	0.334	0.849	0.694
	N	7	7	7	7
WEEK 12 TO 13	MEAN	49.88 d	48.53	48.84	50.66
	S.E.	0.801	0.651	0.756	0.700
	N	7	7	7	7

Statistical key: d= ANOVA & Dunnett test * = p<0.05 ** = p<0.01
N=Number of cages

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 30.1 MEAN FOOD CONSUMPTION -- (g/animal/day)

FEMALES

				A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
WEEK	0 TO	1	MEAN	13.92 d	14.16	13.45	14.06
			S.E.	0.238	0.287	0.274	0.282
			N	7	7	7	7
WEEK	1 TO	2	MEAN	13.87 d	14.49	13.79	14.03
			S.E.	0.310	0.316	0.298	0.249
			N	7	7	7	7
WEEK	2 TO	3	MEAN	13.95 d	14.52	13.86	13.64
			S.E.	0.271	0.383	0.277	0.256
			N	7	7	7	7
WEEK	3 TO	4	MEAN	13.86 d	14.40	13.72	13.79
			S.E.	0.208	0.412	0.238	0.201
			N	7	7	7	7
WEEK	4 TO	5	MEAN	13.64 d	14.12	13.89	13.77
			S.E.	0.188	0.332	0.159	0.199
			N	7	7	7	7
WEEK	5 TO	6	MEAN	13.16 d	13.57	13.45	13.39
			S.E.	0.137	0.285	0.172	0.252
			N	7	7	7	7
WEEK	6 TO	7	MEAN	12.59 d	12.84	12.83	12.78
			S.E.	0.211	0.252	0.210	0.270
			N	7	7	7	7
WEEK	7 TO	8	MEAN	12.82 d	12.84	12.78	13.03
			S.E.	0.121	0.293	0.144	0.202
			N	7	7	7	7
WEEK	8 TO	9	MEAN	12.61 d	12.50	13.02	12.90
			S.E.	0.164	0.273	0.150	0.237
			N	7	7	7	7
WEEK	9 TO	10	MEAN	12.49 d	12.89	12.76	12.99
			S.E.	0.130	0.289	0.141	0.236
			N	7	7	7	7

Statistical key: d= ANOVA & Dunnett test
N=Number of cages

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 30.2 MEAN FOOD CONSUMPTION -- (g/kg/day)

FEMALES

				A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
WEEK	0 TO	1	MEAN	112.01 d	113.83	109.57	117.27
			S.E.	2.207	2.477	1.489	2.767
			N	7	7	7	7
WEEK	1 TO	2	MEAN	98.23 d	101.69	98.80	102.11
			S.E.	2.423	2.339	1.273	1.416
			N	7	7	7	7
WEEK	2 TO	3	MEAN	89.22 d	91.58	89.64	89.86
			S.E.	1.733	1.974	1.824	0.878
			N	7	7	7	7
WEEK	3 TO	4	MEAN	81.92 d	84.02	81.97	84.35
			S.E.	1.268	1.925	1.156	0.587
			N	7	7	7	7
WEEK	4 TO	5	MEAN	76.16 d	77.83	78.47	79.79
			S.E.	1.202	1.421	1.738	1.096
			N	7	7	7	7
WEEK	5 TO	6	MEAN	70.74 d	72.16	73.23	74.63
			S.E.	0.902	1.144	1.719	0.526
			N	7	7	7	7
WEEK	6 TO	7	MEAN	65.92 d	66.37	68.04	69.11
			S.E.	0.810	0.950	2.518	0.971
			N	7	7	7	7
WEEK	7 TO	8	MEAN	65.26 d	64.61	65.81	68.39
			S.E.	0.664	1.060	1.627	0.798
			N	7	7	7	7
WEEK	8 TO	9	MEAN	62.36 d	61.74	65.49	65.93
			S.E.	0.874	0.998	1.894	0.630
			N	7	7	7	7
WEEK	9 TO	10	MEAN	60.31 d	62.18	62.60	64.79**
			S.E.	0.708	1.031	1.270	0.531
			N	7	7	7	7

Statistical key: d= ANOVA & Dunnett test ** = p<0.01
N=Number of cages

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:31.1 MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- g/animal/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 0 TO 7	MEAN	14.21 d	14.85	14.61	14.49
	S.E.	0.217	0.284	0.261	0.257
	N	23	25	25	25
DAYS 7 TO 14	MEAN	16.26 d	16.33	16.33	16.48
	S.E.	0.272	0.250	0.339	0.273
	N	23	25	25	25
DAYS 14 TO 21	MEAN	11.73 d	10.83	12.54	12.30
	S.E.	0.442	0.352	0.471	0.432
	N	22	24	25	23

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:31.2 MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION -- g/kg/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 0 TO 7	MEAN	66.16 d	68.21	68.59	68.71
	S.E.	0.835	0.928	1.313	0.927
	N	23	25	25	25
DAYS 7 TO 14	MEAN	69.82 d	69.41	70.13	72.08
	S.E.	1.178	0.655	1.169	0.739
	N	23	25	25	25
DAYS 14 TO 21	MEAN	45.62 d	42.28	48.83	49.10
	S.E.	1.575	1.286	1.809	1.705
	N	22	24	25	23

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:32.1 MEAN MATERNAL FOOD CONSUMPTION DURING LACTATION -- g/animal/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 1 TO 7	MEAN	20.19 d	19.63	21.32	21.89
	S.E.	0.672	1.101	0.779	0.533
	N	22	24	24	25
DAYS 7 TO 14	MEAN	39.11 d	39.48	40.61	41.58
	S.E.	1.005	1.521	1.287	1.175
	N	22	24	24	25
DAYS 14 TO 21	MEAN	55.58 d	54.89	57.30	56.89
	S.E.	1.280	2.077	1.667	1.475
	N	22	24	24	25

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:32.2 MEAN MATERNAL FOOD CONSUMPTION DURING LACTATION -- g/kg/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 1 TO 7	MEAN	95.56 d	92.06	100.14	105.07
	S.E.	3.436	4.459	4.077	2.769
	N	22	24	24	25
DAYS 7 TO 14	MEAN	168.95 d	166.85	171.48	178.04
	S.E.	4.432	5.545	5.835	4.960
	N	22	24	24	25
DAYS 14 TO 21	MEAN	227.18 d	218.52	229.71	232.11
	S.E.	5.294	7.522	7.347	5.726
	N	22	24	24	25

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 33.1 MEAN SUBSTANCE INTAKE -- g/kg body weight/day

MALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
				TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	0.00	2.45	4.92	9.73
			S.E.	0.000	0.024	0.061	0.076
			N	7	7	7	7
WEEK	1 TO	2	MEAN	0.00	2.10	4.15	8.43
			S.E.	0.000	0.013	0.029	0.090
			N	7	7	7	7
WEEK	2 TO	3	MEAN	0.00	1.85	3.76	7.42
			S.E.	0.000	0.012	0.029	0.103
			N	7	7	7	7
WEEK	3 TO	4	MEAN	0.00	1.66	3.38	6.69
			S.E.	0.000	0.015	0.037	0.029
			N	7	7	7	7
WEEK	4 TO	5	MEAN	0.00	1.53	3.08	6.03
			S.E.	0.000	0.030	0.031	0.102
			N	7	7	7	7
WEEK	5 TO	6	MEAN	0.00	1.42	2.90	5.65
			S.E.	0.000	0.015	0.029	0.060
			N	7	7	7	7
WEEK	6 TO	7	MEAN	0.00	1.36	2.77	5.51
			S.E.	0.000	0.016	0.030	0.087
			N	7	7	7	7
WEEK	7 TO	8	MEAN	0.00	1.36	2.76	5.46
			S.E.	0.000	0.013	0.021	0.076
			N	7	7	7	7
WEEK	8 TO	9	MEAN	0.00	1.28	2.55	5.20
			S.E.	0.000	0.014	0.027	0.075
			N	7	7	7	7
WEEK	9 TO	10	MEAN	0.00	1.24	2.38	4.89
			S.E.	0.000	0.018	0.028	0.078
			N	7	7	7	7

N=Number of cages

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 33.1 MEAN SUBSTANCE INTAKE -- g/kg body weight/day MALES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
WEEK	13 TO 14	MEAN 0.00	1.22	2.36	4.75
		S.E. 0.000	0.018	0.058	0.084
		N 7	7	7	7
WEEK	14 TO 15	MEAN 0.00	1.14	2.23	4.53
		S.E. 0.000	0.012	0.042	0.079
		N 7	7	7	7

N=Number of cages

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 33.2 MEAN SUBSTANCE INTAKE -- g/kg body weight/day

FEMALES

				A	B	C	D
				CONTROL	2.5%	5%	10%
					TREHALOSE	TREHALOSE	TREHALOSE
WEEK	0 TO	1	MEAN	0.00	2.22	4.53	9.19
			S.E.	0.000	0.021	0.053	0.089
			N	7	7	7	7
WEEK	1 TO	2	MEAN	0.00	2.06	4.24	8.41
			S.E.	0.000	0.016	0.082	0.069
			N	7	7	7	7
WEEK	2 TO	3	MEAN	0.00	1.94	3.95	7.84
			S.E.	0.000	0.036	0.049	0.069
			N	7	7	7	7
WEEK	3 TO	4	MEAN	0.00	1.82	3.72	7.17
			S.E.	0.000	0.036	0.040	0.188
			N	7	7	7	7
WEEK	4 TO	5	MEAN	0.00	1.70	3.48	7.12
			S.E.	0.000	0.024	0.043	0.106
			N	7	7	7	7
WEEK	5 TO	6	MEAN	0.00	1.57	3.34	6.57
			S.E.	0.000	0.015	0.054	0.074
			N	7	7	7	7
WEEK	6 TO	7	MEAN	0.00	1.56	3.12	6.23
			S.E.	0.000	0.026	0.040	0.057
			N	7	7	7	7
WEEK	7 TO	8	MEAN	0.00	1.50	2.98	6.06
			S.E.	0.000	0.025	0.045	0.080
			N	7	7	7	7
WEEK	8 TO	9	MEAN	0.00	1.47	2.91	6.04
			S.E.	0.000	0.017	0.048	0.093
			N	7	7	7	7
WEEK	9 TO	10	MEAN	0.00	1.47	2.88	5.94
			S.E.	0.000	0.024	0.047	0.093
			N	7	7	7	7

N=Number of cages

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:33.3 SUMMARY OF SUBSTANCE INTAKE IN FOOD DURING GESTATION -- g/kg body weight/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 0 TO 7	MEAN	0.00	1.62	3.26	6.67
	S.E.	0.000	0.028	0.110	0.096
	N	23	25	24	27
DAYS 7 TO 14	MEAN	0.00	1.70	3.48	6.87
	S.E.	0.000	0.020	0.042	0.084
	N	23	25	24	27
DAYS 14 TO 21	MEAN	0.00	1.10	2.22	4.40
	S.E.	0.000	0.031	0.091	0.110
	N	22	24	23	26

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:33.4 SUMMARY OF SUBSTANCE INTAKE IN FOOD DURING LACTATION -- g/kg/body weight/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 1 TO 7	MEAN	0.00	2.51	4.98	10.10
	S.E.	0.000	0.093	0.231	0.281
	N	21	26	22	26
DAYS 7 TO 14	MEAN	0.00	4.34	8.94	17.94
	S.E.	0.000	0.114	0.207	0.421
	N	21	26	22	26
DAYS 14 TO 21	MEAN	0.00	5.00	10.42	20.99
	S.E.	0.000	0.144	0.161	0.364
	N	21	26	22	26

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 34.1 MEAN SUBSTANCE INTAKE -- g/kg body weight/day

MALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	0.00	2.90	5.65	12.43
			S.E.	0.000	0.060	0.095	0.224
			N	7	7	7	7
WEEK	1 TO	2	MEAN	0.00	2.47	4.84	10.35
			S.E.	0.000	0.042	0.078	0.136
			N	7	7	7	7
WEEK	2 TO	3	MEAN	0.00	2.15	4.26	9.05
			S.E.	0.000	0.037	0.050	0.119
			N	7	7	7	7
WEEK	3 TO	4	MEAN	0.00	1.89	3.78	7.95
			S.E.	0.000	0.031	0.047	0.084
			N	7	7	7	7
WEEK	4 TO	5	MEAN	0.00	1.72	3.34	7.19
			S.E.	0.000	0.025	0.087	0.094
			N	7	7	7	7
WEEK	5 TO	6	MEAN	0.00	1.61	3.19	6.68
			S.E.	0.000	0.023	0.092	0.074
			N	7	7	7	7
WEEK	6 TO	7	MEAN	0.00	1.48	3.00	6.23
			S.E.	0.000	0.021	0.033	0.077
			N	7	7	7	7
WEEK	7 TO	8	MEAN	0.00	1.40	2.92	5.98
			S.E.	0.000	0.032	0.045	0.079
			N	7	7	7	7
WEEK	8 TO	9	MEAN	0.00	1.34	2.75	5.61
			S.E.	0.000	0.016	0.027	0.075
			N	7	7	7	7
WEEK	9 TO	10	MEAN	0.00	1.30	2.58	5.34
			S.E.	0.000	0.012	0.049	0.061
			N	7	7	7	7

N=Number of cages

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 34.1 MEAN SUBSTANCE INTAKE -- g/kg body weight/day MALES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
WEEK 11 TO 12	MEAN	0.00	1.23	2.45	5.09
	S.E.	0.000	0.008	0.042	0.069
	N	7	7	7	7
WEEK 12 TO 13	MEAN	0.00	1.21	2.44	5.07
	S.E.	0.000	0.016	0.038	0.070
	N	7	7	7	7

N=Number of cages

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 34.2 MEAN SUBSTANCE INTAKE -- g/kg body weight/day

FEMALES

			A	B	C	D	
			CONTROL	2.5%	5%	10%	
			TREHALOSE	TREHALOSE	TREHALOSE	TREHALOSE	
WEEK	0 TO	1	MEAN	0.00	2.85	5.48	11.73
			S.E.	0.000	0.062	0.074	0.277
			N	7	7	7	7
WEEK	1 TO	2	MEAN	0.00	2.54	4.94	10.21
			S.E.	0.000	0.058	0.064	0.142
			N	7	7	7	7
WEEK	2 TO	3	MEAN	0.00	2.29	4.48	8.99
			S.E.	0.000	0.049	0.091	0.088
			N	7	7	7	7
WEEK	3 TO	4	MEAN	0.00	2.10	4.10	8.43
			S.E.	0.000	0.048	0.058	0.059
			N	7	7	7	7
WEEK	4 TO	5	MEAN	0.00	1.95	3.92	7.98
			S.E.	0.000	0.036	0.087	0.110
			N	7	7	7	7
WEEK	5 TO	6	MEAN	0.00	1.80	3.66	7.46
			S.E.	0.000	0.029	0.086	0.053
			N	7	7	7	7
WEEK	6 TO	7	MEAN	0.00	1.66	3.40	6.91
			S.E.	0.000	0.024	0.126	0.097
			N	7	7	7	7
WEEK	7 TO	8	MEAN	0.00	1.62	3.29	6.84
			S.E.	0.000	0.027	0.081	0.080
			N	7	7	7	7
WEEK	8 TO	9	MEAN	0.00	1.54	3.27	6.59
			S.E.	0.000	0.025	0.095	0.063
			N	7	7	7	7
WEEK	9 TO	10	MEAN	0.00	1.55	3.13	6.48
			S.E.	0.000	0.026	0.064	0.053
			N	7	7	7	7

N=Number of cages

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:34.3 SUMMARY OF SUBSTANCE INTAKE IN FOOD DURING GESTATION -- g/kg body weight/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 0 TO 7	MEAN	0.00	1.71	3.43	6.87
	S.E.	0.000	0.023	0.066	0.093
	N	23	25	25	25
DAYS 7 TO 14	MEAN	0.00	1.74	3.51	7.21
	S.E.	0.000	0.016	0.058	0.074
	N	23	25	25	25
DAYS 14 TO 21	MEAN	0.00	1.06	2.44	4.91
	S.E.	0.000	0.032	0.090	0.171
	N	22	24	25	23

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:34.4 SUMMARY OF SUBSTANCE INTAKE IN FOOD DURING LACTATION -- g/kg/body weight/day

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
DAYS 1 TO 7	MEAN	0.00	2.30	5.01	10.51
	S.E.	0.000	0.111	0.204	0.277
	N	22	24	24	25
DAYS 7 TO 14	MEAN	0.00	4.17	8.57	17.80
	S.E.	0.000	0.139	0.292	0.496
	N	22	24	24	25
DAYS 14 TO 21	MEAN	0.00	5.46	11.49	23.21
	S.E.	0.000	0.188	0.367	0.573
	N	22	24	24	25

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:35 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Females placed with males	N	28	28	28	28
Females mated	N	28	28	28	28
Pre-coital time (days)	MEAN S.E.	2.26 u 0.217	3.11 0.515	2.18 0.242	3.21 0.629
Day 1 to 4	N %	26 f 96	25 89	27 96	24 86
Day 5 to 7	N %	1 f 3.7	2 7.1	1 3.6	2 7.1
Day 8 to 14	N %	0 f 0.0	0 0.0	0 0.0	2 7.1
Day 15 to 21	N %	0 f 0.0	1 3.6	0 0.0	0 0.0
Day 1 to 21	N %	27 f 100	28 100	28 100	28 100
Females pregnant	N	24 f	26	24	27
Females with liveborn	N	24 f	26	24	27
Mating index	%	100	100	100	100
Female fecundity index	%	86	93	86	96
Male fertility index	%	86	93	86	96
Female fertility index	%	86	93	86	96
Gestation index	%	100	100	100	100
Duration of gestation	MEAN S.E.	21.39 u 0.122	21.23 0.115	21.46 0.120	21.44 0.111
Females surviving delivery as % of pregnant females	N %	24 f 100	26 100	24 100	27 100
Females with stillborn pups as % of pregnant females	N %	7 f 29	6 23	4 17	4 15
Females with all stillborn pups as % of pregnant females	N %	0 f 0.0	0 0.0	0 0.0	0 0.0

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

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:35 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Pups delivered (total)	N	259	271	266	296
	MEAN	10.79 u	10.42	11.08	10.96
	S.E.	0.493	0.356	0.474	0.408
Liveborn	N	237 f	255	248	288**
Live Birth Index	%	92	94	93	97
Stillborn	N	22 f	16	18	8**
Pup mortality day 1	%	8.5	5.9	6.8	2.7
Number of pups lost (dying, missing, and/or cannibalized) on the following days:					
days 1-4	N	26 f	1#	23	10**
Pup mortality day 4	%	11	0.4	9.3	3.5
Culled day 4		53	58	54	76
Number alive, after culling at day 4	N	158	196	171	202
Number of pups lost (dying, missing, and/or cannibalized) on the following days:					
days 5-7	N	2 f	0	0	0
days 8-14	N	1 f	0	3	0
days 15-21	N	0 f	0	0	0
Pups alive day 21	N	155 f	196	168	202
Viability index day 4-21	%	98	100	98	100
Number of litters lost entirely (Stillborn, dying, missing, cannibalized, and/or culled) in the period between:					
days 0- 4	N	3 f	0	2	1
	%	13	0.0	8.3	3.7
days 5- 7	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 8-14	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 15-21	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 0-21	N	3 f	0	2	1
	%	13	0.0	8.3	3.7

Statistical key: f= Fishers exact test u= Kruskal-Wallis & Mann-Whitney U ** = p<0.01 # = p<0.001

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:35 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Live Pups/litter					
day 1	MEAN	9.88 u	9.81	10.33	10.67
	S.E.	0.615	0.514	0.517	0.430
day 4 preculling	MEAN	10.05 u	9.77	10.23	10.69
	S.E.	0.607	0.518	0.460	0.453
day 4 postculling	MEAN	7.52 u	7.54	7.77	7.77
	S.E.	0.298	0.305	0.130	0.139
day 7	MEAN	7.43 u	7.54	7.77	7.77
	S.E.	0.335	0.305	0.130	0.139
day 14	MEAN	7.38 u	7.54	7.64	7.77
	S.E.	0.334	0.305	0.168	0.139
day 21	MEAN	7.38 u	7.54	7.64	7.77
	S.E.	0.334	0.305	0.168	0.139
Number of male pups at day 1	N	127 f	126	126	159
Sex ratio at day 1	%	54	49	51	55
Number of male pups at day 21	N	83 f	97	81	110
Sex ratio at day 21	%	54	49	48	54
No of lost implantations	N	46	47	40	49
Post implantations loss %	N	16.05 u	14.84	12.87	13.91
	S.E.	3.866	4.293	3.685	3.230

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

Mating index : number of females mated * 100 / number of females placed with males
 Female fecundity index : number of females pregnant * 100 / number of females mated
 Male fertility index : number of males that became sire * 100 / number of males placed with females
 Female fertility index : number of females pregnant * 100 / number of females placed with males
 Gestation index : number of females with live pups * 100 / number of pregnant females
 Live birth index : number of pups born alive * 100 / total number of pups born
 Pup mortality day n : number of dead pups on day n * 100 / total number of pups on day n
 Viability index day 4-21: number of pups surviving 21 days / number of liveborn after culling at day 4
 Sex ratio : number of male pups date n * 100 / total number of pups
 Post-implantation loss : number of implantation sites - number of pups born alive * 100 / number of implantation sites

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:36 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Females placed with males	N	28	28	28	28
Females mated	N	28	28	28	28
Pre-coital time (days)	MEAN S.E.	2.68 u 0.212	2.18 0.225	2.64 0.305	2.25 0.234
Day 1 to 4	N %	26 f 93	28 100	25 89	27 96
Day 5 to 7	N %	2 f 7.1	0 0.0	3 11	1 3.6
Day 8 to 14	N %	0 f 0.0	0 0.0	0 0.0	0 0.0
Day 15 to 21	N %	0 f 0.0	0 0.0	0 0.0	0 0.0
Day 1 to 21	N %	28 f 100	28 100	28 100	28 100
Females pregnant	N	23 f	25	25	25
Females with liveborn	N	23 f	25	25	25
Mating index	%	100	100	100	100
Female fecundity index	%	82	89	89	89
Male fertility index	%	82	89	89	89
Female fertility index	%	82	89	89	89
Gestation index	%	100	100	100	100
Duration of gestation	MEAN S.E.	21.26 u 0.094	21.12 0.088	21.40 0.100	21.44 0.101
Females surviving delivery as % of pregnant females	N %	23 f 100	25 100	25 100	25 100
Females with stillborn pups as % of pregnant females	N %	3 f 13	3 12	3 12	2 8.0
Females with all stillborn pups as % of pregnant females	N %	0 f 0.0	0 0.0	0 0.0	0 0.0

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

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:36 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Pups delivered (total)	N	217	244	245	237
	MEAN	9.43 u	9.76	9.80	9.48
	S.E.	0.457	0.380	0.374	0.523
Liveborn	N	197 f	223	236*	233#
Live Birth Index	%	91	91	96	98
Stillborn	N	20 f	21	9*	4#
Pup mortality day 1	%	9.2	8.6	3.7	1.7
Number of pups lost (dying, missing, and/or cannibalized) on the following days:					
days 1-4	N	7 f	15	10	2
Pup mortality day 4	%	3.6	6.7	4.2	0.9
Culled day 4		26	36	45	46
Number alive, after culling at day 4	N	164	172	181	185
Number of pups lost (dying, missing, and/or cannibalized) on the following days:					
days 5-7	N	0 f	1	0	0
days 8-14	N	0 f	0	0	0
days 15-21	N	0 f	0	0	0
Pups alive day 21	N	164 f	170	181	185
Viability index day 4-21	%	100	99	100	100
Number of litters lost entirely (Stillborn, dying, missing, cannibalized, and/or culled) in the period between:					
days 0- 4	N	1 f	1	1	0
	%	4.3	4.0	4.0	0.0
days 5- 7	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 8-14	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 15-21	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 0-21	N	1 f	1	1	0
	%	4.3	4.0	4.0	0.0

Statistical key: f= Fishers exact test u= Kruskal-Wallis & Mann-Whitney U * = p<0.05 # = p<0.001

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:36 NATURAL DELIVERY DATA AND LITTER DATA -- SUMMARY

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
Live Pups/litter					
day 1	MEAN	8.57 u	8.88	9.44	9.32
	S.E.	0.474	0.555	0.462	0.547
day 4 preculling	MEAN	8.64 u	8.63	9.42	9.24
	S.E.	0.434	0.567	0.492	0.552
day 4 postculling	MEAN	7.45 u	7.13	7.54	7.40
	S.E.	0.252	0.342	0.295	0.294
day 7	MEAN	7.45 u	7.08	7.54	7.40
	S.E.	0.252	0.361	0.295	0.294
day 14	MEAN	7.45 u	7.08	7.54	7.40
	S.E.	0.252	0.361	0.295	0.294
day 21	MEAN	7.45 u	7.08	7.54	7.40
	S.E.	0.252	0.361	0.295	0.294
Number of male pups at day 1	N	95 f	114	120	124
Sex ratio at day 1	%	48	51	51	53
Number of male pups at day 21	N	77 f	85	94	96
Sex ratio at day 21	%	47	50	52	52
No of lost implantations	N	36	47	30	32
Post implantations loss %	N	13.81 u	15.78	11.32	11.69
	S.E.	4.345	5.065	3.537	3.403

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

Mating index : number of females mated * 100 / number of females placed with males
 Female fecundity index : number of females pregnant * 100 / number of females mated
 Male fertility index : number of males that became sire * 100 / number of males placed with females
 Female fertility index : number of females pregnant * 100 / number of females placed with males
 Gestation index : number of females with live pups * 100 / number of pregnant females
 Live birth index : number of pups born alive * 100 / total number of pups born
 Pup mortality day n : number of dead pups on day n * 100 / total number of pups on day n
 Viability index day 4-21: number of pups surviving 21 days / number of liveborn after culling at day 4
 Sex ratio : number of male pups date n * 100 / total number of pups
 Post-implantation loss : number of implantation sites - number of pups born alive * 100 / number of implantation sites

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE
IN RATS (F0-GENERATION)

TABEL:37 SUMMARY OF CLINICAL OBSERVATIONS IN PUPS RECORDED ON DAYS 1,4,7,14 AND 21 OF LACTATION

	GROUP A CONTROL	GROUP B 2.5% TREHALOSE	GROUP C 5% TREHALOSE	GROUP D 10% TREHALOSE
Number of pups showing the observation				
DAY 1 LACTATION				
Pale	19(2) ¹	7* (6)	9 (3)	6**(6)
Cold	8(2)	2 (2)	23**(3)	2 (1)
Small pup ²	22(3)	6**(2)	16 (6)	7# (5)
Large pup ³	1	0	6 (2)	4 (1)
Cyanosis	0	0	0	1
Subcutaneous haemorrhage(s):head	2(2)	2 (2)	9 (6)	4 (3)
dorsal	7(2)	0	7 (5)	11 (2)
ventral	2(1)	0	0	1
leg(s)	2(2)	0	0	0
tail	1	0	0	0
Wound	1	1	2 (2)	1
No milk in stomach	7(1)	1*	2 (2)	1*
Missing/dead tailtip	0	2(2)	2 (2)	2 (2)
Missing tail	2(2)	0	0	0
Missing toe(s)	0	0	0	1
Missing leg(s)	2(2)	0	1	1
DAY 4 LACTATION				
Pale	0	1	1	0
Small pup	24(4)	3# (2)	14 (4)	6# (3)
Large pup	2(1)	0	7 (1)	6 (1)
Subcutaneous haemorrhage(s):tail	0	0	1	0
Haemorrhagis discharge nose	0	0	0	1
Missing/dead tailtip	2(1)	2 (2)	2 (2)	2 (2)
Missing leg(s)	1	0	0	1
DAY 7 LACTATION				
Small pup ²	2(1)	1	3 (1)	0
Large pup ³	0	0	7* (1)	6* (1)
Missing/dead tailtip	1	2 (2)	1	2 (2)
Missing leg(s)	1	0	0	0
Statistical key: Fishers exact test * p< 0.05 ** p< 0.01 # p< 0.001				

¹ Figures in brackets represent the number of litters with pups showing the observation² Criterium for small pup: lactation body weight < 75% of the mean body weight of historical control(study 1905F1)
(viz. < 3.8 g day 1; < 5.8 g day 4; < 8.6 g day 7; < 18.8 g day 14; < 32.1 g for male pups and < 30.9 g for female pups day 21).³ Criterium for large pup: lactation body weight > 125% of the mean body weight of historical control(study 1905F1)
(viz. > 6.4 g day 1; > 9.7 g day 4; > 14.3 g day 7; > 31.4 g day 14; > 53.4 g for male pups and > 51.5 g for female pups day 21).

30-JUL-98

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE
IN RATS (F0-GENERATION)

TABEL:37 SUMMARY OF CLINICAL OBSERVATIONS IN PUPS RECORDED ON DAYS 1,4,7,14 AND 21 OF LACTATION

OBSERVATIONS	GROUP A CONTROL	GROUP B 2.5% TREHALOSE	GROUP C 5% TREHALOSE	GROUP D 10% TREHALOSE
----- Number of pups showing the observation				
DAY 14 LACTATION				
Large pup ³	3(1) ¹	5(2)	0	0
Sparsely haired/alopecia	2(1)	0	5(1)	0
Missing/dead tailtip	1	2(2)	1	0
Missing leg(s)	1	0	0	0
DAY 21 LACTATION				
Large pup ³	3(1)	2(1)	10(5)	4(3)
Missing/dead tailtip	1	2(2)	1	0

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

¹ Figures in brackets represent the number of litters with pups showing the observation

³ Criterium for large pup: lactation body weight > 125% of the mean body weight of historical control(study 1905F1)
(viz. > 6.4 g day 1; > 9.7 g day 4; > 14.3 g day 7; > 31.4 g day 14; > 53.4 g for male pups and > 51.5 g for female pups day 21).

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE
IN RATS (F1-GENERATION)

TABEL:38 SUMMARY OF CLINICAL OBSERVATIONS IN PUPS RECORDED ON DAYS 1,4,7,14 AND 21 OF LACTATION

OBSERVATIONS	GROUP A CONTROL	GROUP B 2.5%	GROUP C 5%	GROUP D 10%
Number of pups showing the observation				
DAY 1 LACTATION				
Pale	2(2) ¹	2 (2)	3(3)	0
Cold	3(1)	3 (2)	10(2)	0
Small pup ²	1	37#(7)	7(2)	3(1)
Large pup ³	6(2)	0*	4(1)	12(4)
Subcutaneous haemorrhage(s):head	2(1)	2 (2)	2(2)	1
tail	2(2)	0	0	0
Missing/dead tailtip	1	1	0	1
Leg(s) missing	1	0	0	0
No milk in stomach	0	5(4)	0	0
Wound nose	0	1	0	0
DAY 4 LACTATION				
Cold	0	0	2(1)	0
Small pup	4(1)	28#(6)	2(2)	0*
Large pup	4(1)	0	6(2)	11(3)
Missing/dead tailtip	2(2)	2 (2)	1	1
Leg(s) missing	1	0	0	0
Wound tail	0	0	0	1
DAY 7 LACTATION				
Small pup	2(2)	11*(4)	1	0
Large pup	4(1)	0	4(1)	10(4)
Missing/dead tailtip	2(2)	3(3)	1	2(2)
Leg(s) missing	1	0	0	0
Cyanosis	0	1	0	0
DAY 14 LACTATION				
Small pup	0	2(2)	0	0
Large pup	4(1)	2(1)	5(2)	8(3)
Missing/dead tailtip	2(2)	3(3)	1	2(2)
Leg(s) missing	1	0	0	0
Sparsely haired	0	8**(1)	0	0
DAY 21 LACTATION				
Small pup	1	1	0	0
Large pup	5(2)	5(1)	9(4)	16*(5)
Missing/dead tailtip	2(2)	3(3)	1	2(2)
Leg(s) missing	1	0	0	0
Sparsely haired	0	0	0	2(1)

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

¹ Figures in brackets represent the number of litters with pups showing the observation

² Criterium for small pup: lactation body weight < 75% of the mean body weight of historical control(study 1905F1)
(viz. < 3.8 g day 1; < 5.8 g day 4; < 8.6 g day 7; < 18.8 g day 14; < 32.1 g for male pups and < 30.9 g for female pups day 21).

³ Criterium for large pup: lactation body weight > 125% of the mean body weight of historical control(study 1905F1)
(viz. > 6.4 g day 1; > 9.7 g day 4; > 14.3 g day 7; > 31.4 g day 14; > 53.4 g for male pups and > 51.5 g for female pups day 21).

08-FEB-99

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE
IN RATS (F0-GENERATION)

TABLE:39 SUMMARY OF MACROSCOPIC OBSERVATIONS IN STILLBORN PUPS AND PUPS THAT DIED DURING LACTATION

OBSERVATIONS	GROUP A CONTROL	GROUP B 2.5 % TREHALOSE	GROUP C 5% TREHALOSE	GROUP D 10% TREHALOSE
No abnormalities	11(4) ¹	4(1)	6(3)	8(4)
Autolytic	2(2)	7(5)	0	0
Partly cannibalized	10(4)	5(2)	12(3)	0

¹ Figures in brackets represent the number of litters with pups showing the observation.

08-FEB-99

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:40 SUMMARY OF MACROSCOPIC OBSERVATIONS IN STILLBORN PUPS AND PUPS THAT DIED DURING LACTATION

OBSERVATIONS	GROUP A CONTROL	GROUP B 2.5%	GROUP C 5%	GROUP D 10%
No abnormalities	1	3(1)	3(1)	3(1)
Pale	0	1	0	0
Cold	0	1	0	0
No milk in stomach	0	1	0	0
Wound nose	0	1	0	0
Autolytic	6(1) ¹	9(1)	3(2)	1
Partly cannibalized	13(1)	9(1)	3(1)	0
Very small	0	1	0	0

¹ Figures in brackets represent the number of litters with pups showing the observation.

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:41 SUMMARY OF PUP WEIGHTS (g)

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
day 1 males+females	MEAN	4.74 d	4.90	4.99	4.86
	S.E.	0.148	0.099	0.165	0.122
	N	24	26	24	27
day 4 males+females preculding	MEAN	7.15 d	7.43	7.90	7.42
	S.E.	0.265	0.169	0.282	0.199
	N	21	26	22	26
day 4 males+females postculling	MEAN	7.16 d	7.45	7.91	7.40
	S.E.	0.265	0.169	0.284	0.202
	N	21	26	22	26
day 7 males+females	MEAN	11.59 d	11.83	12.71*	11.87
	S.E.	0.351	0.221	0.325	0.228
	N	21	26	22	26
day 14 males+females	MEAN	26.46 d	26.88	28.50*	27.59
	S.E.	0.518	0.468	0.554	0.368
	N	21	24	22	26
day 21 males	MEAN	44.03 d	44.89	47.04	45.77
	S.E.	0.808	0.798	0.919	0.562
	N	21	26	22	26
day 21 females	MEAN	42.42 d	43.23	45.28	44.43
	S.E.	0.982	0.716	0.778	0.526
	N	21	25	22	24
day 21 males+females	MEAN	43.37 d	43.97	46.07*	45.24
	S.E.	0.799	0.709	0.757	0.518
	N	21	26	22	26

Statistical key: d= ANOVA & Dunnett test * = p<0.05

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE:41 SUMMARY OF PUP BODY WEIGHT CHANGES -- GRAMS

		A	B	C	D
		CONTROL	2.5%	5%	10%
			TREHALOSE	TREHALOSE	TREHALOSE
day 1- 4 males+females preculling	MEAN	2.38 d	2.53	2.83	2.54
	S.E.	0.125	0.087	0.146	0.103
	N	21	26	22	26
day 4- 7 males+females postculling	MEAN	4.43 d	4.37	4.80*	4.48
	S.E.	0.144	0.103	0.078	0.069
	N	21	26	22	26
day 7-14 males+females	MEAN	14.88 d	15.06	15.80	15.72
	S.E.	0.396	0.331	0.369	0.216
	N	21	24	22	26
day 14-21 males+females	MEAN	16.91 d	17.08	17.57	17.65
	S.E.	0.370	0.366	0.333	0.253
	N	21	24	22	26

Statistical key: d= ANOVA & Dunnett test * = p<0.05

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:42 SUMMARY OF PUP WEIGHTS (g)

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
day 1 males+females	MEAN	5.16 d	4.75	5.08	5.38
	S.E.	0.161	0.169	0.144	0.203
	N	23	24	25	25
day 4 males+females preculing	MEAN	8.17 d	7.24	8.14	8.24
	S.E.	0.305	0.283	0.249	0.326
	N	22	24	24	25
day 4 males+females postculling	MEAN	8.18 d	7.23	7.90	8.22
	S.E.	0.307	0.282	0.260	0.327
	N	22	24	24	25
day 7 males+females	MEAN	12.31 d	11.23	12.17	12.67
	S.E.	0.381	0.361	0.314	0.377
	N	22	24	24	25
day 14 males+females	MEAN	27.39 d	25.92	27.47	27.73
	S.E.	0.596	0.662	0.589	0.609
	N	22	24	24	25
day 21 males	MEAN	46.23 d	45.25	45.71	46.96
	S.E.	0.928	0.996	0.833	1.016
	N	22	23	23	25
day 21 females	MEAN	44.58 d	43.18	44.59	45.50
	S.E.	0.961	0.826	0.943	0.984
	N	22	24	24	24
day 21 males+females	MEAN	45.32 d	44.10	45.34	46.12
	S.E.	0.910	0.853	0.891	0.943
	N	22	24	24	25

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE:42 SUMMARY OF PUP BODY WEIGHT CHANGES -- GRAMS

		A	B	C	D
		CONTROL	2.5%	5%	10%
			TREHALOSE	TREHALOSE	TREHALOSE
day 1- 4 males+females preculling	MEAN	2.98 d	2.41*	3.00	2.85
	S.E.	0.163	0.144	0.160	0.147
	N	22	23	24	25
day 4- 7 males+females postculling	MEAN	4.13 d	4.00	4.27	4.45
	S.E.	0.122	0.140	0.106	0.102
	N	22	24	24	25
day 7-14 males+females	MEAN	15.09 d	14.69	15.30	15.06
	S.E.	0.323	0.371	0.424	0.384
	N	22	24	24	25
day 14-21 males+females	MEAN	17.93 d	18.18	17.87	18.39
	S.E.	0.380	0.331	0.402	0.420
	N	22	24	24	25

Statistical key: d= ANOVA & Dunnett test * = p<0.05

28-JUL-98

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 43 MEAN ORGAN WEIGHTS ABSOLUTE (g)

		MALES			
		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	442.8 d	438.7	440.9	450.5
	S.E.	6.49	5.55	7.18	7.03
	N	28	28	28	28
SPLEEN	MEAN	0.659 d	0.635	0.658	0.641
	S.E.	0.0121	0.0119	0.0143	0.0114
	N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 43 MEAN ORGAN WEIGHTS RELATIVE TO TERMINAL BODY WEIGHT (g.kg b.w.) MALES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	442.8 d	438.7	440.9	450.5
	S.E.	6.49	5.55	7.18	7.03
	N	28	28	28	28
SPLEEN	MEAN	1.494 d	1.453	1.495	1.427
	S.E.	0.0301	0.0297	0.0283	0.0227
	N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 44 MEAN ORGAN WEIGHTS ABSOLUTE (g)

		FEMALES			
		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	244.5 d	244.8	251.2	249.6
	S.E.	4.14	2.51	2.83	3.28
	N	24	26	24	27
SPLEEN	MEAN	0.446 d	0.471	0.467	0.454
	S.E.	0.0101	0.0103	0.0106	0.0081
	N	24	26	24	27

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F0 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F0-GENERATION)

TABLE: 44 MEAN ORGAN WEIGHTS RELATIVE TO TERMINAL BODY WEIGHT (g.kg b.w.)

FEMALES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	244.5 d	244.8	251.2	249.6
	S.E.	4.14	2.51	2.83	3.28
	N	24	26	24	27
SPLEEN	MEAN	1.827 d	1.924	1.866	1.825
	S.E.	0.0366	0.0354	0.0511	0.0322
	N	24	26	24	27

Statistical key: d= ANOVA & Dunnett test

28-MAR-00

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 45 MEAN ORGAN WEIGHTS ABSOLUTE (g)

		MALES			
		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	371.0 d	383.6	393.7	374.0
	S.E.	7.43	8.71	6.59	5.67
	N	28	28	28	28
SPLEEN	MEAN	0.576 d	0.628	0.614	0.573
	S.E.	0.0108	0.0301	0.0150	0.0154
	N	28	27	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 45 MEAN ORGAN WEIGHTS RELATIVE TO TERMINAL BODY WEIGHT (g.kg b.w.) MALES

		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	371.0 d	383.6	393.7	374.0
	S.E.	7.43	8.71	6.59	5.67
	N	28	28	28	28
SPLEEN	MEAN	1.562 d	1.643	1.562	1.536
	S.E.	0.0291	0.0671	0.0315	0.0382
	N	28	28	28	28

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 46 MEAN ORGAN WEIGHTS ABSOLUTE (g)

		FEMALES			
		A	B	C	D
		CONTROL	2.5% TREHALOSE	5% TREHALOSE	10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	227.6 d	231.5	231.0	225.1
	S.E.	3.03	3.21	3.50	2.82
	N	23	25	25	25
SPLEEN	MEAN	0.474 d	0.475	0.484	0.483
	S.E.	0.0106	0.0116	0.0145	0.0135
	N	23	25	25	24

Statistical key: d= ANOVA & Dunnett test

STUDY NO.1992F1 ORAL TWO-GENERATION REPRODUCTION STUDY WITH TREHALOSE IN RATS
(F1-GENERATION)

TABLE: 46 MEAN ORGAN WEIGHTS RELATIVE TO TERMINAL BODY WEIGHT (g.kg b.w.)

FEMALES

		A CONTROL	B 2.5% TREHALOSE	C 5% TREHALOSE	D 10% TREHALOSE
TERMINAL BODY WEIGHT (g)	MEAN	227.6 d	231.5	231.0	225.1
	S.E.	3.03	3.21	3.50	2.82
	N	23	25	25	25
SPLEEN	MEAN	2.086 d	2.054	2.096	2.143
	S.E.	0.0430	0.0472	0.0566	0.0555
	N	23	25	25	24

Statistical key: d= ANOVA & Dunnett test

Oral two-generation reproduction study with Trehalose in rats (F0 generation)
TNO Nutrition and Food Research Institute
Study : 1992F0
Table 47 : Summary of macroscopic lesions

CHANGES	TREATMENT	INCIDENCE OF GROSS LESIONS (NUMERIC)							
		MALES				FEMALES			
		2.5% Trehalose lose	5 % Trehalose lose	10 % Trehalose lose	Contr.	2.5% Trehalose lose	5 % Trehalose lose	10 % Trehalose lose	Contr.
HABITUS/CLINICAL SIGNS:									
Malocclusion of incisors		2	2	2			1		

The absence of a numeral indicates that the change specified was not identified

Oral two-generation reproduction study with Trehalose in rats (F0 generation)
TNO Nutrition and Food Research Institute
Study : 1992F0
Table 48 : Summary of microscopic changes

CHANGES	INCIDENCE OF LESIONS (NUMERIC)														
	TREATMENT	MALES							FEMALES						
		Contr.	2.5% Treha lose	5 % Treha lose	10 % Treha lose	#	# Contr.	2.5% Treha lose	5 % Treha lose	10 % Treha lose	#	# Contr.	2.5% Treha lose	5 % Treha lose	10 % Treha lose
ABDOMINAL CAVITY:															
Sinusoidal blood lymph node															
Fat necrosis															
COAGULATING GLANDS:															
No abnormality detected															
EPIDIDYMIDES:															
Devoid of spermatozoa															
Focal interstitial mononuclear cell infiltrate															
EYES:															
Submucosal mononuclear cell infiltrate															
OVARIES:															
Reduced number of corpora lutea															
Focal mineralisation															
Cyst(s)															

Figures in brackets represent the number of animals from which this tissue was examined microscopically. Where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. * P<0.05, ** P<0.01, *** P<0.001. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001. 1992F0

Oral two-generation reproduction study with trehalose in rats (F0 generation)
TNO Nutrition and Food Research Institute
Study : 1992F0
Table 48 : Summary of microscopic changes

CHANGES	INCIDENCE OF LESIONS (NUMERIC)												
	MALES						FEMALES						
	Contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#	#	Contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#	#	
TREATMENT													
OVARIES:													
Increased follicular atresia							(28)	(1)			(28)	(2)	(4)
PITUITARY:													
Pars distalis cyst(s)	4			(28)	1							0	1
PREPUTIAL/CLITORAL GLANDS:													
Adenitis							(28)	(3)	(4)	(28)			
PROSTATE:													
Increased detritus	1	0	0	0									
Focal interstitial mononuclear cell infiltrate	1	1	1	1									
SEMINAL VESICLES:													
No abnormality detected	(28)	(2)	(4)	(28)									
SKIN/SUBCUTIS:													
Keratotic plug(s)	28	2	4	28									
Focal hypotrichosis	(2)	(1)	(1)	(3)								(12)	(3)
	0	0	0	0									
	1	0	1	1									

Figures in brackets represent the number of animals from which this tissue was examined microscopically. Where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001 1992F0

Oral two-generation reproduction study with Trehalose in rats (F0 generation)
TNO Nutrition and Food Research Institute
Study : 1992F0
Table 48 : Summary of microscopic changes

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC)																		
		MALES					FEMALES													
		Contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#	Contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#									
SKIN/SUBCUTIS:																				
Few atrophic hair follicles		(2)	(1)	(1)	(3)		(12)	(3)	(5)	(9)										
Small ulcer(s)		0	0	0	0		0	0	0	1										
Focal acanthosis		0	0	0	2		0	0	0	2										
SPLEEN:		(28)			(28)		(28)			(27)										
Increased extramedullary haematopoiesis		2			6		0			0										
TESTES:		(28)	(2)	(5)	(28)															
Diffuse leydig cell hyperplasia		0	0	1	0															
Seminiferous tubular atrophy		2	0	2	1															
THYMUS:		(3)	(1)	(3)	(2)		(2)		(1)	(1)										
Focal ductular structures		0	0	1	0		0		1	0										
Haemorrhage(s)		0	0	1	1		1		0	1										
UTERUS:							(28)	(2)	(4)	(27)										
Increased brown pigment accumulation							0	0	0	2										

Figures in brackets represent the number of animals from which this tissue was examined microscopically. Where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. * P<0.05, ** P<0.01, *** P<0.001. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001. 1992F0

Oral two-generation reproduction study with Trehalose in rats (F0 generation)
TNO Nutrition and Food Research Institute
Study : 1992F0
Table 48 : Summary of microscopic changes

CHANGES	INCIDENCE OF LESIONS (NUMERIC)												
	TREATMENT	MALES						FEMALES					
		Contr.	2.5% Treha lose	5 % Treha lose	10 % Treha lose	# Contr.	2.5% Treha lose	5 % Treha lose	10 % Treha lose	# Contr.	2.5% Treha lose	5 % Treha lose	10 % Treha lose
UTERUS:					# (28)	(2)	(4)	(27)					
Endometritis					# 1	0	0	0					
VAGINA:					# (28)	(2)	(4)	(27)					
Increased mucification					# 2	0	0	0					
Focal submucosal mononuclear cell infiltrate					# 0	1	0	0					
Inclusion cyst					# 0	0	1	0					

Figures in brackets represent the number of animals from which this tissue was examined microscopically. Where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001 1992F0

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1

Table 49 : Summary of macroscopic lesions

CHANGES	TREATMENT	INCIDENCE OF GROSS LESIONS (NUMERIC)									
		MALES					FEMALES				
		contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	#	2.5% Treha lose	5% Treha lose	10% Treha lose	#
HABITUS/CLINICAL SIGNS:											
Malocclusion of incisors		1	1	1				1			
Missing tail tip		1						1			
ABDOMINAL CAVITY:											
Lymph node(s) red margin(s)		1									
Lymph node(s) yellow enlarged										1	
Yellow nodule(s) parametrial adipose tissue								1			
AXILLARY LYMPH NODES:											
Uni-lateral red margin(s)			1								
COLON:											
Gaseous content									1		
Patches of peyer enlarged			1								
EPIDIDYMIDES:											
(Uni-lateral) hyalin tissue			1							1	
(Uni-lateral) small			1							1	

The absence of a numeral indicates that the change specified was not identified

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1
Table 49 : Summary of macroscopic lesions

CHANGES	INCIDENCE OF GROSS LESIONS (NUMERIC)									
	MALES					FEMALES				
	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#
EYES:										
(Uni-lateral) (red) encrustations	1	2	3				1	1		
LIVER:										
Granular aspect							1	1		
Pronounced lobular pattern							1	1		
MEDIASTINAL LYMPH NODES:										
Uni-lateral red spot(s)/discoloured							1	4		
Uni-lateral granular surface parathyroid lymphnodes									1	
(Uni-lateral) enlarged parathyroid lymphnodes		2					1	5	1	
OVARIES:										
Small								1		
PANCREAS:										
Petechiation								2		
SKIN/SUBCUTIS:										
Sparsely haired region	4	3	6	2			11	11	13	

The absence of a numeral indicates that the change specified was not identified.

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1
Table 49 : Summary of macroscopic lesions

CHANGES	INCIDENCE OF GROSS LESIONS (NUMERIC)									
	MALES					FEMALES				
TREATMENT	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#
SKIN/SUBCUTIS:										
Alopecic area		1								
Encrustations									1	
SMALL INTESTINES:										
Gaseous content	1									
Patches of peyer enlarged		4		1						
SPLEEN:										
White thickening		1								
STOMACH:										
Pale thickened mucosa						1				
Gaseous content	1									
Haemorrhagic content								1		
TESTES:										
(Uni-lateral) flabby	1	1		1						
Uni-lateral cryptorchism									1	
(Uni-lateral) small	1	1	2	1						

The absence of a numeral indicates that the change specified was not identified

Oral two-generation reproduction study with trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1
Table 49 : Summary of macroscopic lesions

CHANGES	TREATMENT	INCIDENCE OF GROSS LESIONS (NUMERIC)																			
		MALES					FEMALES														
		contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#	#	#	#	#	#	contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#	#	#	#	#	
TESTES:																					
Uni-lateral enlarged		1																			
THYMUS:																					
Red spot(s)												3	1	3							
UTERUS:																					
Pyometra																					1

The absence of a numeral indicates that the change specified was not identified

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1
Table 50 : Summary of microscopic changes

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC)													
		MALES						FEMALES							
		contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	#	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	#		
ABDOMINAL CAVITY:			(1)					(1)	(1)					(1)	
Sinusoidal blood lymph node			1					0	0					0	
Fat necrosis			0					1	1					0	
AXILLARY LYMPH NODES:			(1)												
Uni-lateral sinusoidal blood			1												
COAGULATING GLANDS:		(28)	(3)	(2)	(28)										
No abnormality detected		28	3	2	28										
COLON:			(1)		(1)										
GALT lymphoid hyperplasia			1		0										
EPIDIDYMIDES:		(28)	(3)	(4)	(28)										
Devoid of spermatozoa		1	2	1	1										
Focal interstitial mononuclear cell infiltrate		3	0	0	4										
EYES:		(1)	(2)	(3)										(1)	
Conjunctivitis		0	0	0										0	1

Figures in brackets represent the number of animals from which this tissue was examined microscopically where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001 1992F1

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1
Table 50 : Summary of microscopic changes

CHANGES	INCIDENCE OF LESIONS (NUMERIC)											
	MALES						FEMALES					
	contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#	#	contr.	2.5% Trehalose	5% Trehalose	10% Trehalose	#	#
LIVER:												
Kupffer-cell brown pigment accumulation										(1)		
Multifocal hepatocellular vacuolation										1		
Small centrilobular hepatocytes										1		
MEDIASTINAL LYMPH NODES:												
Sinusoidal blood parathymic lymphnodes		(2)					(1)			(5)		(1)
Germinal centre development parathymic lymphnodes		0					1			5		1
Lymphoid hyperplasia parathymic lymphnodes		0					0			3		0
OVARIES:												
Reduced number of corpora lutea		2					0			1		2
Focal mineralisation												
Cyst(s)												
Increased follicular atresia							(28)			(3)		(28)
							0			0		1
							1			0		2
							4			1		0
							1			0		1

Figures in brackets represent the number of animals from which this tissue was examined microscopically. Where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001 1992F1

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1

Table 50 : Summary of microscopic changes

CHANGES	INCIDENCE OF LESIONS (NUMERIC)											
	MALES						FEMALES					
	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	#	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	#
PANCREAS:												
Hyperaemia in/around endocrine areas										(2)		
										1		
PITUITARY:												
Pars distalis cyst(s)	(28)			(28)			(28)				(28)	
	2			0			0				1	
PROSTATE:												
Focal interstitial mononuclear cell infiltrate	(28)	(3)	(3)	(28)								
	1	0	0	0								
RECTUM:												
No abnormality detected				(1)								
				1								
SEMINAL VESICLES:												
No abnormality detected	(28)	(3)	(3)	(28)								
	28	3	3	28								
SKIN/SUBCUTIS:												
Keratotic plug(s)	(4)	(4)	(6)	(2)			(4)	(11)	(11)	(13)		
	0	2	1	0			0	0	0	0		
Focal hypotrichosis	0	1	0	1			1	0	1	1		
	0	0	1	0			0	0	0	1		
Small ulcer(s)												

Figures in brackets represent the number of animals from which this tissue was examined microscopically. Where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001 1992F1

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1
Table 50 : Summary of microscopic changes

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC)									
		MALES					FEMALES				
		contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	# contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#
SKIN/SUBCUTIS:		(4)	(4)	(6)	(2)		(4)	(11)	(11)	(13)	
Focal acanthosis		2	2	1	1		1	0	0	1	
SMALL INTESTINES:		(1)	(4)		(1)						
GALT lymphoid hyperplasia		0	2		1						
SPLEEN:		(28)	(1)		(28)		(28)			(28)	
Increased extramedullary haematopoiesis		3	1		5		0			0	
Increased brown pigment accumulation		0	0		0		9			7	
Multifocal necrosis		0	1		0		0			0	
STOMACH:		(1)					(1)				
No abnormality detected		1					1				
TESTES:		(28)	(4)	(5)	(28)						
Diffuse leydig cell hyperplasia		1	0	0	0						
Seminiferous tubular atrophy		1	2	0	2						
Uni-lateral sloughing of germ cells		0	0	2	1						

Figures in brackets represent the number of animals from which this tissue was examined microscopically
Where no figures in brackets are indicated the tissue was not examined microscopically
Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations
Statistical analysis was only performed on protocol organs to be examined in all animals of a group
Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001
1992F1

Oral two-generation reproduction study with Trehalose in rats (F1 generation)
TNO Nutrition and Food Research Institute
Study : 1992F1

Table 50 : Summary of microscopic changes

CHANGES	INCIDENCE OF LESIONS (NUMERIC)												
	TREATMENT	MALES						FEMALES					
		contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	#	contr.	2.5% Treha lose	5% Treha lose	10% Treha lose	#	#
TESTES:		(28)	(4)	(5)	(28)								
Multinucleate giant cell(s)		0	1	2	1								
THYMUS:							(3)	(1)	(2)	(3)			
Focal ductular structures							1	0	0	0			
Haemorrhage(s)							2	1	2	3			
UTERUS:							(28)	(3)	(3)	(28)			
Squamous metaplasia							0	0	1	0			
Luminal dilatation							6	0	0	3			
VAGINA:							(26)	(3)	(3)	(28)			
Focal submucosal mononuclear cell infiltrate							0	0	1	0			

Figures in brackets represent the number of animals from which this tissue was examined microscopically. Where no figures in brackets are indicated the tissue was not examined microscopically. Low numbers of animals between brackets (non-protocol organs) represent the microscopic verification of gross observations. Statistical analysis was only performed on protocol organs to be examined in all animals of a group. Statistics: two-sided Fisher's exact test between the control and each of the treatment groups. * P<0.05, ** P<0.01, *** P<0.001 1992F1