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Chemical Category	1,3,5-TRIGLYCIDYL ISOCYANURATE		

OFFICE OF TOXIC SUBSTANCES
CODING FORM FOR GLOBAL INDEXING

REV. 7/27/82

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Ciba Specialty Chemicals Corporation
USA

Performance
Polymers

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Ciba



July 14, 1999

MR 24475

Attn: Section 8(e) Coordinator
Document Processing Center (7407)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

pacn 88990000070

Re: TSCA Section 8(e) Notice, reference 8EHQ-99-14351

Ciba Specialty Chemicals claims no information in this letter as Confidential Business Information.

In accordance with the EPA policy statement and reporting guide on TSCA Section 8(e), Ciba Specialty Chemicals is submitting the enclosed carcinogenicity study of 1,3,5-triglycidylisocyanurate (CAS registry number 2451-62-9). A preliminary report was submitted in December 29, 1998 and assigned the number 8EHQ-99-14351. We have now received the full report and are submitting it to the agency.

The conclusions of this study remain the same as previously reported, and are attached. Please contact me if there are any questions on this matter.

Sincerely,

Dr. Jonas Weiss
Director, Product Safety

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8EHQ-99-14351

Resubmission

281 Fields Lane
Brewster, New York 10509
Tel. 914 785 3000



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Value beyond chemistry

Conclusion

The test substance, TGIC (1,3,5-triglycidyl isocyanurate) was administered by dietary admixture to male Sprague-Dawley rats at concentrations of 10, 30, 100 and 300 ppm. Marked signs of toxicity and a rapid onset of mortality were noted in animals given the test substance at the concentration of 300 ppm, and treatment was stopped in week 63 for this group, and the animals were sacrificed. For the dose-groups at 10, 30 and 100 ppm the treatment was continued up to 98 weeks to cover the life-span of these animals.

At 300 ppm, laboratory and histopathological investigations revealed mastocytosis in the lymph nodes and depletion of lymphoid cells in the spleen. At the lower dose-levels, the principal effect was a slightly lower food consumption at 100 ppm, resulting in a terminal mean group body weight which was 9% lower than the control animals.

The test substance did not show a carcinogenic potential or any effect on the incidence of spontaneously occurring tumours at any dose-level. Furthermore, the test substance did not induce a decrease in the latency of the appearance of spontaneous tumours.

The dose-level of 300 ppm (13.6 mg/kg) clearly exceeded the maximum tolerated dose (MTD). On the basis of the results generated in the course of this study, the NOAEL is considered to be 100 ppm (4.36 mg/kg) and the NOEL is considered to be 30 ppm (1.30 mg/kg).



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Performance Polymers Division
P.O. Box, R-1002.7.62
CH-4002 Basel
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SPONSOR

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Chiyoda - Ku, Tokyo
Japan 101

TEST SUBSTANCE

TGIC (1, 3, 5 - triglycidyl isocyanurate)

STUDY TITLE

CARCINOGENICITY STUDY IN MALE RATS

DATA REQUIREMENT

- . US-EPA (TSCA) Fed Reg 50, 138, September 27, 1985,
- . OECD 451, adopted May 12, 1981

AUTHOR

Catherine Fabreguettes

EXPERIMENTAL COMPLETION DATE

17 November 1997

STUDY COMPLETION DATE

4 June 1999

PERFORMING LABORATORY

CIT

Centre International de Toxicologie
B.P. 563 - Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER

11117 TCR

Page 1 of 1487

Volume 1

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CIT/Study No. 11117 TCR

STATEMENT OF CONFIDENTIALITY OR NO CONFIDENTIALITY CLAIMS

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STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- . OECD principles of Good Laboratory Practice, C(81)50(final) Annex 2. May 12, 1981,
- . US Environmental Protection Agency, Federal Register, 40 CFR Part 792; Toxic Substances Control Act; Good Laboratory Practice Standards, November 29, 1983 (and subsequent amendments).

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, B.P. 563, Miserey, 27005 Evreux, France, except the preparation of all the slides including trimming of organs, embedding in paraffin wax, sectioning, microtomy and staining, which was performed by Histotox (rue Fleming, 17000 La Rochelle, France) according to the Good Laboratory Practice Regulations specified in the protocol.

C. Fabreguettes

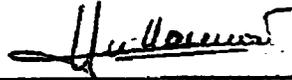
C. Fabreguettes Date: 4 June 1999
Study Director
Doctor of Pharmacy, Ph.D.
Toxicologist

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CIT/Study No. 11117 TCR

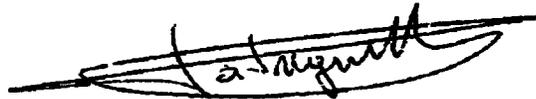
SCIENTISTS INVOLVED IN THE STUDY

Pharmacy



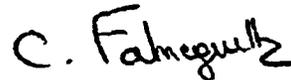
P.O. Guillaumat Date: 4 June 1999
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Head of Pharmacy

Analytical Chemistry



G. Fabreguettes Date: 4 June 1999
D.E.S.S. (Analytical Chemistry)

Toxicology



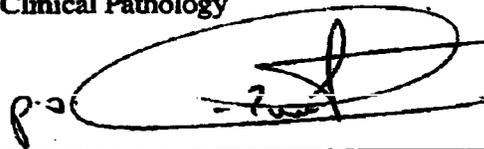
C. Fabreguettes Date: 4 June 1999
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Toxicologist

**Clinical Pathology /
Macroscopic and microscopic examinations /
CIT Management**



M. Attia Date: 4 June 1999
B.V.Msc., D.V.P., Ph.D.
**Director of Histopathology and
Clinical Pathology**

CIT Management



S. de Jouffrey Date: 4 June 1999
Doctor of Veterinary Medicine
Director of Operations

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspection	Dates		
	Inspection	Reported to Study Director (*)	Reported to Management (*)
Protocol	8 December 1995	11 December 1995	12 December 1995
Study	28 December 1995	19 January 1996	24 January 1996
Study	5 January 1996	1 February 1996	1 February 1996
Study	19 March 1996	21 March 1996	21 March 1996
Study	21 March 1996	21 March 1996	22 March 1996
Study	24 April 1996	26 April 1996	26 April 1996
Study	20 May 1996	23 May 1996	23 May 1996
Study	11 June 1996	17 June 1996	17 June 1996
Study	20 June 1996	21 June 1996	21 June 1996
Study	21 June 1996	25 June 1996	26 June 1996
Study	27 June 1996	2 July 1996	2 July 1996
Study	4 September 1996	9 September 1996	9 September 1996
Study	7 October 1996	17 November 1996	19 November 1996
Study	16 October 1996	18 October 1996	21 October 1996
Study	31 October 1996	5 November 1996	5 November 1996
Study	2 December 1996	4 December 1996	5 December 1996
Study	11 December 1996	16 December 1996	16 December 1996
Study	23 December 1996	30 December 1996	31 December 1996
Study	6 March 1997	17 March 1997	17 March 1997
Study	7 March 1997	17 March 1997	17 March 1997
Study	10 March 1997	13 March 1997	13 March 1997
Study	20 March 1997	24 March 1997	24 March 1997
Study	18 April 1997	15 May 1997	15 May 1997

STATEMENT OF QUALITY ASSURANCE UNIT (continued)

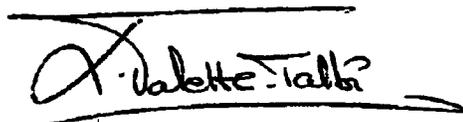
Type of inspection	Dates		
	Inspection	Reported to Study Director (*)	Reported to Management (*)
Study	18 June 1997	12 August 1998	14 August 1998
Study	19 June 1997	26 June 1997	26 June 1997
Study	19 June 1997	26 June 1997	26 June 1997
Study	20 June 1997	23 June 1997	23 June 1997
Study	29 August 1997	4 September 1997	4 September 1997
Study	16 September 1997	18 September 1997	18 September 1997
Study	13 November 1997	4 November 1997	21 November 1997
Study	26 November 1997	15 December 1997	15 December 1997
Study	11 December 1997	18 December 1997	18 December 1997
Study	20 February 1998	12 August 1998	14 August 1998
Study	18 August 1998	7 September 1998	7 September 1998
Report	1 September 1998	14 September 1998	17 September 1998

At about the same time as this study, described in the present report, process-based and routine facility inspections of critical procedures relevant to this study were made by the Quality Assurance Unit.

The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the principles of Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



L. Valette-Talbi Date: 4 June 1999
 Doctor of Biochemistry, Ph.D.
 Head of Quality Assurance Unit
 and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

La Rochelle, September 18 th 1998

QUALITY ASSURANCE ASSESSMENT

Reference: **C.I.T. study number 11117 TCR**

At the Request of the " **CENTRE INTERNATIONAL DE TOXICOLOGIE** ",

the Histological Trimming of the Study bearing the Reference Number **C.I.T. 11117 TCR** was sub-contracted : the Preparation of the Blocks and the Preparation of the Slides, including Microtomy, Staining and Checking, and the Pathology were performed by:

HISTOTOX

Rue Fleming, 17000 La Rochelle (France)

The Quality Assurance Audits realised by HISTOTOX Quality Assurance:

allow to state that this Trimming and the Pathology were performed in Accordance with the *Standard Operating Procedures* of HISTOTOX Facilities and in Compliance with *Good Laboratory Practice International Regulations*.

Nadine FERRE
Quality Assurance Officer



A 13

CIT/Study No. 11117 TCR

PAGE RESERVED FOR 40 CFR 158.34 (c) (1) CERTIFICATION

(to be completed by the Sponsor)

CIT/Study No. 11117 TCR

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SUMMARY

The objective of this study was to evaluate the carcinogenic potential of the test substance, TGIC (1, 3, 5 - triglycidyl isocyanurate) when administered by dietary admixture to male Sprague-Dawley rats. The study was restricted to male rats, because there is no evidence that females react differently to treatment with this test material.

The toxicity of TGIC was evaluated over a 26-week treatment period and the findings are presented in Volume 6.

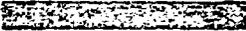
The test substance is a cross-linking agent for epoxy and polyester powder coatings.

Methods

A total of 250 male Sprague-Dawley rats were randomly allocated to four treated and one control group of 50 males. Treated males received the test substance, TGIC (1, 3, 5 - triglycidyl isocyanurate) by dietary admixture at the constant concentrations of 10, 30, 100 or 300 ppm for 63 weeks (300 ppm group) or 98/99 weeks (10, 30 and 100 ppm groups). Control males received the untreated diet (A04 C rodent maintenance diet, type 2.5, UAR) for 98/99 weeks. The treatment groups in this study as well as the accompanying satellite study, together with the duration of treatment, are summarised in the table below:

Satellite study

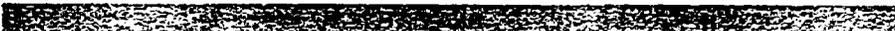
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0 ppm			
100 ppm			
300 ppm			
	↑	↑	↑
weeks	0	13	27

- . week 13: blood biochemistry (all groups)
- . week 27: haematology and blood biochemistry (all groups)

Principal study

(volumes 1-5)

0 ppm									
10 ppm									
30 ppm									
100 ppm									
300 ppm									
	↑	↑	↑	↑	↑	↑	↑	↑	↑
weeks	0	13	27	52	59/60	63	78		98/99

- . weeks 13, 26, 52 and 63: haematology (0 and 300 ppm groups)
- . week 59/60: blood pressure (0 and 300 ppm groups)
- . week 78: haematology (0 - 100 ppm)
- . week 98/99: haematology (0, 10, 30 and 100 ppm groups)

The animals were checked daily for clinical signs and mortality. After six months of treatment, the animals were palpated regularly in order to monitor palpable masses. Body weight and food consumption were recorded once and twice a week respectively during the first 13 weeks of treatment and then once a month. Achieved dosage was calculated throughout the treatment period and the efficiency of food utilisation was estimated from the food conversion ratio during the period of maximal growth of the animals. Blood pressure was recorded in 20 control animals and all surviving animals of the 300 ppm group on one occasion in weeks 59/60. The Differential White Blood Cell Count (DWBC) was investigated in weeks 13, 26, 52 and 78 in the control and highest dose-level group; haematological investigations were carried out on all surviving animals at the end of the treatment period. DWBC was also evaluated in all moribund animals.

Any animal found dead or killed prematurely during the treatment period was subjected to a full macroscopic *post-mortem* examination and a full list of tissues was preserved for microscopic examination. Due to the high rate of mortality noted in the 300 ppm group (44% in week 62) and the rapid clinical degradation of these animals, the treatment of this group was stopped in week 63 and the animals killed.

The study was scheduled for at least 104 weeks. At the request of the Sponsor's Monitoring Scientist, since a survival rate of 40% was observed in the low dose-level group in week 98, the study was terminated in weeks 98/99.

At the end of the treatment period, all surviving animals were killed and submitted to a detailed macroscopic *post-mortem* examination. Tissue specimens and any masses or lesions were preserved. A microscopic examination was performed on all macroscopic lesions, masses and almost all tissues in control, 100 and 300 ppm groups and on all macroscopic lesions, masses, kidneys, liver and lungs in the 10 and 30 ppm groups.

Results

Clinical signs

Signs of poor clinical condition (round back, piloerection and emaciation associated in some animals with swollen and/or hard abdomen, coldness to the touch, hypokinesia, dyspnea, pallor of eyes and body extremities, chromodacryorrhea) were noted in the 300 ppm group and attributed to treatment with the test substance.

All the other clinical signs noted in treated groups were similar to those of control group.

Palpable masses

The incidence, time of onset, multiplicity, location and size of palpable masses were similar in all treated and control groups and consistent with those commonly recorded in rats of this strain and age.

Morbidity and mortality

At 300 ppm, there was a rapid onset of mortality and low terminal survival rate. The cause of death was possibly a histamine-related hypotension.

At 10, 30 and 100 ppm, the onset of mortality, the nature and frequency of the factors contributing to death or premature killing were similar to those in the controls and similar to those commonly recorded in rats of this strain and age and did not show any indication of dose- or treatment-relationship.

Food consumption

Slight to markedly lower food consumption was recorded in males given 100 or 300 ppm.

CIT/Study No. 11117 TCR

Body weight

At 100 ppm, a slightly lower terminal body weight was noted (-9%).

At 300 ppm, a body weight loss or a lower body weight gain was recorded during the treatment period with a marked degradation over the last nine months of dosing.

Achieved dosages

The achieved dosages based on the nominal concentrations of 10, 30, 100 and 300 ppm were as follows:

- . 0.43 mg/kg/day (0.85 mg/kg/day to 0.32 mg/kg/day) for the 10 ppm concentration,
- . 1.30 mg/kg/day (2.65 mg/kg/day to 1.01 mg/kg/day) for the 30 ppm concentration,
- . 4.36 mg/kg/day (8.47 mg/kg/day to 3.41 mg/kg/day) for the 100 ppm concentration,
- . 13.6 mg/kg/day (20.2 mg/kg/day to 12.4 mg/kg/day) for the 300 ppm concentration

Efficiency of food utilisation

The efficiency of food utilisation was markedly lower in the 300 ppm group than in the control or other treated groups during the first four weeks of treatment.

Blood pressure

There was no difference in blood pressure measurements.

Haematology

- . 300 ppm group (weeks 13, 26, 52 and 63)

A higher neutrophil percentage and a lower lymphocyte percentage were noted in week 52.

In week 63, at the time of final sacrifice, a slightly lower total leucocyte count, mainly attributable to a lower lymphocyte count, was noted.

No biologically relevant abnormalities were noted in animals killed prematurely.

- . 10, 30 and 100 ppm groups (weeks 78 and 98/99 and animals killed prematurely)

No treatment-related differences from control animals were noted.

Macroscopic examination

Non-neoplastic findings

The incidence and nature of the non-neoplastic changes observed in all organs were similar in control and treated animals and showed no indication of treatment or dose-relationship. These changes were typical for rats of this strain and age.

Neoplastic findings

The masses found in some organs and tissues were equally distributed between control and treated animals and showed no influence of treatment, either in size or multiplicity.

Microscopic examination

Non-neoplastic findings

In the 300 ppm group, the following findings were attributed to treatment with the test substance.

- . high incidence of mastocytosis, haemosiderosis and sinusal haemorrhage in the mesenteric lymph nodes,
- . high incidence of lymphoid depletion in the spleen,
- . moderate to marked dilatation of some intestinal segments,
- . hyopsecretion together with small tubulo-alveolar units in the prostate.

In the other treated groups, the incidence, severity and morphological characteristics of the non-neoplastic microscopic findings showed no indication of treatment or dose-relationship and were similar to spontaneous lesions described for rats of this strain and age.

Neoplastic findings

The incidence and morphological types of neoplastic lesions were similar in control and treated animals and showed no indication of treatment or dose-relationship.

The test substance showed neither a carcinogenic potential, nor an effect on the incidence of spontaneously occurring tumours at 300 ppm (up to week 63) or 100 ppm, 30 and 10 ppm (up to week 98/99). Moreover, the test substance did not induce a decrease in the latency of tumour appearance.

Conclusion

The test substance, TGIC (1, 3, 5 - triglycidyl isocyanurate), was administered by dietary admixture to male Sprague-Dawley rats at concentrations of 10, 30, 100 and 300 ppm. Marked signs of toxicity and a rapid onset of mortality were noted in animals given the test substance at the concentration of 300 ppm, and treatment was stopped in week 63 for this group, and the animals were sacrificed. For the dose-groups at 10, 30 and 100 ppm the treatment was continued up to 98 weeks to cover the life-span of these animals.

At 300 ppm, laboratory and histopathological investigations revealed mastocytosis in the lymph nodes and depletion of lymphoid cells in the spleen. At the lower dose-levels, the principal effect was a slightly lower food consumption at 100 ppm, resulting in a terminal mean group body weight which was 9% lower than the control animals.

The test substance did not show a carcinogenic potential or any effect on the incidence of spontaneously occurring tumours at any dose-level. Furthermore, the test substance did not induce a decrease in the latency of the appearance of spontaneous tumours.

The dose-level of 300 ppm (13.6 mg/kg/day) clearly exceeded the maximum tolerated dose (MTD). On the basis of the results generated in the course of this study, the NOAEL is considered to be 100 ppm (4.36 mg/kg/day) and the NOEL is considered to be 30 ppm (1.30 mg/kg/day).

1. INTRODUCTION

This study was performed at the request of Ciba Specialty Chemical Inc., Basel, Switzerland and Nissan Chemical Industries, Tokyo, Japan.

The aim of this study was to evaluate the carcinogenic potential of TGIC on male rats when administered by dietary exposure. The study was restricted to male rats, because there is no evidence that females react differently to treatment with this test material.

The toxicity of TGIC was evaluated over a 26-week treatment period and the findings are presented in Volume 6.

The test substance is a cross-linking agent for epoxy and polyester powder coatings.

The rat was chosen because it is a rodent species generally accepted by regulatory authorities and the Sprague-Dawley strain was selected because background data from previous studies is available at our laboratory.

The concentrations (in the dietary admixture) to be used in this study were selected on the basis of a 13-week toxicity study in male rats (*CIT/Study No. 11099 TCR*), performed using the dose-levels of 10, 30 and 100 ppm.

The oral route was selected since it is a possible route of exposure in man.

This study was designed to comply with:

- . US-EPA (TSCA) Fed Reg 50, 188, September 27, 1985,
- . OECD 451, adopted May 12, 1981.

2. MATERIALS AND METHODS

2.1. TEST AND CONTROL SUBSTANCES

2.1.1 Test substance

2.1.1.1 Identification

The test substance, TGIC (1, 3, 5 - triglycidyl isocyanurate), used in the study was supplied by Ciba Specialty Chemicals Inc.

Documentation supplied by the Sponsor identified the test substance as follows:

- . denomination:
 - protocol: TGIC (1, 3, 5 - triglycidyl isocyanurate)
 - labelling: ARALDITE® PT 810 PASTILLEN
- . batch number: 407923.48
- . description: white disks
- . quantity and container: two cardboard boxes each containing 25 kg of test substance in plastic bags
- . date of receipt: 11 May 1994
- . storage conditions: at +4 °C away from light and humidity (since 26.12.95).

At the request of the Sponsor's Monitoring Scientist, the analytical certificate is only filed in the raw data of the study.

2.1.1.2 Diet

The diet was A04C P 2.5 diet, supplied by U.A.R. (Villemoisson-sur-Orge, France). The batches and dates of utilisation are presented in Appendix 2.

2.1.2 Formulation procedure

The test substance was given by dietary admixture in A04C P 2.5 powdered diet.

The test substance was mixed with a small quantity of diet using a Braun mixer for 5 minutes; this premix was then transferred to a Lödige FM 50 mixer (A.T.R., Paris, France) with the required total quantity of diet in order to achieve the appropriate concentration of 640 ppm and then mixed for 15 minutes.

The appropriate quantity of premix at 640 ppm for each group was transferred into a Lödige FM 50 mixer (A.T.R., Paris, France) with the total required quantity of diet in order to achieve the appropriate concentration of 10 ppm, 30 ppm, 100 ppm and 300 ppm and then mixed for 15 minutes.

The 640 ppm premix was not given to the animals.

The formulations were made for one week of treatment and stored at +4 °C, in two closed plastic bags (the second placed in a bag containing silica gel).

2.1.3 Chemical analysis of dietary mixtures

The homogeneity and stability of dietary mixtures containing 10 ppm and 640 ppm of test substance was determined in a previous study (*CIT/Study No. 12289 TSR*) for dietary mixtures prepared under the same conditions.

During the treatment period, a check of the concentration was performed on dietary mixtures prepared for use in the study.

2.1.3.1 Homogeneity

For *CIT/Study No. 12289 TSR*, two dietary mixtures were made, under conditions representative of those of the study:

- . a dietary mixture at the lowest concentration i.e. 10 ppm,
- . a dietary mixture at the highest concentration i.e. 640 ppm.

The results of these analyses are presented in the study report but the corresponding raw data are filed with *CIT/Study No. 12289 TSR*.

During the present study (*CIT/Study No. 11117 TCR*), additional checks of homogeneity were performed in week 60 (all dietary mixtures prepared for treatment and pre-mixture), due to the variability observed for diet formulations analysed during the study.

From each dietary mixture, samples were taken at three different levels (top, middle, bottom) and analysed in duplicate (or more to improve the coefficient of variation) for homogeneity.

2.1.3.2 Stability

For *CIT/Study No. 12289 TSR*, the two dietary mixtures prepared for homogeneity analysis were stored as follows:

- . in closed bags and sampled after 0 and 4 days storage at +4 °C,
- . in open feeders and sampled after 0, 3 and 5 days storage at room temperature,
- . in closed bags for 4 days at +4 °C and then in open feeders and sampled after 0, 3 and 5 days storage at room temperature, to evaluate the highest risk of degradation.

The results of these analyses are presented in the study report but the corresponding raw data are filed with *CIT/Study No. 12289 TSR*.

During the present study (*CIT/Study No. 11117 TCR*), the stability of the test substance in dietary mixtures was checked again in week 60 to confirm the results obtained previously. All dietary mixtures prepared for treatment of the animals and analysed for homogeneity were stored and sampled as follows:

- . in closed bags and sampled after 0 and 4 days storage at +4 °C,
- . in open feeders and sampled after 0, 3 and 5 days storage at room temperature,
- . in closed bags for 4 days at +4 °C and then in open feeders and sampled after 3 and 5 days storage at room temperature to evaluate the cumulated storage.

Stability of the premix was checked at times 0 and 4h after preparation in weeks 18 and 60.

Each analytical sample was analysed in duplicate (or more to improve the coefficient of variation) as soon as possible after sampling.

In each case the mean results of the homogeneity analyses were taken as the initial values for the stability test.

2.1.3.3 Achieved concentration

The concentration of samples taken from each dietary mixture (including control along the study and premix in week 16 then from week 24) prepared for use in weeks 1, 4 and then every four weeks until the end of the study was determined. In addition, the dietary mixtures prepared for use in week 65 were analysed because of the unexpected values measured in week 64.

Furthermore, from week 76, the concentration of samples taken from open feeders at the end of period of distribution to the animals (each treated group) was determined.

The analytical procedure and results are presented in Appendix 1.

2.2. TEST SYSTEM

2.2.1 Animals

A total of 300 male Sprague-Dawley rats of the CrI CD[®] (SD) BR strain, *Caesarian Obtained, Barrier Sustained-Virus Antibody Free (COBS-VAF[®])* were supplied by Charles River France (Saint-Aubin-lès-Elbeuf, France) and received at CIT on 7 December 1995.

On arrival, the animals were given a complete examination to ensure that they were in good clinical condition.

A 22-day acclimatisation period to the conditions of the study preceded the beginning of the treatment period. A larger number of animals than necessary was acclimatised in case additional animals were needed to replace any animal showing signs of poor clinical condition.

The required number of animals (280 males) was selected according to body weight and clinical examination and allocated, by sex to the groups, according to a computerised randomization procedure. Subsequently, each group was assigned to a different dose-level. The remaining spare animals were progressively removed from the study room.

Each animal was identified by an individual ear tattoo and received an unique study number.

On the first day of treatment, the animals were approximately eight weeks old and had a mean body weight of 319 g (range: 241 g to 364 g) for the principal animals and 319 g (range: 286 g to 344 g) for the satellite animals.

2.2.2 Environmental conditions

From the moment of their arrival at CIT and throughout the study, the animals were housed in a barred rodent unit.

The environmental conditions in the animal room were set as follows:

- . temperature : 21 ± 2 °C,
- . relative humidity : 50 ± 20 %,
- . light/dark cycle : 12h/12h (07:00 - 19:00),
- . ventilation : approximately 12 cycles/hour of filtered, non-recycled air.

The temperature and relative humidity were under continuous control and recording and the ventilation was checked every two months. The records were checked daily and filed. In addition to these daily checks, the housing conditions and corresponding instrumentation and equipment are verified and calibrated at regular intervals.

The animal room was disinfected before the arrival of the animals and then cleaned regularly. Microbiological analyses of the air and the surfaces of the walls and floor of the animal room are performed regularly by an external laboratory and the results are archived at CIT.

The animals were kept in suspended wire-mesh cages (43.0 x 21.5 x 18.0 cm) containing two animals of the same group during the treatment period.

A metallic tray, containing autoclaved sawdust for the collection of urine and faeces (SICSA, Alfortville, France), was placed under each cage. Sawdust was changed once a week and trays, cages and racks once every four weeks.

Bacteriological and chemical analyses of the sawdust, including the detection of possible contaminants (pesticides, heavy metals) are performed periodically by external laboratories and the results are archived at CIT.

In order to avoid a bias in the study, the cages were placed in numerical order, vertically on the racks. Every fortnight, all the racks were moved clockwise around the room, rack by rack. In this way, each group had identical exposure to environmental conditions.

2.2.3 Food and water

The animals had free access to A04 C pelleted maintenance diet (type P2.5), batch Nos. 50727, 51025, 51221, 60129, 60603, 60304, 60322, 60613, 60717, 60918, 61122, 70108, 70410 and 70520 (U.A.R., Villetta-sur-Orge, France) distributed weekly during the acclimatisation period. During the treatment period, it was distributed twice a week according to the stability of the test substance in the diet. During periods of fasting, food, but not water, was removed. The diet formula is presented in Appendix 2. The batch of food was analysed by the supplier for composition and contaminant levels. The results of these analyses are archived at CIT.

The animals had free access to bottles containing tap water, filtered using a 0.22 µm filter.

Bottles were changed once a week, feeders twice a week.

Bacteriological and chemical analyses of diet and water, including the detection of possible contaminants (pesticides, heavy metals and nitrosamines) are performed regularly by external laboratories and the results are archived at CIT.

No contaminants were known to have been present in the diet or drinking water or sawdust at levels which may be expected to have interfered with or prejudiced the outcome of the study.

2.3. TREATMENT

2.3.1 Treatment-groups

Rationale for dose-level selection

The concentrations (in the dietary admixture) to be used in this study were selected on the basis of a 13-week toxicity study in male rats (CIT/Study No. 11099 TCR), performed using the dose-levels of 10, 30 and 100 ppm.

In the 13-week study no toxicologically relevant clinical or pathological findings were observed at 10 and 30 ppm. At 100 ppm, the principal observations were lower body weight gain over the first six weeks of dosing and lower leucocyte and lymphocyte counts in 2/10 males. A slight but dose-related decrease in the mean number of spermatozoa was observed over the range of concentrations used, but no effect was observed on male fertility or embryonic development and pup development after mating with untreated females.

On the basis of these findings, the same treatment-levels were selected for the present study. In addition, since these findings did not permit the establishment of an MTD level, a further treatment group was added at 300 ppm, to ensure that the range of treatment-levels covered the MTD value. The intention was to ensure that three dose groups would be available for evaluation at the end of the study.

After 26 weeks of treatment a decision was taken in consultation with the Sponsor's Monitoring Scientist, to continue the rest of the study on the the four dose groups (10, 30, 100 and 300 ppm).

Satellite groups at 0, 100 and 300 ppm were used to assess the toxicity of TGIC after six months (satellite study).

The groups, dose-levels and animal numbers are detailed in the following table:

Group	Treatment	Animals per group	Nominal concentrations (ppm*)	Animal numbers
1	Principal	50 males	0	N20671 to N20720
	Satellite	10 males		N20721 to N20730
2	Principal	50 males	10	N20731 to N20780
3	Principal	50 males	30	N20781 to N20830
4	Principal	50 males	100	N20831 to N20880
	Satellite	10 males		N20881 to N20890
5	Principal	50 males	300	N20891 to N20940
	Satellite	10 males		N20941 to N20950

* ppm: part per million or, milligrams per kilogram of diet

2.3.2 Administration

The test substance mixed with the diet was supplied *ad libitum* for a period of 63 weeks (300 ppm principal group) 98/99 weeks (10, 30 and 100 ppm principal groups) and 26 weeks (all satellite groups).

The concentration of test substance in the diet was constant throughout the study. Control animals were received the untreated diet *ad libitum*.

2.3.3 Serum samples

Due to lack of method for measuring TGIC in serum, all blood sampling intended for this purpose was cancelled.

2.3.4 Duration and criteria for acceptance of the study

The study was scheduled for at least 104 weeks.

Following the high rate of mortality noted in animals of the high dose-level group (44% on 3 March 1997 - week 62), in agreement with the Sponsor's Monitoring Scientist, the treatment of this group was stopped on 9 March 1997 (week 63) and the animals killed. In accordance with the decision of the Sponsor to terminate the study when the survival rate of any group was close to 40% (obtained in the low dose-level group on 12 November 1997 - week 98), the final necropsy was scheduled from the 13th of November 1997 (week 98).

2.4. CLINICAL EXAMINATIONS**2.4.1 Clinical signs**

Each animal was observed at least once a day, at approximately the same time, for the recording of clinical signs.

2.4.2 Palpation of masses (principal groups)

After six months of treatment period, all animals were palpated every two weeks in order to record the date of appearance and any change of palpable masses.

2.4.3 Morbidity and mortality

Each animal was checked at least twice a day (once a day during weekends and public holidays), for mortality or signs of morbidity.

The animals showing signs of poor clinical condition, especially if death appeared imminent, were killed after a blood smear.

A macroscopic *post-mortem* examination was performed on the animals found dead and those killed due to poor clinical condition during the study and the required tissues were preserved for a microscopic examination (see § 2.6. Pathology).

2.4.4 Food consumption

The quantity of food consumed by the animals of each cage was recorded twice a week, over a three or four-day period during the first 13 weeks of treatment and then twice a week every four weeks until the end of the treatment period.

Food intake per cage and per day was calculated by subtracting the amount of food left in each food-hopper, from that given.

When one of the two animals from the same cage died, the number of days for which that animal was present in the cage was taken into consideration for the calculation of the food consumption.

2.4.5 Body weight

The body weight of each animal was recorded once before allocation of the animals to groups, on the first day of treatment, once a week during the first 13 weeks of treatment and then once every four weeks until the end of the study.

2.4.6 Achieved dosages

Achieved intake of test substance (in terms of mg/kg/day) was calculated on a weekly basis for each treated group during the first 13 weeks of treatment and then once every four weeks until the end of the study as follows:

$$D = C \times \frac{FC}{BW}$$

D = achieved dosage (mg/kg/day)

C = nominal concentration (ppm)

FC = mean food consumption (g/animal/day)

BW = mean body weight (g)

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2.4.7 Efficiency of food utilisation

The food conversion ratio was calculated on a weekly basis for each group during the maximum growth period (first 13 weeks of treatment), as follows:

$$\text{FCR} = \frac{\text{BWG}}{\text{FC}} \times 100$$

FCR = food conversion ratio
FC = mean food consumption (g/animal/week)
BWG = mean body weight gain (g/animal/week)

2.4.8 Blood pressure

In order to investigate the high death rate of animals without relevant clinical or necropsy findings in the male 300 ppm group (highest dose-level), at the request of the Sponsor's Monitoring Scientist, blood pressure was measured in 20 control males and in 30 surviving males of the high-dose group.

Systolic blood pressure was determined using an indirect blood pressure system physiograph Letica (Letica, 08907 Hospitalet, Barcelona, Spain) based on sphygmomanometric measure, the pneumatic cuff being placed around the tail of the animal.

This was performed at one occasion in week 59/60 (on 11,12, 13 or 14 February 1997).

2.5. LABORATORY INVESTIGATIONS

2.5.1 Blood collection

A blood smear was taken from the caudal vein for all moribund animals killed in extremis (except for animal N20851, 4M group, which was sampled at the scheduled time week 73) for the first ten surviving unfasted animals of each principal group during weeks 13 and 26, and in all unfasted surviving animals of each principal group during weeks 52 and 78. The Differential White Cell Count (Apparatus/Method: Microscopic/May Grunwald staining, Unit: %) was evaluated in control and 300 ppm groups (except during week 78, evaluated in control and 100 ppm groups).

Blood samples were taken from the orbital sinus of surviving satellite and principal animals under light ether anaesthesia and collected into tubes containing the appropriate anticoagulant (see below).

Prior to blood samples, the animals were deprived of food for an overnight period of at least 14 hours.

2.5.2 Haematology

The following parameters were determined for the following animals:

- . all surviving animals of the satellite groups (control, 100 and 300 ppm groups) during week 27,
- . in the first ten principal animals of the control group and all surviving principal animals of principal 300 ppm group, in week 63,
- . in all surviving animals of the principal groups in week 98/99 (control, 10, 30 and 100 ppm groups).

Parameter	Apparatus/Method	Units
<u>EDTA tubes</u>		
Erythrocytes (RBC)	Bayer Diagnostics H1 (1) Haematology Analyzer/laser	T/l
Haemoglobin (HB)	Bayer Diagnostics H1 Haematology Analyzer/Drabkin	g/dl
Mean Cell Volume (MCV)	Bayer Diagnostics H1 Haematology Analyzer/laser	fl
Packed Cell Volume (PCV)	Bayer Diagnostics H1 Haematology Analyzer/calculated	l/l
Mean Cell Haemoglobin Concentration (MCHC)	Bayer Diagnostics H1 Haematology Analyzer/calculated/laser	g/dl
Mean Cell Haemoglobin (MCH)	Bayer Diagnostics H1 Haematology Analyzer/calculated	pg
Thrombocytes (PLAT)	Bayer Diagnostics H1 Haematology Analyzer/laser	G/l
Leucocytes (WBC)	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry	G/l
Differential White Cell count with cell morphology	Bayer Diagnostics H1 Haematology Analyzer/ peroxidase cytochemistry/laser morphometry (a)	
. neutrophils (N)		% and G/l
. eosinophils (E)		% and G/l
. basophils (B)		% and G/l
. lymphocytes (L)		% and G/l
. monocytes (M)		% and G/l

(a) Blood smears were prepared from all sampled animals. If the samples were not accepted by the H1 Analyser, a microscopic evaluation was performed after May Grünwald Giemsa staining (2).

(1) Bayer Diagnostics (92807 Puteaux, France)

(2) Merck Clévenot (77500 Chelles, France)

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2.5.3 Blood biochemistry

The following parameters were determined for all surviving animals of the satellite groups (control, 100 and 300 ppm groups) during weeks 13 and 27.

Parameter	Apparatus/Method	Unit
<u>Lithium heparinate tubes</u>		
Sodium (Na ⁺)	Hitachi 717 Selective electrode (Boehringer) (1)	mmol/l
Potassium (K ⁺)	Hitachi 717 Selective electrode (Boehringer)	mmol/l
Chloride (Cl ⁻)	Hitachi 717 Selective electrode (Boehringer)	mmol/l
Calcium (Ca ⁺⁺)	Hitachi 717 Ortho-cresolphthalein (Boehringer)	mmol/l
Inorganic phosphorus (I.PHOS)	Hitachi 717 Phosphomolybdic reaction (Boehringer)	mmol/l
Glucose (GLUC)	Hitachi 717 GOD-PAP (Boehringer)	mmol/l
Urea (UREA)	Hitachi 717 Urease UV (Boehringer)	mmol/l
Creatinine (CREAT)	Hitachi 717 Jaffé without deproteinisation (Boehringer)	µmol/l
Total Bilirubin (TOT.BIL)	Hitachi 717 Jendrassik (Boehringer)	µmol/l
Total Proteins (PROT)	Hitachi 717 Biuret (Boehringer)	g/l
Albumin (ALB)	Hitachi 717 Bromocresol green (Boehringer)	g/l
Albumin/globulin ratio (A/G)	Hitachi 717 Calculated	
Cholesterol (CHOL)	Hitachi 717 CHOD-PAP (Boehringer)	mmol/l
Triglycerides (TRIG)	Hitachi 717 GPO-PAP (Boehringer)	mmol/l

(1) Boehringer (38242 Meylan, France)

Parameter	Apparatus/Method	Unit
Alkaline phosphatase (ALP)	Hitachi 717 DGKC Standard/30 °C (Boehringer)	IU/l
Aspartate aminotransferase (ASAT)	Hitachi 717 IFCC Standard/30 °C (Boehringer)	IU/l
Alanine aminotransferase (ALAT)	Hitachi 717 IFCC Standard/30 °C (Boehringer)	IU/l

2.6. PATHOLOGY

2.6.1 Sacrifice (principal and satellite groups)

On completion of the treatment period, after at least 14 hours fasting, all surviving animals were asphyxiated using carbon dioxide and killed by exsanguination. Any moribund animal found during the study was killed in the same way.

2.6.2 Organ weights (satellite groups, see volume 6)

The body weight of all animals killed after a 26-week treatment period was recorded before necropsy and the following organs were weighed wet as soon as possible after dissection:

adrenals	mesenteric lymph nodes	testes
brain	mandibular lymph nodes	thymus
heart	prostate	
kidneys	seminal vesicles	
liver	spleen	

Paired organs were weighed separately except for seminal vesicles and mandibular lymph nodes which were weighed together.

2.6.3 Macroscopic *post-mortem* examination (principal and satellite groups)

A complete macroscopic *post-mortem* examination was performed on all animals including any that died during the study or were killed prematurely. All gross observations were recorded individually.

2.6.4 Preservation of tissues (principal and satellite groups)

All the macroscopic lesions and the following tissues from all animals, including any that died during the study or were killed prematurely, were preserved in 10% buffered formalin (except for the eyes and Harderian glands which were fixed in Davidson fixative for the animals killed in week 27 or at the end of the treatment period) and embedded in paraffin wax.

The testes and epididymides from the animals of the satellite group killed after six months, and from all animals of the principal groups found dead or killed prematurely during the study were fixed in 10% buffered formalin as all other tissues.

The testes and epididymides from the principal animals of the high dose-level group killed on week 63 and from the animals of the principal groups killed on completion of the study were fixed in Bouin's fluid.

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The following organs/tissues were preserved in fixative:

adrenals	liver	skin
aorta*	lungs with bronchi	spinal cord
brain including medulla/ pons, cerebellar and cerebral cortex	lymph nodes (mandibular and mesenteric)	(cervical, thoracic and lumbar)
caecum	oesophagus	spleen
colon	pancreas	sternum with bone marrow
duodenum	pituitary gland	stomach and forestomach
eyes with Harderian glands	prostate	testes and epididymides
femoral bone with articulation	rectum	thymus
heart	salivary glands (sublingual and submaxillary)	thyroids with parathyroids
ileum	sciatic nerve	tongue*
jejunum	seminal vesicle	trachea
kidneys	skeletal muscle	urinary bladder

2.6.5 Microscopic examination (principal and satellite groups)

All tissues required for microscopic examination were sectioned at approximately four microns in thickness and stained with hematoxylin-eosin.

The preparation of all the slides including trimming of organs, embedding in paraffin wax, sectioning, microtomy and staining, was performed by Histotox (rue Fleming, 17000 La Rochelle, France) according to the Good Laboratory Practice Regulations specified in the protocol.

This subcontracted work was under the responsibility of CIT.

Microscopic examination was performed on tissues listed above (except those marked by *), macroscopic lesions and palpable masses:

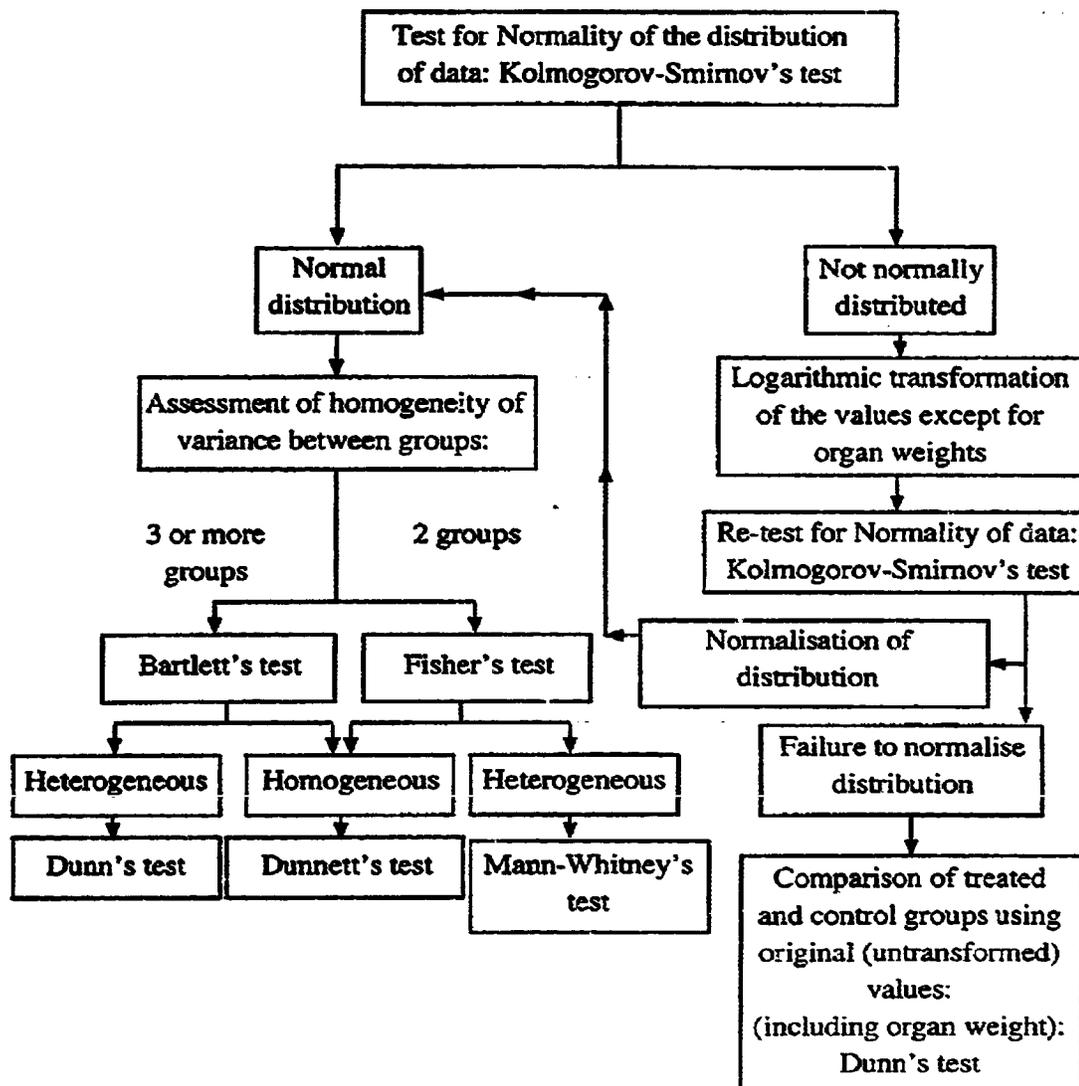
- from animals of the control and high dose-level groups killed after a 26-week treatment period (groups 1 and 5: 0 and 300 ppm),
- from animals of the high dose-level group killed on week 63 (group 5: 300 ppm),
- from animals of the control and high dose-level groups killed at the end of the treatment period (week 98/99, groups 1 and 4: 0 and 100 ppm),
- from any animals that died or were killed prematurely.

Microscopic examination was performed on lungs, liver, kidneys, macroscopic lesions and palpable masses:

- from all animals of the intermediate dose-level group killed after a 26-week treatment period (group 4: 100 ppm),
- from all animals of the low and intermediate dose-level groups killed at the end of the treatment period (week 98/99, groups 2 and 3: 10 and 30 ppm).

2.7. STATISTICAL ANALYSIS

The following sequence was used for the statistical analysis of body weight, food consumption, haematology, blood biochemistry and organ weight data:



The statistical analysis of any neoplastic lesions was performed according to the I.A.R.C. recommendation (Peto and al., 1980).

2.8. ARCHIVING

The study documentation and specimens generated during the course of the study are archived at CIT (27005 Miserey, Evreux, France) for ten years after the end of the *in vivo* phase of the study.

The archived study materials include:

- . protocol and amendments,
- . raw data,
- . correspondence,
- . final report and possible amendments,
- . blood pressure recordings,
- . tissues in preservative, blocks and histological slides,
- . haematological slides,
- . samples of the test substance and of each batch of untreated diet.

On completion of this period, the archived study materials will be returned to the Sponsor, or may be archived at CIT for a further period.

2.9. CHRONOLOGY OF THE STUDY

The chronology of the study is summarised as follows:

Procedures	Study groups	Dates	Study days
Protocol approved by:			
. Study Director		11.12.95	
. Study Monitor		27.12.95	
Arrival of the animals			
. Preidentification and weighing		7.12.95	-22
. Randomisation		13.12.95	-16
. Identification		14.12.95	-15
		18, 21 and 22.12.95	-11, -8 and -7
Carcinogenicity study (Principal groups)			
First day of treatment		29.12.95	1
Week 13			
. Haematology	0-300 ppm	28.3.96	91
Week 26			
. Haematology	0-300 ppm	27.6.96	182
Week 52			
. Haematology	0-300 ppm	26.12.96	364
Week 59/60			
. Blood pressure	0-300 ppm	11 to 14.2.97	411 to 414
Week 63			
. Haematology	0-300 ppm	7.3.97	435
Final sacrifice	300 ppm	10.3.97	438
Week 78			
. Haematology	0 and 100 ppm	26.6.97	546
Week 98/99			
. Haematology	0, 10, 30 and 100 ppm	13, 14 and 17.11.97	686, 687 and 690
Final sacrifice	0, 10, 30 and 100 ppm	13, 14 and 17.11.97	686, 687 and 690
Satellite study (Satellite groups)			
First day of treatment		29.12.95	1
Week 13			
. Blood biochemistry	0, 100 and 300 ppm	28.3.96	91
Week 27			
. Haematology and blood biochemistry	0, 100 and 300 ppm	28.6.96	183
Final sacrifice	0, 100 and 300 ppm	28.6.96	183

2.10. PROTOCOL ADHERENCE

The study was performed in accordance with Study Protocol No. 11117 TCR and subsequent amendments, with the following deviations from the agreed Study Protocol:

- . on some occasions, spillage was noted in the 300 ppm group,
- . in week 59/60 blood pressure was measured in 20 control males and in all surviving males of the high dose level group except for the male N20929,
- . for male N20851 (4M group) killed in extremis on 27 June 1997, no blood smear was performed just before sacrifice according to the sampling performed on 26 June 1997 with all other surviving principal animal (week 78),
- . the blood samples taken from satellite animals were performed in week 27 instead of week 26,
- . minor variations were noted on some occasions in the temperature and hygrometry records,
- . stability of the premix was checked at times 0 and +4h after preparation in week 18 (analysis not scheduled in the study protocol) and checked again in week 60 (times 0 and 4h after preparation), not at the same time that the dietary mixtures (as indicated in amendment No. 3),
- . on some occasions during the study (check of homogeneity, stability or concentration), the mean concentration was calculated from two to five analyses and not by analysis in duplicate,
- . the results of unscheduled check of concentration performed in week 2 on all groups were not presented in the study report,
- . as the analysis of concentration was not undertaken on dietary mixture (group 2) remaining in the open feeders in week 96, a supplementary check was performed in week 98 for the 10 ppm dietary mixture which was analysed after preparation and after treatment of the animals (open feeders),
- . due to an error in fixative (water instead of 10% buffered formalin) the tissues of animal N20819 (principal group 3) were not preserved and no microscopic examination were performed (only the eyes and Harderian gland and testes with epididimides, fixed respectively in Davidson fixative or Bouin's fluid, were preserved for this animal but not examined).

These minor deviations were not considered to have compromised the validity or integrity of the study.

3. RESULTS

3.1. CHEMICAL ANALYSIS OF THE PREPARATIONS (Appendix 1)

3.1.1 Homogeneity

The results of analyses performed in *CIT/Study No. 12289 TSR* revealed a poor homogeneity and a deviation between the nominal and the measured concentrations of the test material (-16%) for the low-dose dietary mixture (10 ppm). This can be explained by the very low concentration studied. For the high dose-level, the homogeneity was satisfactory but the mean concentration measured was higher than the nominal concentration (+16%).

The additional results obtained in week 60 of the study confirmed the poor homogeneity for the low-dose preparation (10 ppm) and demonstrated that the dietary mixture was homogeneous at the intermediate and high concentrations. The measured concentrations of the test material in the diet varied approximately from -9% to -22% of the nominal concentrations.

3.1.2 Stability

The results of analyses performed in *CIT/Study No. 12289 TSR* revealed an acceptable stability of investigated dietary mixtures (10 and 640 ppm) since the loss in the concentration measured was approximately -10%, under the storage conditions previously described.

In week 60 of the study, the loss in concentration was found in the same range (-11 to -15%) for dietary mixtures containing 30 ppm to 300 ppm of test substance. For the 10 ppm dietary mixture, the loss measured was higher (approximately -20% except for the last time point -38%) but the individual values presented a wide variability.

Concerning the premix, no significant decrease in concentration was demonstrated over the period of use (4 hours).

3.1.3 Achieved concentration

During the study, the test substance concentrations in administered dietary mixtures were found generally lower than expected with most of the deviations observed within a range of $\pm 20\%$. However, from individual analytical results obtained during the study (one result every four weeks), a mean concentration of TGIC in administered dietary mixture was evaluated for each treated group and the following deviations were calculated:

Nominal concentration (ppm)	Mean concentration calculated (ppm)	CV%	Deviation (%) from nominal
10	9.2	17	-8
30	25.2	17	-16
100	86.2	18	-14
300	262	15	-13

The mean exposure levels could be estimated to be in a range from -10% to -20% from nominal levels; trials undertaken to improve the formulation procedure and the analytical method reduced but did not eliminate the loss of concentration.

3.2. CLINICAL EXAMINATIONS

3.2.1 Clinical signs (Table 1, Appendix 3)

In the 300 ppm group for which a terminal sacrifice was performed on week 63, the principal clinical signs observed were those of poor clinical condition (round back, piloerection and emaciation, associated in some animals with swollen and/or hard abdomen, coldness to the touch, hypokinesia, dyspnea, pallor of eyes and body extremities, chromodacryorrhea). They were noted from week 34 for the first animal and often before the death or premature killing of the animals. This poor clinical status which was noted early in this group than in the other treated groups and with a higher incidence is attributed to treatment with the test substance. Nevertheless, a large number of animals found dead at the high dose-level showed no toxicologically relevant clinical signs before the time of death. These above-mentioned observations justified the premature final killing of this group in week 63.

In the other treated groups, signs of poor clinical condition (observed later and included round back, piloerection, emaciation, coldness to the touch, hypokinesia, dyspnea, pallor of eyes and body extremities, chromodacryorrhea, chromorhinorrhoea, loss of balance, bent head, decrease in grasping reflex, soiled urogenital area and eyes half-closed) and signs typically seen in the ageing rat (such as swollen limbs, abscess, nodosities, immobility, alopecia, cutaneous lesions) were noted with an incidence similar to that of controls with no indication of treatment or dose-relationship either in nature, incidence, onset or severity.

3.2.2 Palpable masses (Appendix 4)

The number of palpable masses (confirmed as tumours at microscopic examination) recorded during the course of the study were as follows:

Dose-levels (ppm)	0	10	30	100	300
Number of animals in study	50	50	50	50	50
Number of palpable masses	3	0	3	3	1
Number of animals bearing palpable masses	3	0	2	3	1
Mean per animal	1	0	1.5	1	1
Week of appearance of the first mass	32	-	57	87	63

The onset, incidence, morphological type and the multiplicity of the masses observed were similar in both control and treated animals and showed no effect of treatment.

3.2.3 Morbidity and mortality (Figure 1, Tables 2 to 8, Appendix 5)

3.2.3.1 Survival rate (SR)

Dose-levels (ppm)	0	10	30	100	300
Onset of mortality of the first 4 to 5 animals	week 63	week 63	weeks 61 to 68	weeks 70 to 75	weeks 45 to 52
SR after 52 weeks	94%	96%	98%	96%	78%
SR after 62 weeks	90%	90%	92%	96%	56%
- found dead (FD)	4	4	3	2	20
- killed prematurely (KP)	1	1	1	0	2
SR after 78 weeks	80%	82%	78%	88%	-
SR after 97 weeks	60%	44%	46%	48%	-
- found dead (FD)	15	20	19	17	
- killed prematurely (KP)	5	8	8	9	
- ratio FD/KP	3	2.5	2.4	1.9	

After 52 weeks of treatment the survival rate in animals given 300 ppm was lower than that of the other treated groups. According to the rapid increase of mortality noted at 300 ppm, the surviving animals of this group were killed in week 63. In all groups, the number of animals found dead was higher than the number of animals killed prematurely.

At 10, 30 and 100 ppm, the incidence and nature of the factors contributing to death were similar to those in the controls. The progressive nephropathy and the pituitary tumours were the predominant factors among these animals.

At 300 ppm, although the cause of death of the animals was not evident from the systemic examination, the presence of marked mastocytosis in the mesenteric lymph node together with sinusal haemorrhage, indicates that a histamine-related hypotension might have been the cause of death.

3.2.3.2 Onset of mortality

There were no noteworthy differences from controls concerning the onset of mortality at 10, 30 and 100 ppm groups but it was significantly earlier at 300 ppm. This last observation correlated with the differences noted in the clinical observations.

3.2.4 Food consumption (Table 9, Appendix 6)

There were no noteworthy differences from controls in the mean food consumption of males given 10 or 30 ppm.

Lower mean food consumption was recorded in males given 100 or 300 ppm as follows:

- at 100 ppm, slightly lower mean values were noted throughout the treatment period with a maximal difference from controls of -11% on some occasions; most values were not statistically significant,
- at 300 ppm, lower mean values were recorded throughout the treatment period; they were statistically significant at all times and slightly more marked during the first eight weeks of treatment (between -27% and -42% until week 8 and between -18% and -34% from week 9 to week 63).

These differences from controls were attributed to treatment with the test substance.

3.2.5 Body weight (Figure 2, Table 10, Appendix 7)

There were no noteworthy differences from controls in males given 10 or 30 ppm.

At 100 ppm, a lower mean body weight gain (-33% when compared to controls) was recorded during the first two weeks of dosing; thereafter, the absolute body weight gain was often slightly lower than that of controls but the body weight gain evolution over the different periods was similar to that of controls. Over the whole treatment period, the mean body weight gain was similar to that of controls. Consequently the low values noted at the beginning of the treatment period were attributed to the adaptation of the animals to a treated diet, without any toxicological consequence.

At 300 ppm, a mean body weight loss or a lower mean body weight gain was recorded during the treatment period with a marked degradation over the last nine months of dosing. These differences from controls, correlated with a lower mean food consumption, were attributed to treatment with the test substance.

Mean body weight evolution during the study

Dose-levels (ppm)	0	10	30	100	300
Mean body weight: day 1 (g)	320	317	323	320	316
Mean body weight: week 3 (g)	403	395	398	376**	306**
Mean body weight gain (or loss) after 2 weeks of treatment (g and % from day 1)	83 +26%	78 +25%	75 +23%	56 +18%	-10 -3%
Mean body weight: week 9 (g)	536	532	543	502**	378**
Mean body weight gain (weeks 3 to 9) (g and % from week 3)	133 +33%	137 +35%	145 +36%	126 34%	72 +24%
Mean body weight: week 26 (g)	679	674	677	633*	462**
Mean body weight gain (weeks 9 to 26) (g and % from week 9)	143 +27%	142 +27%	134 +25%	131 +26%	84 +22%
Mean body weight: week 62 (g)	837	819	842	779	479**
Mean body weight gain (weeks 26 to 62) (g and % from week 26)	158 +23%	145 +22%	165 +24%	146 +23%	17 +4%
Mean body weight gain (day 1 to week 62) (g)	517 x 2.6				163 x 1.5
Differences from controls (%)					-68
Mean body weight: week 98 (g)	815	790	793	771	
Mean body weight loss (weeks 62 to 98) (g and % from week 62)	-22 -3%	-29 -4%	-49 -6%	-8 -1%	
Mean body weight gain (day 1 to week 98) (g)	495 x 2.5	473 x 2.5	470 x 2.5	451 x 2.4	
Differences from controls (%)		-4	-5	-9	

3.2.6 Achieved dosages (Table 11)

The achieved dosages based on the nominal concentrations of 10, 30, 100 and 300 ppm were as follows:

- . 0.43 mg/kg/day (0.85 mg/kg/day to 0.32 mg/kg/day) for the 10 ppm concentration,
- . 1.30 mg/kg/day (2.65 mg/kg/day to 1.01 mg/kg/day) for the 30 ppm concentration,
- . 4.36 mg/kg/day (8.47 mg/kg/day to 3.41 mg/kg/day) for the 100 ppm concentration,
- . 13.6 mg/kg/day (20.2 mg/kg/day to 12.4 mg/kg/day) for the 300 ppm concentration.

3.2.7 Efficiency of food utilisation (Table 12)

The efficiency of food utilisation was similar to that of controls in the 10 and 30 ppm groups. The efficiency of food utilisation was slightly lower than in the control or other treated groups, over the first week of treatment at 100 ppm and over the first four weeks of treatment at 300 ppm; this correlated with the low body weight gain or the body weight loss recorded over these periods. Thereafter, there were no noteworthy differences from controls.

3.2.8 Blood pressure (Table 13, Appendix 8)

The blood pressure measurement did not show any difference between the control and 300 ppm groups.

3.3. LABORATORY INVESTIGATIONS: Haematology (Tables 14 to 21, Appendix 9)

. 300 ppm group (weeks 13, 26, 52 and 63)

There were no noteworthy differences from controls in the mean differential white cell percentages performed in weeks 13 and 26.

In week 52, a higher mean neutrophil (+41%) and a lower mean lymphocyte (-23%) percentage was noted.

In week 63, at the time of final sacrifice, a slightly lower mean total leucocyte count was noted (9.31 G/l vs. 12.12 G/l in controls, $p < 0.01$, -23%), mainly attributable to a lower lymphocyte count (5.88 G/l vs. 8.82 G/l in controls, $p < 0.01$; -33%). These differences from controls were consistent with the changes noted in the 26-week treatment period and were attributed to treatment with the test substance. No other relevant differences from controls were noted in this high dose-level group and some differences (namely eosinophil and basophil counts) were considered to be of no toxicological importance, since they were minor and in the range of those commonly occurring in aged rats.

There were no notable differences in the relative percentage of neutrophils and lymphocytes between control and treated animals killed prematurely during the study. The increase in the relative percentage of lymphocytes noted in male N20926 was due to the presence of malignant lymphoma and consequently of no toxicological importance.

. 10, 30 and 100 ppm groups (weeks 78 and 98/99)

In week 78 (0 and 100 ppm groups) and week 98/99 (0, 10, 30 and 100 ppm groups), the mean differential leucocyte count showed a considerable variability in the relative percentage of neutrophils and lymphocytes among the control and treated individuals without any indication of treatment or dose-relationship. The variability in the differential leucocyte count is an expected change in the ageing rat of this strain and related to the variation in the incidence and severity of the spontaneous inflammatory changes.

There were no noteworthy differences in the relative percentage of neutrophils and lymphocytes between control and treated animals killed prematurely during the study.

The few abnormalities observed in all groups during the morphological examination of the blood smears such as anisocytosis, anisochromia, myelemia the presence of normoblasts, lymphoblasts, hypersegmented neutrophils and erythrocytes in rouleaux are all expected geriatric changes in rats and occurred with similar incidence in both control and treated animals and showed no indication of treatment- or dose-relationship.

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3.4. PATHOLOGY**3.4.1 Macroscopic *post-mortem* examination (Table 22, Appendix 10)****1) Non-neoplastic findings**

All the findings encountered were either related to the method of sacrifice (red discolouration due to congestion or haemorrhage) or were those which are common geriatric changes in male rats of this strain (brownish/blackish discolouration of different organs due to pigment deposition, enlargement and cystic appearance of the kidneys, etc...). All these changes were of similar incidence and severity in both control and treated animals and showed no indication of treatment- or dose-relationship.

2) Neoplastic findings

The few masses found in some organs and tissue were equally distributed between control and treated animals and showed no indication of treatment- or dose-relationship either in size, location and multiplicity.

3.4.2 Microscopic findings (Tables 23 to 28, Appendix 10)**1) Non-neoplastic findings****Haemolymphoreticular system**

There was a remarkably high incidence of mastocytosis, haemosiderosis and sinusal haemorrhage in the mesenteric lymph node (see table below) of the animals given 300 ppm.

Incidence of findings in mesenteric lymph nodes

Findings	Number of animals bearing the findings				
	0 ppm (48)	10 ppm (28)	30 ppm (28)	100 ppm (50)	300 ppm (49)
Mastocytosis	1	0	1	4	44
Sinusal haemorrhage	1	1	2	0	24
Haemosiderosis	7	3	2	3	22

(..): number of animals examined.

The higher incidence of mastocytosis, haemosiderosis and sinusal haemorrhage was considered to be treatment-related. This is consistent with the findings observed in the same dose group of the satellite study in week 26.

In addition, the higher incidence of lymphoid depletion in the spleen in the animals given 300 ppm is also considered to be treatment-related.

High incidence of thymic involution was found with similar incidence and severity in both control and treated animals. Myeloid cell hyperplasia, lipomatosis and osteofibrosis were found with equal incidence and severity in the sternal and femoral bone of both control and treated animals.

These changes and the other non-neoplastic findings encountered in the haemolymphoreticular system were recognised as those commonly occurring in aged rats and are regarded as being of no toxicological importance.

Gastro-intestinal tract

A high incidence of dilated lumen in the duodenum, jejunum and ileum (see table below) was found in the animals given 300 ppm. This is considered to be treatment-related.

Incidence of dilated lumen

Organs / Findings	Number of animals bearing the findings				
	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm
Duodenum (dilated lumen)	0/46	0/25	0/25	0/49	32/44
Jejunum (dilated lumen)	0/41	0/22	0/17	0/46	30/45
Ileum (dilated lumen)	0/37	0/12	0/13	0/38	13/36

(..): number of animals examined.

All the other findings noted in the gastro-intestinal tract were recognised as commonly occurring changes in the aged rat and their incidence, severity and morphological characteristics were similar in both control and treated animals, showing no indication of treatment or dose-relationship.

Male genital organs

Hyposecretion and small tubulo-alveolar units were found with higher incidence (see table below) in the prostate of animals of the top dose-group (300 ppm). This is considered to be treatment-related.

Incidence of prostatic changes

Organs / Findings	Number of animals bearing the findings				
	0 ppm (50)	10 ppm (31)	30 ppm (32)	100 ppm (50)	300 ppm (50)
Hyposecretion	3	2	3	3	14
Small tubulo-alveolar units	3	2	3	3	12

(..): number of animals examined.

The incidence, severity and morphological characteristics of the other changes encountered in the male genital organs were similar in both control and treated animals and showed no effects resulting from treatment.

Heart

The expected finding of myocardial degeneration and fibrosis was observed in both control and treated animals with marginally greater incidence in control males. The incidence of this finding and the others affecting the heart showed no effect of treatment.

Liver

The terminology and criteria for the morphological diagnosis of the non-neoplastic proliferative hepatic lesions were as described by Goodman et al. (1993). The diagnostic term used was hepatocellular foci (acidophilic, basophilic, vacuolated and mixed). The foci of cellular alteration which were found among rats of all groups were within the range described for the rat strain found either in the reference documentation (Lang, 1992) or in our historical background data (up to 89%), and showed no indication of treatment- or dose-relationship.

The other non-neoplastic lesions found such as biliary proliferation, pericholangitis, steatosis, cystic degeneration, focal or multifocal coagulative hepatic cell necrosis, showed no indication of treatment- or dose-relationship. Moreover, their incidence, severity and morphological characteristics were similar to those reported spontaneously in the rat of this strain and age. Consequently, they were regarded as being of no toxicological importance.

Kidneys

All the non-neoplastic changes encountered in the kidneys of the control and treated animals were those which are commonly recorded geriatric findings in aged rats. Moreover, their incidence, severity and morphological characteristics were similar in control and treated animals and showed no indication of treatment or dose-relationship.

Other organs and tissues

The non-neoplastic findings observed in the other organs and tissues were those which are commonly recorded geriatric changes in the CrI:CD(SD)BR rat and were regarded as being of no toxicological importance.

In addition, phenomena due to trauma resulting from the identification procedure, fighting and/or animal husbandry were found among both control and treated animals with a similar incidence and severity.

These changes comprised lesions of the limbs and tail (ulceration, cellulitis and proliferative osteitis).

2) Neoplastic findings

The number of animals with neoplasms and the number of animals with more than one primary neoplasm and the number of benign and malignant tumours were similar in all groups, including the control group. In addition, the analysis of incidental tumours, as assessed from the evaluation of incidental neoplastic lesions observed at each 200 day-interval in the animals that died or were sacrificed prematurely before the end of the study, showed that the test substance did not produce a decrease in the latency of tumour appearance in the treated animals when compared with the controls.

Statistical analysis (Trend test) according to Peto et al., 1980, was performed. A trend was considered positive if the P value was less than 0.05 for a rare neoplastic finding and less than 0.01 for a common finding.

As all neoplastic lesions observed were common ones, no positive trend was found except for the pituitary adenomas ($P = < 0.016$ for acidophilic cell adenoma and < 0.008 for pooling pituitary tumours).

Since this P value was based on all dose-groups, the dose-dependency statistical analysis was performed and the statistical NOEL was determined by excluding groups 5, 4, 3, successively, until no further positive trend was detected.

The results of this analysis showed that the positive trend was mainly due to a higher number of fatal pituitary tumours (18/39) in examined animals of group 3 (30 ppm) when compared to the control group (5/49, fatal tumours).

Consequently, in the absence of positive trend in the other two high dose groups (100 or 300 ppm), it was concluded that there was no treatment-related effect.

Liver

The terminology and criteria used for the morphological diagnosis of the neoplastic lesions are described by Goodman et al., 1993. The diagnostic terms used were "hepatocellular adenoma" and "hepatocellular carcinoma".

Incidence of hepatic neoplastic lesions

Organs / Lesions	0 ppm (50)		10 ppm (50)		30 ppm (48)		100 ppm (50)		300 ppm (49)	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Hepatocellular adenoma	4	8	3	6	1	2	6	12	0	-
Hepatocellular carcinoma	0	-	0	-	0	-	3	6	0	-

(.): number of animals examined.

When these data were compared with the spontaneous incidence of similar neoplastic lesions in aged Sprague-Dawley rats (adenoma up to 18.2% and carcinoma up to 9.1%) found either in reference documentation (Lang, 1992) or from our historical control data, no significant difference was noted.

Consequently it was concluded that there was no treatment-related effect on the distribution of hepatic neoplastic lesions.

Intestines

Adenocarcinoma was noted in the jejunum of one animal of the low-dose group (10 ppm) and in two animals of the top-dose group (300 ppm).

Incidence of adenocarcinoma

Organs / Lesions	0 ppm (41)	10 ppm (22)	30 ppm (24)	100 ppm (46)	300 ppm (45)
Adenocarcinoma	0		0	0	2

There have been reports of short-lasting outbreaks of higher incidence of spontaneous adenocarcinoma in rat intestines. For example, Heslop (1969) observed an outbreak of spontaneous adenocarcinoma; tumours were found in 44 out of 314 rats examined within less than a year. The majority of tumours were found in young males (3-5 months old). Within the following four years, not a single adenocarcinoma was detected in the rats examined.

This fact, besides the absence of a dose-relationship argue against a treatment-relationship.

Pituitary gland

The adenomas found in the pituitary gland were classified according to their tinctorial properties and/or the size of the cells, as small cell adenomas, acidophilic cell adenomas, and mixed cell adenomas.

The incidence and morphological types of the pituitary tumour in the present study were similar in both control and treated animals (see neoplastic findings page 104) and are not considered to be treatment-related. In addition, the incidence and morphological types of the pituitary neoplastic lesions reported in this study are similar to those reported in geriatric rats (Anver et al., 1982; Attia, 1996).

Adrenals and thyroids

The incidence and morphological types of the adrenal and thyroid neoplastic lesions in this study were similar in both control and treated animals and were similar to those reported spontaneously in Sprague-Dawley rats (Lang, 1992; Attia, 1996).

Haemolymphoreticular system

Leukaemia and lymphoma were found in the following organs: liver, spleen, thymus, lymph nodes and sometimes heart, kidneys, lungs and adrenals.

These neoplastic lesions occurred with similar incidence in both control and treated animals and are not considered to be treatment-related.

Other organs and tissues

The few other neoplastic lesions encountered in this study, namely in the forestomach, skin, pancreas, spleen, lungs, prostate, kidneys, testes, abdominal cavity, lips and lower jaw, were recognised as commonly occurring neoplastic findings in geriatric rats and showed no difference in incidence or morphological type that were considered to be treatment-related.

Conclusion

Non-neoplastic findings

The administration of the test substance in dietary admixture at the dose-level of 300 ppm caused:

- . high incidence of mastocytosis, haemosiderosis and sinusal haemorrhage in the mesenteric lymph nodes,
- . high incidence of lymphoid depletion in the spleen,
- . moderate to marked dilatation of some intestinal segments,
- . hyosecretion together with small tubulo-alveolar units in the prostate.

In the other treated groups, the incidence, severity and morphological characteristics of the non-neoplastic microscopic findings showed no indication of treatment or dose-relationship and were similar to spontaneous lesions described for rats of this strain and age.

Neoplastic findings

The test substance showed neither carcinogenic potential nor an effect on the incidence of spontaneously occurring tumours at 300 ppm (up to week 63) or 100 ppm, 30 and 10 ppm (up to week 98/99). Moreover, the test substance did not induce a decrease in the latency of tumour appearance.

4. CONCLUSION

The test substance, TGIC (1, 3, 5 - triglycidyl isocyanurate), was administered by dietary admixture to male Sprague-Dawley rats at concentrations of 10, 30, 100 and 300 ppm. Marked signs of toxicity and a rapid onset of mortality were noted in animals given the test substance at the concentration of 300 ppm, and treatment was stopped in week 63 for this group, and the animals were sacrificed. For the dose-groups at 10, 30 and 100 ppm the treatment was continued up to 98 weeks to cover the life-span of these animals.

At 300 ppm, laboratory and histopathological investigations revealed mastocytosis in the lymph nodes and depletion of lymphoid cells in the spleen. At the lower dose-levels, the principal effect was a slightly lower food consumption at 100 ppm, resulting in a terminal mean group body weight which was 9% lower than the control animals.

The test substance did not show a carcinogenic potential or any effect on the incidence of spontaneously occurring tumours at any dose-level. Furthermore, the test substance did not induce a decrease in the latency of the appearance of spontaneous tumours.

The dose-level of 300 ppm (13.6 mg/kg/day) clearly exceeded the maximum tolerated dose (MTD). On the basis of the results generated in the course of this study, the NOAEL is considered to be 100 ppm (4.36 mg/kg/day) and the NOEL is considered to be 30 ppm (1.30 mg/kg/day).

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