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Re: Supplemental Submission of the Final Report to TSCA Section 8(e) Notification of Substantial Risk: 8EHQ-99-14591, Hexamethyldisiloxane: A 24-Month combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study in Fischer-344 Rats.

Dear Docket Clerk:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act (TSCA), as interpreted in the Statement of Interpretation and Enforcement Policy (40 FR 11110, 16 March 1978), Dow Corning is submitting the following recently issued final study report as a supplemental submission to our initial TSCA Section 8(e) notification of November 8, 1999 (8EHQ-99-14591). The information presented in this submission was generated as part of our Siloxane Research Program. This program was the subject of a memorandum of understanding, dated April 9, 1996, between Dow Corning and EPA.

Chemical Substance: 107-46-0

Manufacturer:

8EHQ-99-14591

Dow Corning Corporation
2200 West Salzburg Road
Midland, Michigan 48686-0994

Final Study Report:

HEXAMETHYLDISILOXANE: A 24-MONTH COMBINED CHRONIC TOXICITY AND ONCOGENICITY WHOLE BODY VAPOR INHALATION STUDY IN FISCHER-344 RATS.

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Phone: (989) 496-4000
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Dow Corning Corporation
2004-I0000-53896

Summary:

Preliminary results from an ongoing 24-month combined chronic/oncogenicity study with hexamethyldisiloxane (HMDS) in Fischer 344 rats were submitted to the Agency as a TSCA Section 8(e) notification (November 10, 1999). The preliminary results indicated an increase in Leydig cell hyperplasia and Leydig cell tumors (LCTs) in male rats following one year of exposure. The Leydig cell hyperplasia occurred in 20/20 males in all exposure groups including controls (i.e., 0 ppm) and tended to increase in severity as the exposure concentration increased. The incidence of LCTs was reported as 4/20, 10/20, 9/20, 12/20 and 15/20 in the 0, 100, 400, 1600, and 5000 ppm exposure groups respectively. Dow Corning is now submitting the final study report for the recently completed 24-month combined chronic/oncogenicity study as a supplemental submission to our previous TSCA Section 8(e) notification.

Inhalation exposure of Fischer 344 rats to HMDS for up to 24-months resulted in notable affects in the liver, testes, kidney, and nasal cavity. In a 24-month combined chronic/oncogenicity inhalation study, male and female Fischer 344 rats were exposed to vapor concentrations of 0, 100, 400, 1600, and 5000 ppm of hexamethyldisiloxane (HMDS) for six hours/day, five days/week.

In males exposed for one year to 1600 ppm increases in relative liver weight, organ to body weight, were recorded. For male animals exposed to 5000 ppm an increase in both absolute and relative liver weights was evident. However, there were no correlated findings at histopathological examination. Therefore, the increased liver weight in males but not in females after one year of exposure could be the consequence of a transient metabolic adaptation without any toxicological relevance. This finding was not diagnosed in either male or female animals exposed for two years.

Following one year of exposure to HMDS an increase in relative testes weights for male animals exposed to 5000 pm was noted. Histopathological evaluation performed on male rats following one year of exposure showed an increase in Leydig cell hyperplasia and Leydig cell tumors (LCTs). The Leydig cell hyperplasia occurred in 20/20 males in all exposure groups including controls (i.e., 0 ppm) and tended to increase in severity with an increased exposure concentration. Following a histopathology peer review, the final incidence of LCTs was 4/20, 13/20, 13/20, 15/20, and 18/20 in the 0, 100, 400, 1600, and 5000 ppm exposure groups respectively. Statistical significant increases in relative and absolute testes weights were not noted in those animals exposed for one year and given a one year recovery period. Leydig cell hyperplasia was found to be present in one male animal of the control group, 0 ppm and one male exposed to 5000 ppm. The incidence of LCTs was 20/20, 20/20, 19/20, 19/20 and 19/20 in the 0, 100, 400, 1600, and 5000 ppm

exposure groups respectively.

After two years of exposure increases in both the absolute and relative testes weights in male animals exposed to 1600 and 5000 ppm were observed. Leydig cell hyperplasia was found to be present in male animals of the control group, 0 ppm and the first three groups, 100, 400 and 1600 ppm. The incidence of Leydig cell hyperplasia was 4/65, 2/65, 3/65 and 1/65. The incidence of LCTs was 62/65, 64/65, 62/65, 63/65, 64/64 in the 0, 100, 400, 1600, and 5000 ppm exposure groups respectively.

After one year of exposure, an increase in the relative kidney weight, organ/body weight ratio, was seen in the males exposed to 5000 ppm HMDS. Non-neoplastic changes in the kidney, predominantly associated with the male animals, included an increased incidence of calcified cylinders in the tubules of the papilla, 19/20 and 19/20 for the 1600 and 5000 ppm exposure groups respectively. Further, tubular degeneration/regeneration was evident in 12/20, 15/20, 14/20, 17/20 and 15/20 male animals exposed to 0, 100, 400, 1600, and 5000 ppm, respectively. However, these changes were not statistically significant given that this finding was also present in the control group. Cortical mineralization was evident in these male animals at incidence rates of 18/20, 19/20, 19/20, 20/20 and 20/20 for exposed animals at 0, 100, 400, 1600, and 5000 ppm, respectively. Also present in the kidneys was an increase in the incidence of tubular hyaline casts in male animals, 10/20, of the high dose group, 5000 ppm.

After one year of exposure and one year of recovery, non-neoplastic changes in the kidney, predominantly associated with the male animals, included an increased incidence of calcified cylinders in the tubules of the papilla, 4/20, 19/20 and 19/20 for the 400, 1600 and 5000 ppm exposure groups respectively. Further, transitional cell hyperplasia was evident in 7/20 and 12/20 male animals exposed to 1600 and 5000 ppm, respectively. Hyaline casts were also increased, 7/20, for the male animals exposed to 5000 ppm.

After two years of exposure, there were no differences in either absolute or organ/body weight ratio for the kidney in the male animals exposed to HMDS. Nonneoplastic changes in the kidney, predominantly associated with the male animals, included an increased incidence of calcified cylinders in the tubules of the papilla, 24/65, 63/65 and 65/65 for the 400, 1600 and 5000 ppm exposure groups respectively. Further, transitional cell hyperplasia and corticial mineralization was evident in 32/65 and 49/65 and 59/65 and 60/65 for those male animals exposed to 1600 and 5000 ppm, respectively. Also present in the kidneys was an increase in the incidence of tubular hyaline casts in male animals, 25/65, of the low dose group. Tubular degeneration/regeneration of the kidney was not statistically relevant in the male animals of this exposure duration. Neoplastic findings included a statistically significant increase in trend (0/65, 0/65, 0/65, 3/65 and 6/65 for 0, 100, 400, 1600 and 5000 ppm respectively) in the male animals for combined tubular cell adenoma

and carcinoma. The combined (6/65) incidence in the 5000 ppm group was also significantly different (<0.05) from control by the Fisher's Exact test. This finding was not evident in the female animals of either exposure group.

After one year of exposure, an increase in the incidence of eosinophilic inclusions was recorded in the nasal cavity, predominately in male animals of all exposure groups. Empirically, this finding is represented as 7/20, 7/20, 14/20 and 9/20 for the male animals exposed to 100, 400, 1600 and 5000 ppm, respectively.

After one year of exposure and one year of recovery, an increase in the incidence of eosinophilic inclusions was recorded in the nasal cavity. The incidence rate for the male animals exposed to 1600 ppm was 17/20, while the incidence rate for male and female animals exposed to 5000 ppm was 18/20 and 18/20 respectively.

After two years of exposure, an increase in the incidence of eosinophilic inclusions was recorded in the nasal cavity, for the male animals exposed to 1600 and 5000 ppm, these incidence rates were 64/65 and 64/65, respectively.

Discussion:

The increased liver weight in the absence of histopathological findings in males but not in females after one year of exposure is most likely the consequence of a transient metabolic adaptation without any toxicological relevance.

LCTs are commonly encountered in one year old F344 rats and become increasingly more frequent with age (Boorman, et. al. 1990). The incidence of spontaneous LCTs is generally greater than 90% in the F344 strain of rat at 2 years of age (Iwata, et. al. 1991). Virtually all plausible modes-of -action for the chemical induction of LCTs involve the elevation of serum luteinizing hormone (LH) and/or Leydig cell response to LH (Cook, et. al. 1999). There are several lines of evidence that suggest human Leydig cells are quantitatively less sensitive than rats in their proliferative response to LH and hence their sensitivity to chemically induced LCTs (Clegg, et. al. 1997; Quigley, et. al. 1995; Shenker, et. al. 1993). Further, epidemiology studies available on a number of compounds that induce LCTs in rats do not demonstrate an association between human exposure to these compounds and induction of Leydig cell hyperplasia or adenomas (Himmelstein, et. al. 1996; Longnecker, et. al., 1995; IARC, 1986; Preston-Martin, et. al. 1991; and Wilmer, et. al. 1994).

Crissman, et. al. (2003, Dow Corning Corporation report number 2002-I0000-51723) tested the hypothesis that the alpha 2u-globulin mechanism was responsible for the nephropathy and kidney neoplasia observed in male rats on studies with HMDS. Seven male Fischer 344 rats per group were exposed via a nose-only vapor inhalation system to HMDS vapors at 5000 ppm HMDS or to air 6 hours per day for 6 days. At the end of the exposure period, kidneys were harvested and embedded in methyl methacrylate, and duplicate slides stained either immunohistochemically using a mouse monoclonal antibody against alpha

2u-globulin or with Lee's methylene blue/basic fuchsin. There were HMDS exposure induced renal effects characteristic of alpha 2u-globulin nephropathy. HMDS exposure clearly increased the amount and altered the morphology of alpha 2u-globulin stained material. Quantitatively, both the proportional area and density of positively stained material in the renal cortex was increased in exposed rats. Further, occasional tubules in alpha 2u-globulin accumulation areas of HMDS-exposed rats showed sloughing of necrotic epithelial cells into the tubular lumen, and consequent thinning of the epithelium. These results provide mechanistic support for the hypothesis.

It is common for a number of age-related changes to occur in the nasal cavity of the Fischer 344 rat (Boorman, et. al. 1990). Among these changes are the visualization of eosinophilic or hyaline globules in either the respiratory or olfactory epithelium.

Because the findings related to the testes, kidney, nasal cavity, and liver are of doubtful toxicological relevance to humans and that potential human exposure to HMDS is low, this material does not represent a risk to humans; however we are reporting our findings to ensure compliance with both the letter and spirit of TSCA Section 8(e).

Actions:

These findings from the aforementioned study will be communicated to appropriate internal and external audiences. Dow Corning will notify EPA of any further relevant information that may be developed concerning this material.

If you have any questions concerning any of the aforementioned studies, please contact Dr. Robert G. Meeks, Scientific Director of Toxicology and Risk Assessment, at 989-496-8629 or at the address provided herein. If you require further general information regarding this submission, please contact Michael E. Thelen, Manager of U.S. EPA Regulatory Affairs, at 989-496-4168 or at the address provided herein.

Sincerely,

Kathy P. Blaszko for Laura L. Perkins

Laura L. Perkins, Ph.D.
Director, Health, Environment and Safety
(989) 496-8568

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Dow Corning Report No.:	2004-I0000-53896
Title:	Hexamethyldisiloxane: A 24-Month Combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study in Fischer-344 Rats
Dow Corning Study Number:	8788
External Testing Facility Number:	655997
Test item:	Hexamethyldisiloxane (HMDS)
Study Director:	P. A. Smith
Authors:	A. Dotti, P. A. Smith and H. J. Chevalier
Sponsor:	Dow Corning Corporation, Auburn, Michigan 48611 / U.S.A.
Sponsor Representative:	Steven D. Crofoot
Test Facilities:	RCC Ltd, Toxicology Division (RCC TOX) Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland and Zelgliweg 1, CH-4452 Itingen, Switzerland RCC Ltd, Biotechnology and Animal Breeding Division (RCC BAB), Wölferstrasse 4, CH-4414 Füllinsdorf RCC Ltd, Environmental Chemistry and Pharmanalytics Division (RCC ECP), Zelgliweg 1, CH-4452 Itingen, Switzerland EPS Experimental Pathology Services (EPS) CH-4132 Muttenz, Switzerland Propath UK Ltd. Willow Court, Netherwood Road, Hereford, HR2 6JU, U.K. EPL, Experimental Pathology Laboratories, Inc. (EPL) P.O. Box 474, Herndon, VA 20172-0474 Dow Corning Corporation 2200 W. Salzburg Road, C03101 Auburn, Michigan 48611, U.S.A. Environ International Corp. (Environ) 602 East Georgia Avenue Ruston, Louisiana 71270, U.S.A.
Study Completion Date:	22-DEC-2005
Security Classification Statement:	DOW CORNING INTERNAL – This report may be reproduced and shared with any Dow Corning employee. Distribution outside the corporation must be approved by the Director of Health and Environmental Sciences. When this INTERNAL report is no longer needed dispose in a manner that safeguards against unauthorized disclosure

1 CONFIDENTIALITY STATEMENT

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2 STATEMENT OF COMPLIANCE

RCC Study Number	655997
Dow Corning Study Number	8788
Test Item Dow Corning Product Name	Hexamethyldisiloxane (HMDS) Dow Corning® OS-10
Study Director	P. A. Smith
Title	Hexamethyldisiloxane: A 24-Month Combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study in Fischer-344 Rats

This study was performed in compliance with:

- Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986
- OECD Principles of Good Laboratory Practice, Paris, France, [C(81)30(Final)], adopted by the Council 12th May 1981, re-published as document OECD/GD(92)32 in 1992.

and met the requirements of:

- EPA - TSCA - Good Laboratory Practice Standard. Code of Federal Regulations, 40 CFR, Part. 792 (Published in Federal Register).

Note that technical trials performed without animals prior to the start of the study were not covered by GLP Regulations. The data is retained in a separate section of the raw data but is not included in the final report.

This statement does not include the reporting of the pathology data performed by EPS, the peer review of pathology data performed by EPL, the purity verification report of the test item and the morphometric testes evaluation performed by Dow Corning Corporation.

The GLP compliant performance of the analytical chemistry was the responsibility of RCC Ltd, Environmental Chemistry & Pharmanalytics Division.

Propath was responsible for GLP compliance of the work performed at their facility.

The statistical analysis of the pathological findings performed by ENVIRON and part of the microbiological investigations were not formally conducted according to GLP.

There were no circumstances that may have affected the quality or integrity of the study.

STUDY DIRECTOR P. A. Smith

Paul Smith
.....
Date 22-DEC-2005

3 DOW CORNING APPROVAL PAGE

Steven D. Crofoot

Dow Corning Corporation
2200 W. Salzburg Road, CO 3101
Auburn, Michigan 48611, U.S.A.

.....
Date:

4 QUALITY ASSURANCE GLP TOXICOLOGY

RCC Ltd, Toxicology, CH-4452 Itingen, Switzerland

STATEMENT

RCC Study Number	655997
Dow Corning Study Number	8788
Test Item Dow Corning Product Name	Hexamethyldisiloxane (HMDS) Dow Corning® OS-10
Study Director	P. A. Smith
Title	Hexamethyldisiloxane: A 24-Month Combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study in Fischer-344 Rats.

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures, with the exception of the pre-test(s), were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below:

Dates and type of QA Inspections		Dates of Reports to the Study Director and Test Facility Management
18/20-AUG-1997	Study plan	20-AUG-1997
21-AUG-1997	Randomisation	21-AUG-1997
12-SEP-1997	Test system, test item, treatment, raw data, sample taking, sample handling	12-SEP-1997
25-SEP-1997	Test system, measurement, sample taking, sample handling	25-SEP-1997
18-NOV-1997	Test system treatment, measurement, raw data	18-NOV-1997
29-NOV-1997	Test system, blood sampling	29-NOV-1997
30-NOV-1997	Clinical laboratory investigations	30-NOV-1997
01-DEC-1997	Clinical signs, body weight	01-DEC-1997
13-JAN-1998	Test system, treatment, sample taking sample handling, raw data	13-JAN-1998
15-JAN-1998	Raw data	15-JAN-1998
26-FEB-1998	Microbiology, raw data, sample handling	26-FEB-1998
28-FEB-1998	Test system, blood sampling, raw data	28-FEB-1998
19/20-MAY-1998	Test system, test item, treatment, raw data, measurement, sample taking	20-MAY-1998
11-AUG-1998	Test system, treatment, raw data	11-AUG-1998
02-SEP-1998	Necropsy, raw data	02-SEP-1998
23-SEP-1998	Test system, treatment, measurement, sample taking, raw data	23-SEP-1998

Dates and type of QA Inspections		Dates of Reports to the Study Director and Test Facility Management
15-DEC-1998	Test system, test item, treatment, raw data, measurements	15-DEC-1998
22-JAN-1999	Histotechnique, raw data	05-FEB-1999
18-FEB-1999	Histotechnique	19-FEB-1999
25-MAR-1999	Measurements, raw data	25-MAR-1999
07-JUN-1999	Test system, raw data, clinical signs	07-JUN-1999
14-JUN-1999	Test system, raw data, observations	14-JUN-1999
21-JUN-1999	Test system, test item, treatment, raw data	21-JUN-1999
12-JUL-1999	Test system, palpation, raw data, body weights	12-JUL-1999
15-JUL-1999	Test system, raw data, test item, measurements	15-JUL-1999
05-AUG-1999	Test system, ophthalmoscopic examination, raw data	05-AUG-1999
11-AUG-1999	Measurements, raw data	11-AUG-1999
25-AUG-1999	Test system, blood sampling, raw data	25-AUG-1999
30-AUG-1999	Necropsy	30-AUG-1999
01-SEP-1999	Test system, raw data, blood sampling, body weight, clinical signs	01-SEP-1999
26-OCT-1999	Histotechnique, raw data	05-NOV-1999
22-APR-2004	Test item, raw data	22-APR-2004
27-30-DEC-2001 03/07-10/12-20-JAN-2002	Draft report	20-JAN-2002
29/31-DEC-2004 03-05/10/19-JAN-2005	Draft report	19-JAN-2005
12-16-DEC-2005	Report	16-DEC-2005

This statement also confirms that this final report reflects the raw data.

This final report includes further QA-Statements issued by the Test Site Quality Assurance Units.

Quality Assurance

Th. Frei

Date: 22 December 2005

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LAST PAGE OF REPORT 5254

5 SIGNATURE PAGE

Study Director P. A. Smith

.....
Date: 22-DEC-2005

Management (for) S. J. Corney

.....
Date: 22-DEC-2005

6 PREFACE

6.1 GENERAL INFORMATION

Title	Hexamethyldisiloxane: A 24-Month Combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study in Fischer-344 Rats.
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Sponsor Representative and Addressee	Steven D. Crofoot Dow Corning Corporation 2200 W. Salzburg Road, CO 3101 Auburn, Michigan 48611, U.S.A.
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6.2 PROJECT STAFF

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Necropsy (RCC Itingen)	Dr. H.-J. Chevalier (EPS), Dr. K. Weber (RCC TOX)
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Microbiological Investigations	S. Kehl (RCC BAB)
Histotechnology	I. J. Stiff (Propath)
Peer reviewing pathologist	Dr. P. Mann (EPL)
Histopathology	Dr. H.-J. Chevalier (EPS)
Morphometric testes evaluation	Dr. J.W. Crissman (Dow Corning)
Statistics of Histopathology Data	Dr. Annette Shipp (Environ)

6.3 SCHEDULE

Experimental starting date (Delivery of animals)	20-AUG-1997
Acclimatisation	21 to 31-AUG-1997
Start of exposure	01-SEP-1997
Sacrifice after 1 year	Subgroup A: 31-AUG to 04-SEP-1998
Last day of exposure (Subgroup B)	28-AUG-1998
Last day of exposure (Subgroup C)	01-SEP-1999 to 13-SEP-1999 The animals were exposed until the day before necropsy.
Terminal sacrifice	Subgroup B: 30-AUG to 03-SEP-1999 Subgroup C: 02 to 14-SEP-1999
Experimental completion date	30-AUG-2005
Final report	22-DEC-2005

6.4 TEST GUIDELINES

The study procedures described in this report are in compliance with the following Guidelines:

- OECD Guidelines for Testing of Chemicals, Section 4, number 453, "Combined Chronic Toxicity/Carcinogenicity Studies", adopted May 12, 1981.
- U.S. Environmental Protection Agency, Toxic Substances Control Act (TSCA), 40 CFR (Edition July 1, 1991) Ch. 1, § 798.3320: Combined Chronic Toxicity/Oncogenicity Studies.

6.5 ARCHIVING

RCC Ltd, CH-4452 Itingen, Switzerland will archive all the material, specimens and documents generated at RCC, Propath and EPS (with exceptions listed below), for at least 10 years. Archiving at RCC includes:

- all study related documents
- all raw data
- RCC protocol and report and possible amendments to these documents
- EPS pathology report and possible amendments to this document
- all specimens from diagnostics
- photographs, specimens, tissue blocks and slides of pathology and histopathology
- the test item reference sample

Archiving at RCC Ltd will be performed according to the Swiss GLP Guidelines under RCC responsibility. After 10 years, the Sponsor will be contacted for the disposal of the archived material. However on his request, part or all archived material and documents can be sent to the Sponsor prior to the end of the 10-year-period. Transportation and further archiving will be under the responsibility of the Sponsor.

EPS, Experimental Pathology Services, Muttenz (Switzerland), will archive:

- all computer data generated at EPS, on magnetic diskettes stored in a bank safe,
- hard copy of the data generated at EPS
- study-related correspondence (at EPS facility).

Archiving at EPS will be performed according to the Swiss GLP Guidelines under EPS responsibility.

7 SUMMARY

This combined toxicity / oncogenicity study was comprised of five groups of 105 male and 105 female Fischer 344 rats each, giving a total of 1050 rats. The animals were treated with hexamethyldisiloxane (HMDS) by whole body exposure. The target exposure levels were: 0 ppm (0 mg/L, control Group 1), 100 ppm (= 0.7 mg/L Group 2), 400 ppm (= 2.7 mg/L, Group 3), 1600 ppm (= 10.6 mg/L, Group 4), and 5000 ppm (= 33.2 mg/L, Group 5). At all these concentrations the test atmosphere was in the vapor phase (saturated vapor concentration of the test item: 55'500 ppm (370 mg/L air). The animals were exposed for 6 hours/day, 5 days/week.

The allocation of the animals in the different subgroups was as follows: subgroup A (20 males and 20 females at each exposure concentration) was sacrificed after one year of exposure, subgroup B (20 males and 20 females at each exposure concentration) was sacrificed after one year of exposure and one year of recovery, subgroup C (65 males and 65 females at each exposure concentration) was sacrificed after two years of exposure.

During the exposure period temperature, relative humidity, air flow and the test item concentration in the exposure chambers were monitored continuously. The oxygen concentration was recorded once daily in the HMDS groups and continuously in the control group. The following biological parameters were recorded during the study: mortality, clinical signs, ophthalmoscopic changes, body weights and food consumption. Clinical laboratory investigations (hematology, clinical biochemistry and urinalysis) were performed after 3, 6 and 12 months. Terminal macroscopic examination, organ weights and organ collection were performed on all animals. All collected organs in all control and high dose rats and in the animals which prematurely died were histopathologically examined. From the surviving rats of the intermediate groups 2 – 4, liver, lungs, kidneys, nasal cavities, testes and gross lesions were examined microscopically.

EXPOSURE CONCENTRATIONS

The concentration of HMDS in the exposure chambers was continuously measured by gas chromatographic (GC) analysis and recorded. The nominal (gravimetric) concentration was determined daily from measurement of the quantity of test item used and the air flow rate through the chamber. The overall mean nominal chamber concentrations (calculated as mean of the weekly means) for males were 103.9, 409.9, 1639.4 and 5118.1 ppm (0.727, 2.767, 10.861, and 33.984 mg/L) HMDS for target exposure concentrations of 100 (0.7 mg/L), 400 (2.7 mg/L), 1600 (10.6 mg/L) and 5000 ppm (33.2 mg/L), respectively. The GC analysis of these chamber concentrations for males yielded 98.6, 396.1, 1588.1 and 4900.5 ppm (0.690, 2.674, 10.522, and 32.539 mg/L HMDS, respectively). The overall mean nominal chamber concentrations for females were 103.6, 411.0, 1630.8 and 5110.1 ppm (0.725, 2.774, 10.804, and 33.931 mg/L HMDS, respectively). The GC analysis of these chamber concentrations for females yielded 98.6, 395.9, 1589.0 and 4908.9 ppm (0.690, 2.672, 10.528, and 32.595 mg/L HMDS, respectively).

The mean chamber concentrations determined by GC analysis (Week 1 to 107) were within 2% of target. For all groups the coefficient of variation (SD/mean × 100) of the weekly means was less than 1.7%. This demonstrates excellent control over the vapor generation and inhalation throughout the duration of the study. The mean chamber concentration measured by gravimetry ranged from 2.6 % to 6.1 % higher than measurement by GC analysis.

MORTALITY

There were no treatment related deaths observed during the study.

During the first year 12 animals out of 1050 [two in group 2 (100 ppm), three in each of groups 3 (400 ppm) and 4 (1600 ppm), and four in group 5 (5000 ppm)] prematurely died. Although there were no deaths in the control group and the number of deaths was marginally higher in the high dose group, the distribution was considered to be incidental. Of the 12 animals that died prematurely during the first year 1 animal was from subgroup A (one year exposure), 2 animals were from subgroup B (one year exposure and one year recovery) and 9 animals were from subgroup C (two year exposure). During the second year, the mortality rate was similar in control and treated animals and there were no differences between subgroup B and subgroup C animals.

CLINICAL SIGNS AND MASSES

There were no clinical signs related to HMDS exposure. At weekly palpation of the animals no masses were noted which were considered to be related to the exposure with HMDS.

Neither the types nor the incidence or severity of the clinical signs observed – mainly hair loss and crusts on the eyelid – gave any indications of test item related effects.

OPHTHALMOSCOPIC EXAMINATION

No test item related findings were noted. After one year and towards the end of the two year exposure, all examined animals (100% incidence) showed corneal opacity.

FOOD CONSUMPTION

In male animals the differences in food consumption in test item exposure groups compared to controls were considered unrelated to test item exposure.

In female animals from groups 4 (1600 ppm) and 5 (5000 ppm) of subgroups A (one year of exposure) and C (two years of exposure), the slight reduction in food consumption (-3.5 to -8.8%) was considered to be test item related. The mean food consumptions were statistically significantly reduced from the respective control values on 16 and 21 (N=33) occasions in subgroup A (one year of exposure), and 23 and 40 occasions (N=60) in subgroup C (two years of exposure) for groups 4 (1600 ppm) and 5 (5000 ppm), respectively. The mean food consumption during the exposure year for subgroup B (one year of exposure and one year of recovery), group 5 (5000 ppm) females was 4.5% lower than control. This is indicative of the effect recorded for subgroup A (one year of exposure) and C (two years of exposure) females but the differences from control for subgroup B (one year of exposure and one year of recovery) food consumption values were statistically significant on only 8 out of the 33 measurements during the exposure year.

BODY WEIGHTS

In males, the differences in mean body weight noted between the exposed animals and the respective controls were considered to be unrelated to the test item treatment. In female animals from groups 4 (1600 ppm) and 5 (5000 ppm), the mean body weights were statistically significantly reduced from the respective control values on 27 (N=33) occasions for subgroup A (one year of exposure) and 29 (N=60) occasions for subgroup C (two years of exposure). However, the differences were marginal (-2 to -8% from control) and there was up to -4% difference in body weight from control before the start of exposures at Week 1. In addition, there was no dose-relationship in body weight between the groups, and there was no confirmation of a similar response in the subgroup B (one year of exposure and one year of recovery) females or male animals in all groups. This finding is probably associated with a slight reduction in food consumption noted in these animals, which was considered to be test-item related.

HEMATOLOGY, CLINICAL BIOCHEMISTRY AND URINALYSIS

The changes noted in the hematological parameters between 400 and 5000 ppm consisted of occasional, slightly reduced, hemoglobin concentration, hematocrit, and mean cell hemoglobin concentration, and increased mean cell volume.

The increase in urea and creatinine and the imbalances of electrolytes may be related to the morphological tubular changes noted in the kidneys. Other findings in the clinical laboratory investigations comprising of an increase in lipids and proteins and changes in enzyme activity, suggest metabolic adaptive changes, primarily related to the liver.

ORGAN WEIGHTS

The increased relative testes weights in group 5 (5000 ppm) subgroup A (one year of exposure) males and of the absolute and relative testes weights in subgroup C (two years of exposure) males of the mid-high (1600 ppm) and high dose levels (5000 ppm) were considered to be test item related. These findings also correlated with the results of the histopathological examination.

In males from subgroup A (one year of exposure), a test item related effect could not be ruled out for the increase in absolute and relative liver weight at 5000 ppm or the relative liver weight at 1600 ppm. However, there were no correlated findings at histopathological examination. Therefore, the increased liver weight in males but not in females after one year of exposure could be the consequence of a transient metabolic adaptation without any toxicological relevance. This finding was not diagnosed in subgroup C (two years of exposure) animals of both sexes.

MACROSCOPIC EXAMINATION

In subgroup A (one year of exposure), a macroscopic observation of enlarged liver was recorded in group 5 (5000 ppm) males. This finding correlated with the increased liver weight.

In subgroup C (two years of exposure), nodules in the kidneys were present in 3 males from group 4 (1600 ppm) and 7 males from group 5 (5000 ppm). This finding correlated with the results of the histopathological examination.

In subgroup C (two years of exposure), the incidence of enlarged testes was higher in all treated male groups than in the control group. The observation in groups 4 (1600 ppm) and 5 (5000 ppm) correlated with the recorded increase in testicular weight for these groups and the results of the histopathological examination.

HISTOPATHOLOGY

At interim sacrifice after one year exposure as well as at terminal sacrifice after two years, a variety of non-neoplastic and neoplastic findings diagnosed in this study which were considered to be incidental findings commonly observed in rats of this age and strain and which did not distinguish exposed animals from controls.

The following findings and/or increased incidences of findings were considered to be related to the exposure to the test item:

NON-NEOPLASTIC FINDINGS

Sacrifice after one year of exposure (subgroup A)

Kidneys: An increased incidence of calcified cylinders in the tubules of the papilla was noted in males of groups 4 (1600 ppm) and 5 (5000 ppm). Further, tubular granular casts were present in 2 male animals of group 5 (5000 ppm). Increased severity of cortical mineralization was recorded in males of groups 3 (400 ppm), 4 (1600 ppm) and 5 (5000 ppm). Also present in the kidneys was an increase in the incidence of tubular hyaline casts in male animals of group 5 (5000 ppm) and female animals of group 4 (1600 ppm).

Testes: Multifocal Leydig cell hyperplasia was found to be present in all male animals regardless of dose by using morphometric methods. An increased severity of Leydig cell hyperplasia was noted in all groups exposed to HMDS.

Nasal cavity: An increased incidence of eosinophilic inclusions was recorded in the olfactory and respiratory epithelium in males of all exposure groups.

All other findings diagnosed after one year of exposure were considered to be incidental findings commonly observed in rats of this age and strain.

Sacrifice after two years of exposure (subgroup C)

Kidneys: Calcified cylinders were noted in the tubules of the papilla in males of groups 3 (400 ppm), 4 (1600 ppm), and 5 (5000 ppm). Further, transitional cell hyperplasia was observed in male animals of groups 4 (1600 ppm) and 5 (5000 ppm). An increased incidence of tubular pigment deposits was noted in male animals of groups 4 (1600 ppm) and 5 (5000 ppm) whereas the incidence was decreased in female animals of groups 2 (100 ppm) and 5 (5000 ppm). Similarly, an increased incidence of cortical mineralization was recorded in males of groups 4 (1600 ppm) and 5 (5000 ppm) whereas a decreased incidence was recorded in females of all exposure groups.

Lungs: The incidence of alveolar histiocytosis was increased in females of groups 4 (1600 ppm) and 5 (5000 ppm), focal alveolitis was increased in males and females of group 5 (5000 ppm) and foreign body granuloma was increased in males of group 5 (5000 ppm).

Nasal cavity: An increased incidence and severity of eosinophilic inclusions in the olfactory/respiratory epithelium was recorded in males of groups 4 (1600 ppm) and 5 (5000 ppm).

Sternum/Femur: An increased incidence and severity of enchondral hyperostosis was recorded in the sternum of males of group 5 (5000 ppm) whereas a decreased incidence of enchondral hyperostosis was noted in both sternum and femur of females in groups 2 to 5 (100 to 5000 ppm) and in the femur of males in groups 2 to 4 (100 to 1600 ppm).

Bone marrow: An increased incidence of atrophy was recorded in females of group 5 (5000 ppm).

All other findings diagnosed after two years of exposure were considered to be incidental findings commonly observed in rats of this age and strain.

Sacrifice after one year of exposure and one year of recovery (subgroup B)

Kidneys: An increased incidence of calcified cylinders in the tubules of the papilla was noted in males of groups 3 (400 ppm), 4 (1600 ppm) and 5 (5000 ppm). Further, the incidence of transitional cell hyperplasia was increased in male animals of groups 4 (1600 ppm) and 5 (5000 ppm). Also present in the kidneys was an increase in the incidence of tubular hyaline casts in male animals of group 5 (5000 ppm).

Nasal cavity: An increased incidence of eosinophilic inclusions in the olfactory/respiratory epithelium was recorded in males of groups 4 (1600 ppm) and 5 (5000 ppm) and in females of groups 2-5 (100 to 5000 ppm).

Sternum: An increased incidence of enchondral hyperostosis was recorded in males of group 5 (5000 ppm) whereas the incidence was decreased in females of groups 2-5 (100 to 5000 ppm).

All other findings diagnosed after one year of exposure and one year of recovery were considered to be incidental findings commonly observed in rats of this age and strain.

NEOPLASTIC FINDINGS:

In the testes, there was statistically significantly increased incidence of Leydig cell tumors at all dose levels when compared to controls after one year of exposure (subgroup A). The majority of animals in subgroups B (one year of exposure and one year of recovery) and C (two years of exposure) developed Leydig cell tumors regardless of dose.

From the neoplastic findings diagnosed in this study, the occurrence of renal tubular adenomas and carcinomas in males of groups 4 (1600 ppm) and 5 (5000 ppm) after two years of exposure (subgroup C) are not excluded as possibly related to the exposure to the test item, since these findings did not occur in control group 1 (0 ppm), groups 2 (100 ppm) and 3 (400 ppm).

8 PURPOSE

The purpose of this 24 months combined toxicity/carcinogenicity inhalation study was to evaluate the possible chronic and oncogenic effects associated with administration of hexamethyldisiloxane (HMDS) to rats by inhalation (whole body exposure) upon continuous daily vapor inhalation exposure (6 hours/day, 5 days/week) for a period of one or two years.

9 MATERIALS AND METHODS

9.1 TEST SYSTEM

Test System	Albino Rat, Fischer 344, SPF quality (CDF(F-344)/CrlBR). Colony health status: certified to be free of virus. Monitored for bacterial and parasitic pathogens.
Rationale for Selection of Test System	This species and strain of animals is recognized as appropriate for chronic and oncogenicity studies. The Fischer 344 rats were utilised because it is a widely used strain for which significant historical control data are available. A higher number of animals than the minimum required by the referenced test guidelines was used. This was intended to provide a robust data set of increased power to more fully evaluate and interpret results of the study.
Source	Charles River Laboratories; Raleigh, North Carolina, U.S.A.
Number of Groups	5 (1 negative control, 4 treated)
Number of Animals per Group	210 (105 males, 105 females)
Number of Subgroups	3 (A, B and C or K1, R1 and K0 as used in histopathology data, respectively)
Number of Animals per Subgroup per Dose Group	Subgroup A: 40 animals/group for interim sacrifice after 1 year (20 m, 20 f) Subgroup B: 40 animals/group for 1 year of exposure and 1 year of recovery (20 m, 20 f) Subgroup C: 130 animals/group for 2 years of exposure (65 m, 65 f)

Number of animals/Subgroup/Dose group

Subgroup	Group 1 (0 ppm)	Group 2 (100 ppm)	Group 3 (400 ppm)	Group 4 (1600 ppm)	Group 5 (5000 ppm)
A (one year of exposure)	20m, 20f	20m, 20f	20m, 20f	20m, 20f	20m, 20f
B (one year of exposure and one year of recovery)	20m, 20f	20m, 20f	20m, 20f	20m, 20f	20m, 20f
C (two years of exposure)	65m, 65f	65m, 65f	65m, 65f	65m, 65f	65m, 65f
Total number of animals per group	210 (105m, 105f)	210 (105m, 105f)	210 (105m, 105f)	210 (105m, 105f)	210 (105m, 105f)

Additionally, in groups 1 (0 ppm), 2 (100 ppm), 3 (400 ppm), 4 (1600 ppm), and 5 (5000 ppm) of subgroup C (two years of exposure) blood sampling for hematological and clinical biochemistry investigations and urine collection for urinalysis were performed on the first 20 male animals throughout the study. Similarly, the above-mentioned clinical laboratory investigations were performed on the first 20 female animals of groups 1 (0 ppm), 2 (100 ppm), 4 (1600 ppm) and 5 (5000 ppm) and in animals 596 to 615 of group 3 (400 ppm) throughout the study.

Total Number of Animals in the study

1050 (525 males, 525 females)
Additionally, 10 males and 10 females were used for disease surveillance. The fate of these animals was documented such as they were separated from the main study animals.

Total Number of Animals ordered

1120 (560 males, 560 females)

Delivery

Both male and female animals were 4 weeks old and their weight ranged between 30 and 50 grams. The shipment date was 18-AUG-1997, arrival date at RCC was 20-AUG-1997 in the afternoon. Both study director and study veterinarian reviewed the documentation and checked the health status of the animals. All animals were alive and in good health conditions. Overnight the animals were allocated in Makrolon® cages to room 3.11. Food and water were supplied.

Age at the First Exposure

6 weeks (both sexes)

Body Weight at Randomisation

Males			Females		
Minimum	Maximum	Mean	Minimum	Maximum	Mean
32.3	62.2	49.1	32.3	57.6	46.6

Body Weight at Start of treatment

Males		Females	
Minimum	Maximum	Minimum	Maximum
84.9	129.2	73.4	114.8

Randomisation

On the first day of acclimatisation (21-AUG-1997), the animals were randomly assigned to the different groups and subgroups using a computer generated stratified random sampling procedure, stratified by body weight.

Identification

By subcutaneously implanted uniquely numbered transponders and corresponding ear number. The transponders were validated before use. Initially, during the early part of the study, the animals were identified by tail numbers before the individual transponders were implanted. The transponders were implanted between the 16th and 21st of September 1997.

Acclimatisation

From 21 to 31-AUG-1997 under laboratory conditions, after clinical health examination. Only animals without any visible sign of illness were used for the study.

Habituation period: during the last days of the acclimatisation period the animals were accustomed to the exposure conditions (without test item) maintaining them in wire cages and exposure chambers for 5 daily periods of approximately 30 minutes, 1, 2, 4 and 5 hours, respectively.

The remaining animals from the delivery not assigned to the treatment groups or disease surveillance (25 males and 25 females) were retained as reserves during the acclimatisation phase for possible replacement of weak animals prior to the start of the treatment phase. All reserve animals not used for substitution as well as replaced animals were euthanised on the first day of exposure.

9.2 ALLOCATION

Subgroup A - allocated to evaluation of toxic effects over one year
Animals scheduled for interim sacrifice after one year.

Subgroup B - allocated to evaluation of toxic effects over one year of exposure followed by one year of recovery
Animals scheduled for interim sacrifice after two years.

Subgroup C - Allocated to oncogenicity evaluation
Animals scheduled for sacrifice after 2 years of exposure.

Animal identification / Subgroup / Dose group						
Exposure Concentration		**Group 1** Air only	**Group 2** 0.7 mg/L 100 ppm	**Group 3** 2.7 mg/L 400 ppm	**Group 4** 10.6 mg/L 1600 ppm	**Group 5** 33.2 mg/L 5000 ppm
		Animal Numbers				
Males	Subgroup A	1 - 20	211 - 230	421 - 440	631 – 650	841 - 860
	Subgroup B	21 - 40	231 - 250	441 - 460	651 – 670	861 - 880
	Subgroup C*	41 - 105	251 - 315	461 - 525	671 – 735	881 - 945
Females	Subgroup A	106 - 125	316 - 335	526 - 545	736 – 755	946 - 965
	Subgroup B	126 - 145	336 - 355	546 - 565	756 - 775	966 - 985
	Subgroup C*	146 - 210	356 - 420	566 - 630	776 – 840	986 - 1050
Disease surveillance		Males: 1051 - 1060 Females: 1061 - 1070				

* Additionally, in groups 1 (0 ppm), 2 (100 ppm), 3 (400 ppm), 4 (1600 ppm), and 5 (5000 ppm) of subgroup C (two years of exposure) blood sampling for hematological and clinical biochemistry investigations and urine collection for urinalysis were performed in the first 20 male animals throughout the study. Similarly, the above-mentioned clinical laboratory investigations were performed in the first 20 female animals of groups 1 (0 ppm), 2 (100 ppm), 4 (1600 ppm) and 5 (5000 ppm) and in female animals 596 to 615 of group 3 (400 ppm) throughout the study.

9.3 HUSBANDRY

Room numbers

Rooms 3.11 (group 1, air control), 3.13 (groups 2 and 3) and 3.15 (groups 4 and 5) in RCC Ltd, Füllinsdorf were utilised. The animals remained in the respective rooms during exposure as well as during the after exposure period.

All animals in subgroup B (one year of exposure and one year of recovery) were allocated to room 3.11 during the non-exposure (2nd) year.

Laboratory Conditions

The study was conducted under optimum hygienic conditions behind a barrier system. The rooms were air-conditioned with air changes of 10-15 per hour (recorded prior to study initiation and regularly checked thereafter), and had a 12-hour light period (artificial fluorescent light) and a 12-hour dark period. Each room was monitored continuously, using a data logger, for temperature and relative humidity. The target ranges were: for temperature of 22 ± 3°C and for relative humidity of 40 - 70%.

In room 3.11 the temperature was in the given range (mainly between 19.5 and 23°C) except for a very short period on the 24th of October 1997 with a value of 18.2°C,. The humidity was generally within the given range. However, peaks higher than 70% relative humidity (%rel) (but mostly less than 80%rel) were regularly recorded. This was caused by the daily cleaning procedure in the room. Values below 40% were seen for some periods during the winter months, however the values were usually higher than 36%rel and no values below 32%rel were recorded.

In room 3.13 the temperature ranged between 20 and 24 °C as per target (mainly between 20 and 23°C). The humidity was generally between the given range. However, peaks higher than 70%rel (but less than 80%rel except for a single peak of 85%rel on the 22nd of February 1998) were regularly recorded. This was caused by the daily cleaning procedure in the room. Values below 40%rel were seen for some periods during the winter months, however the values were usually higher than 36%rel and no values below 32 %rel were recorded.

In room 3.15 the temperature was in the given range (mainly between 19.5 and 23°C) except for a very short period on the 2nd June 1998 with a value of 18.7°C. On few occasions, values up to 23.7°C were recorded. The humidity was generally in the given range. However, peaks higher than 70%rel (but always less than 80%rel) were regularly recorded. This was caused by the daily cleaning procedure in the room. Values below 40% were seen for some periods during the winter months, however the values were usually higher than 36%rel and no values below 32%rel were recorded.

The room air was sampled with a gas bag (Chrompack, Tedlar Sample Bag) and analysed by GC for test material contamination. The air in the rooms 3.11, 3.13 and 3.15 was analysed each day during the first three treatment days and weekly thereafter. All measurements were below the detection limit of 2-5 ppm.

Accommodation

All periods except during the exposure

Animals were housed in groups of five of the same sex in Makrolon® type-IV cages (approximate dimensions in cm 33.5 Length x 20 Wide x 13.5 Height) with wire mesh tops and standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz, Switzerland). Bedding, cages and bottles were autoclaved prior to use. Results of bedding analyses for contaminants are included in the report (Attachment 3, pp. 2350 to 2379). The placement of each cage in the rack was rotated vertically every month and recorded after rotation. The first rotation was performed in Week 6/7.

Exposure period

During the 6 hours whole body vapor inhalation exposure period (5 days/week), the animals were housed in groups of 2 of the same sex in stainless steel wire cages in sealed chambers used for group isolation (Constructions Inoxydables S.A., CH-1618 Châtel-Saint-Denis, Switzerland). The cages unit was rotated weekly within the chamber. Each rotation was documented. During this period neither water nor food was supplied to the animals. All chambers (including the cage units) were sterilized by 24-hour exposure to formaldehyde vapor and allowed to off gas prior to use.

Diet

Pelleted standard Kliba 343 rat maintenance diet, batch number 88/97 and Kliba 3433 rat maintenance diet, batch nos. 90/97, 94/97, 20/98, 23/98, 24/98, 25/98, 27/98, 28/98, 31/98, 33/98, 35/99, 37/99, 39/99 (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) was provided *ad libitum*, except during exposure. Results of analysis for contaminants with acceptability limits are included in this report (Attachment 3, pp. 2307 to 2334). The composition of 343 and 3433 maintenance food is the same, the code used to describe the diet was changed by the manufacturer from 343 to 3433.

Water

Community tap water from Füllinsdorf chlorinated at approximately 0.5 ppm, was available *ad libitum*, except during exposure. Results of bacteriological, chemical and contaminant analysis with acceptability limits are included in this report (Attachment 3, pp. 2335 to 2349).

None of the contaminants found in food or water are expected to have any impact on the quality or integrity of the study.

9.4 DISEASE SURVEILLANCE

Data provided by the Supplier

Report of animal colony health status was provided by the supplier and filed in the study raw data. No relevant findings were reported.

Standard Disease Surveillance during the Study

The disease surveillance animals were distributed in the different rooms as follows:

	Males	Females
Room 3.11	1051 - 1053	1061 - 1063
Room 3.13	1054 - 1057	1064 - 1066
Room 3.15	1058 - 1060	1067 - 1070

Non-heparinised blood samples (approximately 2 mL) were obtained by retro-orbital puncture under light ether anaesthesia. The blood samples were immediately forwarded to the Department of Microbiology of RCC BAB, Füllinsdorf, attn. S. Kehl. Serum was obtained following centrifugation of blood.

The serum obtained from each animal at each time-point was split in two parts. The first was immediately deep-frozen and stored at -20°C for possible later investigations prescribed by the Sponsor under separate contractual agreement.

The second portion was used for screening for the presence of antibodies to the following rodent pathogens:

Hantaan virus
Lymphocytic choriomeningitis virus (LCM)
Pneumonia virus of mice (PVM)
Sialodacryoadenitis (SDA)/Rat coronavirus (RCV)
Toolan (H-1)
Bacillus piliformis (Tyzzer's)
Kilham rat virus (KRV)
Minute virus of mice (MVM)
Reovirus type 3 (Reo3)
Sendai virus (parainfluenza-1 virus)
Mycoplasma pulmonis
Rat parvovirus (ROPV)

Reference: see "Diagnostic Microbiology for Laboratory Animals" Ivo Kunstyr, Gustav Fischer Verlag (1992).

Blood was collected on 01-OCT-1997, 24-FEB-1998, 25-AUG-1998, 23-FEB-1999 and 25-AUG-1999. For the latter 4 time-points none of the parameters analyzed was positive at any time point. The results for 01-OCT-1997 are not available.

9.5 TEST ITEM

(Data as provided by the Sponsor)

Identification and code	Hexamethyldisiloxane (HMDS)
Supplier	Dow Corning Corporation, Midland, MI, USA
Description	Crystal clear liquid, free of suspended matter and sediment
Batch Number	1st lot: lot #BB096032 2nd lot: lot #AA017006 3rd lot: lot #0000220953 (supplied as Dow Corning® OS-10, study requirement exceeded single lot production capacity)
Purity	1st lot (lot #BB096032): 99.99% 2nd lot (lot #AA017006): 99.95% 3rd lot (lot # 0000220953): 99.998%
Stability	Test item is stable under specified storage conditions. The scheduled purity testing of the first and second lot of test item on samples retained in Midland, MI approximately one year and two years after the start of the study was not performed.
Retention Sample	For each drum in the study a sample of about 20 mL was taken for possible analysis at a later date. Up to 10 mL of this sample were sent to the Sponsor for analysis as described under "Purity Verification" below.
CAS Number	107-46-0
Instructions for Storage	Keep container closed and away from heat, sparks, and open flame. In practice: stored at room temperature.
Expiry Date	1st lot (lot #BB096032): 30-SEP-2001 2nd lot (lot #AA017006): 31-JAN-2002 3rd lot (lot # 0000220953): 01-MAR-2004
Requested Quantity	ca. 5'350 kilograms
Safety precautions	Eye protection, long sleeves (lab coat), face mask and latex or nitrile gloves were required when handling the test item. The face mask was replaced by a full face, filtered (A2-P3), positive air supplied respirator during exposure. As HMDS is a highly flammable substance, all equipment was grounded to earth to minimise potential static charge build up.

Purity Verification

The portions (up to 10 mL) of the drum retention samples (described above under "Retention Sample") sent to Dow Corning were handled as follows:

Three deliveries were made during the course of the study. The first delivery was sent on 24-OCT-1997 (lot #AA017006), the second delivery on 06-OCT-1998 (lot #AA017006), and the third delivery on 01-OCT-1999 (lot #BB096032, lot #AA017006, and lot # 0000220953) at the end of the in-life phase of the study.

Labelling

- Samples shipped to Dow Corning: the samples were labelled with the following information: study number, test item name, drum number and date the samples were taken.
- GC vials for purity determination: the GC vials were labelled and contained the following information: study number, test item name, drum number and date the samples were taken.

Sample Preparation

No sample preparation was required since neat test item was analysed.

GC/TCD Analysis

The conditions were:

Oven Program:	40°C for 3 minutes followed by a 15°C/min ramp to 250°C for 5 minutes
Inlet Temperature:	250 °C
Capillary Column:	HP-5 (5%-Diphenyl-95%-dimethylsiloxane), 320 µm inner diameter, 30 m length, 0.25 µm film thickness
Mode:	Constant flow, Helium carrier
Initial Flow:	1.1 mL/min
Split Ratio:	40:1

The GC was operated and maintained according to Dow Corning SOP 13.15, except prior to test item analysis, the oven temperature was increased to approximately 280°C for at least 3 min to prevent carry over by heating the column to burn off any residual material left from previous analyses. Once a stable baseline was observed (2 to 3 min), the GC performance was considered acceptable and operation was continued. The actual instrument conditions were documented in the laboratory notebook or study file.

An area percent purity of the major component, HMDS was determined based upon the average of 5 replicate injections (approximately 1 µL) of HMDS. Data were collected by GC/TCD for each accession number using an autosampler.

Data Analysis

Calculations of the mean and standard deviation of the GC/TCD area percent results for HMDS were performed.

The results are presented in Attachment 5.

9.6 EXPOSURE PHASE

9.6.1 GENERAL

Room Numbers

Rooms 3.11 (group 1), 3.13 (groups 2 & 3) and 3.15 (groups 4 & 5) *The subgroup B recovery animals of all groups were housed in room* 3.11 during the 2nd (non-exposure) year.

Method

Vapor inhalation by whole-body exposure

Rationale for Method

The route of administration was inhalation of vapor (using whole body exposure methods) since this is a possible route of human exposure and is standard methodology for the assessment of chronic toxicity and oncogenic potential.

Frequency of Exposures

6 hours/day calculated from pump on to pump off (i.e. including the equilibration time but excluding the decay time), 5 days/week

Duration of the Exposure Phase

Each animal was exposed until the day preceding sacrifice (except for necropsies performed on Mondays)

Exposure Phase

Subgroup A (interim sacrifice after 1 year): 52 weeks = at least 260 exposures

Subgroup B (sacrifice after 1 year of exposure and 1 year of recovery): 52 weeks = at least 260 exposures

Subgroup C (oncogenicity phase, sacrifice after 2 years): target exposure duration 104 weeks = at least 520 exposures.

Exposure Concentrations

Group 1	Filtered air only	=	0 ppm	*Air control*
Group 2	0.7 mg/L air	=	100 ppm	Low concentration
Group 3	2.7 mg/L air	=	400 ppm	Low-mid concentration
Group 4	10.6 mg/L air	=	1600 ppm	High-mid concentration
Group 5	33.2 mg/L air	=	5000 ppm	High concentration

Rationale for Selection of Exposure Levels

Selected by the Sponsor on the basis of RCC Study Number 655301: A Thirteen Week Whole Body Vapor Inhalation Study of hexamethyldisiloxane (HMDS) in Fischer-344 Rats. Further, the choice of the highest concentration was based upon safety considerations and was set at half the Lower Explosive Limit (LEL) for HMDS.

9.6.2 EXPOSURE METHODS

Inhalation Exposure System

Vapor inhalation whole body exposure was performed in sealed chambers used for group isolation (Constructions Inoxydables S.A., CH-1618 Châtel-Saint-Denis, Switzerland). These chambers are constructed entirely of stainless steel. Glass doors are equipped with silicone rubber seals.

The chamber can contain 9 stainless steel wire cage units with an excreta pan below each cage unit.

The data of the exposure chambers are summarized in the table below:

Exposure Chambers			
Chamber Dimensions intern: L x W x H (cm) Volume (liter)	Individual Cage Dimensions: L x W x H (cm)	Number of individually housed animals per cage unit (Each cage unit comprises 8 compartments)	Total Animal Housing (9 cage units)
150 x 96 x 140 ca. 2200	136 x 40 x 13	16 maximum Effective 1st year: 11-12 2nd year: 14-15	144 maximum Effective 1st year: 105 2nd year: 130

During the first year, two exposure chambers per group were used exposing the 105 males and the 105 females separately in each group (total number of chambers = 10). During the second year, exposure was performed only for the subgroup C animals (oncogenicity phase, two years of exposure): 65 males and 65 females = 130 animals (maximally). Therefore males and females from the same group were exposed together in the same chamber.

Test Item Generation

Since the saturated vapor concentration of HMDS was experimentally evaluated to be 370 mg/L air (= 55'500 ppm), the test atmosphere was expected to be in the vapor phase without liquid or mist particles. The HMDS was pumped into a glass round bottomed flask with a valveless metering pump. The volume of the container (between 0.5 and 2 litres) and the flow rates were proportional to the atmosphere concentrations to be generated in the different groups and corresponded to the minimal amount necessary to ensure complete vaporisation. Compressed air (40 L/min) was supplied into the glass flasks and allowed the liquid to equilibrate with the temperature of the warm walls of the container. The vapor produced then passed through a pipe filled with specially made small glass tubes to provide a greater surface area for complete vaporisation. Incomplete vaporisation was indicated by the appearance of liquid test material at the bottom or at the top of the pipe. The temperature of the HMDS vapor in the round flasks was approx. 35 - 40°C and generally between 25 - 30°C at the top of the pipe, with a few exceptions when it was outside the range at the top. The air containing HMDS was then mixed and diluted with 380 L/min of the filtered air to the chamber inlet duct.

Chamber Concentration

Chamber concentrations of HMDS, homogeneity and stability of all individual chambers which were used during the study for exposure were determined prior to the start of the exposure phase. The test item was generated in the chamber at the concentrations which were used in the study. Five GC-sample lines were put in each chamber and connected with the GC. Test item concentration in the chamber was determined in each of the 5 sample points for at least three cycles (duration about 2 hours). Confirmation of complete vaporisation at the highest dose level was qualitatively carried out with aerosol measuring equipment (Mini-Ram, PDM-3, CGA Corporation, Technology Division, Bedford, Massachusetts 01730, U.S.A.). Measurement was performed once before start of exposure, once during the first month of exposure, once in the first month of the second year and once toward the end of the 2nd year of exposure.

9.6.3 EXPOSURE SYSTEM MONITORING

The vapor concentration of the test item (analytical), relative humidity, air flow, temperature and oxygen concentration of the chamber test atmosphere were recorded as specified below.

Nominal Concentration

The nominal concentration was determined once daily by weighing the test item container before and after each exposure to determine the quantity of test item used. The weight was then divided by the total air flow volume to give the nominal concentration. The laboratory personnel immediately informed the study director if the daily nominal concentration was outside the following ranges:

Group Number	Nominal Concentration		Target Concentration	
Group 2	97.1-107.1 ppm	(0.68 - 0.75 mg/L)	100 ppm	(0.7 mg/L)
Group 3	391.1-428.1 ppm	(2.64 - 2.89 mg/L)	400 ppm	(2.7 mg/L)
Group 4	1509.4-1720.8 ppm	(10.0 - 11.4 mg/L)	1600 ppm	(10.6 mg/L)
Group 5	4894.6-5241.0 ppm	(32.5 - 34.8 mg/L)	5000 ppm	(33.2 mg/L)

Analytical Concentrations

- Frequency of analysis

  The concentration in each chamber of the dose groups was determined daily at least 6 times during the exposure period. Start of analysis was about 20 to 30 minutes after the beginning of generation and continued until pump off (i.e. about 5½ hours out of 6 hours exposure). GC sample line integrity and losses needed to be checked before the study and at a minimum of every 6 months.

  The air in the chambers of the control group was sampled (with a gas bag) three times per exposure period: at the beginning, at an intermediate time and at the end of the period, and analysed by GC under the same conditions as described below.

- Method of analysis

  Analytical concentrations were determined by GC analysis based upon a method supplied by the Sponsor as following:

Column:	Fused silica DB-5; 10 m / 0.55 m 1.5 µm film thickness
Oven:	50 °C / 4 min.
Runtime:	5 min
Injector:	225 °C
Detector:	250 °C
Carrier gas:	He 0.25 bar
Sample Loop:	150 µL
Retention Time:	ca. 1.1 min

  The target calibration schedule was every two weeks. However, on a few occasions (i.e. on 02-OCT-1997, one week after the last calibration) in accordance with the study director, an additional GC calibration was performed. Daily bag standard at chambers on sequential basis with plot of actual and nominal concentration to assess trends. If the bag standard response was not between 10% range of the anticipated response another standard was prepared. If the second standard was also not between this 10% range the technical co-ordinator was informed to perform correction actions. If necessary, the GC was re-calibrated. The study director was informed if the mean daily analytical concentrations varied by more than ± 5 % from the target.

Oxygen Concentration

In the dose groups 2 to 5, the oxygen concentration was recorded in all chambers once daily during the 6-hour whole body inhalation exposure period by connecting the analogue output to an oxygen sensor. The values were recorded on data-sheet and are presented in Attachment 1. On some occasions oxygen concentration was not recorded manually. Oxygen concentration in the control chambers was continuously monitored using an oxygen sensor (Pewatron AG, CH-8304 Wallisellen/Switzerland) connected to a data logger.

Relative Humidity/Temperature

The relative humidity (%rel) and temperature were continuously monitored over the 6 hour whole body inhalation exposure period by connecting the analogue outputs of a ROTRONIC temperature and humidity probe to the RCC data-logger system which recorded the mean values at five-minute intervals. Controls were set to maintain daily mean chamber temperature and relative humidity at 22 ± 3 °C and 40 - 60 %, respectively. The print-out from the RCC data-logger system is a diagram which is kept in the raw-data, and not in tabulated form. Therefore in order to include these data in the report, three times during each exposure (once during first hour, once in the middle of the exposure period and once towards the end) the laboratory personnel recorded the current value on data-sheet (except for Week 1). These values were typed into an Excel Table and are given in Attachment 1. On some isolated occasions, the temperature and relative humidity were outside the given range.

Exposure Airflow Rate

The exposure airflow rate was continuously monitored using pressure transducer metering devices. The analogue outputs of these devices were connected to the RCC data-logger system and the values were recorded at five-minute intervals. Airflow through the chambers was such that there were 12-15 air changes per hour and a negative pressure of approximately 2-3 mm H_2O.

9.7 CLINICAL OBSERVATIONS

The following observations and data were recorded:

Mortality

All animals were observed daily for mortality/moribundity during the acclimatisation phase, and twice daily seven days per week during the treatment and recovery phases (before the start and after the completion of each exposure on treatment days).

Clinical Signs

Clinical signs were recorded twice a week throughout the study (including acclimatisation, exposure and recovery phases). The last recording was performed on 01-SEP-1999.

Time of onset, intensity and duration of the observation were recorded. Observations included, but were not limited to: changes in behaviour, somatomotor activity, body position, respiration, alterations of the skin (including skin lesions and hair loss), nose and eyes.

Additionally, palpation for early detection of non-visible masses was performed on a weekly basis from 6 months after start of exposure until termination. Special attention was given to mass development for each visible and palpable mass. The following data were recorded: time of onset, location, size, appearance and progression.

Body Weights

During the acclimatisation phase, each animal was weighed on day 1 (used for randomisation) and 7 (27-AUG-1997, start of habituation). On the first day of treatment the animals were weighed before exposure, then once weekly for the first 14 weeks. Thereafter they were weighed every two weeks. The last recording was performed on 01-SEP-1999. The body weight of one female was inadvertently not recorded at Week 64.

Food Consumption

Food consumption was measured for each cage twice during the acclimatisation phase, then once weekly over a 7-day period for the first 14 weeks. Thereafter, food consumption was generally measured over a 7-day period every two weeks. On some occasions, recording was delayed. The last food consumption was measured on 01-SEP-1999.

The daily food consumption (FC, g/animal/day) was calculated by dividing the amount of food consumed over the respective time intervals by the number of days of each interval and the number of animals. Relative food consumption (RFC, g/kg body weight/day) was calculated by dividing the daily food consumption by the group mean body weight of the animals at the end of the interval. As a consequence, the values presented in the food consumption result tables are average values for each animal.

Ophthalmoscopic Examinations

During the acclimatisation phase, the ophthalmoscopic examination was performed on the last 20 animals/group/sex from the allocation subgroup C (two years of exposure) animals (100 males and 100 females).

Animals examined during acclimatisation

Animal Numbers					
	Group1 (0 ppm)	Group 2 (100 ppm)	Group 3 (400 ppm)	Group 4 (1600 ppm)	Group 5 (5000 ppm)
Males	86 - 105	296 - 315	506 - 525	716 - 735	926 - 945
Females	191 - 210	401 - 420	611 - 630	821 - 840	1031 - 1050

Towards the end of the first year and towards study termination, ophthalmoscopic examination was performed on the last 20 surviving animals/group/sex from the allocation subgroup C (two years of exposure) animals. For the group 3 (400 ppm) females the animals 596 to 615 were not considered for ophthalmoscopic examination (due to possible interference from lesions which might have occurred as a result of retro-orbital blood sampling).

Animals examined towards the end of the first year

Animal Numbers					
	Group1 (0 ppm)	Group 2 (100 ppm)	Group 3 (400 ppm)	Group 4 (1600 ppm)	Group 5 (5000 ppm)
Males	86 - 105	296 - 315	505 - 517; 519 - 525	716 - 735	926 - 945
Females	191 - 210	400 - 404; 406 - 420	591 - 595; 616 - 630	820 - 832; 834 - 840	1031 - 1050

Animals examined towards study termination

Animal Numbers		
Group 1 (0 ppm)	Males	73; 75-78; 83; 84; 86; 87; 91-93; 95; 96; 99-105
	Females	187-190; 192; 193; 195-198; 200-207; 209; 210
Group 2 (100 ppm)	Males	284; 285; 287-290; 292; 293; 295-297; 299; 303; 305-307; 310-312; 314
	Females	399; 401-404; 406-420
Group 3 (400 ppm)	Males	496-498; 500; 502; 504-510; 512; 513; 519; 520; 522-525
	Females	589-595; 616-621; 623; 625-630
Group 4 (1600 ppm)	Males	712-714; 717-720; 722-727; 729-735
	Females	816-818; 820; 821; 823-832; 835-838; 840
Group 5 (5000 ppm)	Males	918-920; 922-926; 928; 930-932; 934-936; 939; 940; 942; 943; 945
	Females	1028-1034; 1036; 1037; 1039-1045; 1047-1050

In all animals allocated for ophthalmoscopic examination, after the application of a mydriatic solution (Dispersa AG, Winterthur/Switzerland) the cornea, lens, anterior chamber, vitreous body and ocular fundus of both eyes were examined under standard light conditions using a Heine Miraflex 2 Ophthalmoscope (Eisenhut Vet AG, Allschwil, Switzerland).

Schedule

During acclimatisation phase	25 and 26-AUG-1997
Within one month of the end of the first exposure year	04-AUG-1998
Within one month of termination	05-AUG-1999

10 CLINICAL LABORATORY INVESTIGATIONS

10.1 GENERAL

Collection of Blood and Urine Samples

Blood samples for hematology and clinical biochemistry were collected from the first 20 animals/sex/group (groups 1, 2, 4 and 5, and group 3 males) of the subgroup C (two years of exposure) animals throughout the study under light ether anesthesia. As for group 3 females, animal nos. 596-615 of the subgroup C were used throughout the study for clinical laboratory investigations. The animals were fasted in metabolism cages for approximately 17 hours before blood sampling but allowed access to water *ad libitum*. Blood samples were collected early in the working day between the hours of 05:30 and 08:00 to reduce biological variation caused by circadian rhythms. Blood samples were drawn from the retro-orbital plexus using a micro-hematocrit glass capillary tube. Urine was collected during the 17-hour fasting period into a specimen vial placed underneath the metabolism cage.

Sampling dates

After 3 months of exposure	males	29-NOV-1997
	females	30-NOV-1997
After 6 months of exposure	males	28-FEB-1998
	females	01-MAR-1998
After 12 months of exposure	males	29-AUG-1998
	females	30-AUG-1998
Blood smears (Rats killed in extremis)	09-MAR-1999 – 01-SEP-1999	
Blood smears (before necropsy)	30-AUG-1999 – 14-SEP-1999	

Assays

The assays of blood and urine samples were performed at RCC Ltd, Toxicology Division, Füllinsdorf, under internal laboratory quality control conditions to assure reliable test results. Clinical laboratory data are expressed in general accordance with the International System of Units (SI). In some cases, non-SI units or conventional units may be used.

Key to Abbreviations of Units of Measure

L	=	liter	T	= tera	(10^{12})
kg	=	kilogram	G	= giga	(10^{9})
g	=	gram	m	= milli	(10^{-3})
sec	=	second	µ	= micro	(10^{-6})
mol	=	mole	n	= nano	(10^{-9})
kat	=	katal (*mol/sec*)	f	= femto	(10^{-15})

Presentation of data

The summary and individual tables were generated by a VAX computer. The program used limits the width of each column to 10 characters, therefore the names of some parameters have been abbreviated. Any abbreviated parameter appears in upper-case letters enclosed by parentheses.

The following abbreviations correspond to:

NS = no sample
NV = no value for one or more parameters
LIP = lipemic sample

Explanatory notes for data not presented in the tables are given on p. 1745. Historical reference values for untreated Fischer-344 rats are presented in Attachment 4, pp. 2381 to 2392.

10.2 HEMATOLOGY

The following anticoagulants were used for blood collection:

- Hematology: EDTA-K2
- Coagulation: Sodium citrate 3.8 %, at a 9:1 ratio of blood to anticoagulant

The following commercial reference controls were used to monitor the performance of the methods:

- Hematology: Eightcheck-3WP (normal range)
  Eightcheck-L-3WP (low abnormal range)
  Ret-check (reticulocyte control)
  (TOA Medical Electronics Co., Ltd. Kobe/Japan)
- Coagulation: IL Control Plasma (normal range)
  IL Control Plasma (abnormal range)
  (Instrumentation Laboratory, SpA, Milano/Italy)

The following methods/instrumentation were used to determine the parameters listed:

Instrumentation: Sysmex (TOA) E-4000 MultiParameter Automated Hematology Analyzer

Parameter	Unit	Method
Erythrocyte count (RBC)	T/L	Hydrodynamic focusing electrical resistance detection
Hemoglobin (HB)	mmol/L	Sodium-lauryl-sulfate hemoglobin (SLS-HB)
Hematocrit (HCT)	L/L	Cumulative pulse height detection
Mean corpuscular volume (MCV)	fL	Calculated value based on the following parameter factors: $MCV = \frac{\text{Hematocrit}}{\text{Erythrocyte count}}$
Mean corpuscular hemoglobin (MCH)	fmol	Calculated value based on the following parameter factors: $MCH = \frac{\text{Hemoglobin}}{\text{Erythrocyte count}}$

Parameter	Unit	Method
Mean corpuscular hemoglobin concentration (MCHC)	mmol/L	Calculated value based on the following parameter factors: $MCHC = \frac{\text{Hemoglobin}}{\text{Hematocrit}}$
Platelet count (PLATELETS)	G/L	Hydrodynamic focusing electrical resistance detection
Total leukocyte count (WBC)	G/L	Electrical resistance detection

Instrumentation: Sysmex (TOA) R-1000 Automated Reticulocyte Analyzer

Parameter	Unit	Method
Reticulocyte count (RETIC.)	% (rel.) T/L (abs)	Flow cytometric analysis based on RNA fluorescently labelled cells using Auramine-O and argon laser technology
Reticulocyte fluorescence ratios: HFR = high MFR = middle LFR = low	%	Flow cytometric analysis based on RNA fluorescently labelled cells using Auramine-O and argon laser technology

Instrumentation: Leitz Laborlux 12 or Dialux 22 light microscope, Baumann-Medical Mediff 8000 and/or 9000 DS 40 Computer Printer Blood Cell Calculator

Parameter	Unit	Method
Nucleated erythrocytes, normoblasts (NEN)	NEN/100 WBC	Microscopic examination. Reported as number of nucleated erythrocytes per 100 leukocytes in the differential leukocyte count (see below)
Differential leukocyte count (Diff. WBC Count)		Monocellular layer blood smear produced with an Omron Microx Centrifugal Spinner Blood smear stained with Wright's Eosin Methylene Blue solution using an Omron Microx Auto-Stainer
	1 (rel.) G/L (abs.)	Manual count of 100 leukocytes by light microscopy

Parameter	Unit	Method
Key to abbreviations of white blood cell classification:		
BAND. = Band Neutrophil SEG. = Segmented Neutrophil EO. = Eosinophil BASO. = Basophil LYMPH. = Lymphocyte MONO. = Monocyte PLAS. = Plasma Cell OTHER = Blast Cell (undifferentiated)		
Red blood cell morphology	normal/ abnormal (score)	Microscopic examination of stained blood smear. Erythrocytes that vary from the normal in size, shape and hemoglobin content, or contain greater amounts of nuclear remnants are indicated as abnormal erythrocytes, and are characterized as such.
	Normal: 0 Abnormal: 1 = slight 2 = moderate 3 = marked	
Key to abbreviations of red blood cell abnormalities:		
POLY. = Polychromatophilia		

Coagulation

Instrumentation: Instrumentation Laboratory (IL) ACL 300 Coagulation System

Parameter	Unit	Method
Thromboplastin time (=Prothrombin time) (PT)	sec	Automated laser-nephelometric centrifugal analyzer method, using an IL™ calcium thromboplastin reagent (a lyophilized extract from rabbit brain, certified according to ICSH/ICTH[1] recommendations)
Activated partial thromboplastin time (APTT)	sec	Automated laser-nephelometric centrifugal analyzer method, using an IL™ bovine brain cephalin, ellagic acid activated reagent

[1] ICSH = International Committee for Standardization in Hematology
ICTH = International Committee on Thrombosis and Hemostasis

10.3 CLINICAL BIOCHEMISTRY

The following anticoagulant was used for blood collection:

- Lithium heparin (15 I.U./ml)

The following commercial reference controls were used to monitor the performance of the methods:

- Precinorm U (normal range)
- Precipath U (abnormal range)
  (Roche Diagnostics GmbH, Mannheim/Germany)

- Lyphochek, Level 1 (normal range)
- Lyphochek, Level 2 (abnormal range)
  (Bio-Rad Laboratories, ECS Division, Anaheim, California/USA)

The following methods/instrumentation were used to determine the parameters listed:

Instrumentation: Hitachi 917 Discrete Random-Access Analyzer

Parameter	Unit	Method
Glucose	mmol/L	Ultraviolet, enzymatic, endpoint- (Hexokinase/G-6-PDH)
Urea	mmol/L	Ultraviolet, kinetic- (Urease-GLDH)
Creatinine	µmol/L	Colorimetric, kinetic- (Jaffé-reaction without deproteinization)
Bilirubin, total (BILI.T.)	µmol/L	Colorimetric, endpoint- (DPD)
Cholesterol, total (CHOLEST. T.)	mmol/L	Enzymatic, colorimetric, endpoint- (CHOD-PAP)
Triglycerides (TRIGL.)	mmol/L	Enzymatic, colorimetric, endpoint- (GPO-PAP)
Phospholipids (PHOS. LIPID)	mmol/L	Enzymatic, colorimetric, endpoint- (Phospholipase D/choline oxidase/PAP)
Aspartate aminotransferase (ASAT/GOT)	µkat/L (37°C)	Ultraviolet, kinetic- (acc. to IFCC[1] recommendations [without pyridoxal phosphate activation], NADH/MDH coupled reaction)
Alanine aminotransferase (ALAT/GPT)	µkat/L (37°C)	Ultraviolet, kinetic- (acc. to IFCC[1] recommendations [without pyridoxal phosphate activation], NADH/LDH coupled reaction)

[1] IFCC = International Federation of Clinical Chemistry

Parameter	Unit	Method
Lactate dehydrogenase (LDH)	µkat/L (37°C)	Ultraviolet, kinetic- (acc. to DGKC[1] recommendations, NADH/LDH coupled reaction using pyruvate as substrate, PYR ---> LACT reaction)
Creatine kinase (CK)	µkat/L (37°C)	Ultraviolet, kinetic- (acc. to DGKC[1] recommendations, CK-NAC activated)
Alkaline phosphatase (ALP)	µkat/L (37°C)	Colorimetric, kinetic- (acc. to DGKC[1] recommendations, hydrolysis of p-nitrophenyl-phosphate/diethanolamine buffer pH 9.8)
Gamma-glutamyl-transferase (G-GT)	nkat/L (37°C)	Colorimetric, kinetic- (acc. to IFCC[2] recommendations using L-gamma-glutamyl-3-carboxy-4-nitroanilide as substrate plus glycylglycine)
Calcium	mmol/L	Colorimetric, endpoint- (o-Cresolphthalein complexone)
Phosphorus	mmol/L	Ultraviolet, endpoint- (direct phosphomolybdate reaction)
Sodium	mmol/L	ISE (Ion-Selective Electrode) Indirect potentiometry
Potassium	mmol/L	ISE (Ion-Selective Electrode) Indirect potentiometry
Chloride	mmol/L	ISE (Ion-Selective Electrode) Indirect potentiometry
Albumin	g/L	Colorimetric, endpoint- (BCG, Bromocresol green)
Protein, total	g/L	Colorimetric, endpoint- (Biuret reaction)
Globulin	g/L	Calculated value: (total protein minus albumin = globulin)
Albumin to Globulin ratio (A/G RATIO)		Calculated value: (albumin concentration / globulin concentration)

1 DGKC = Deutsche Gesellschaft für Klinische Chemie
2 IFCC = International Federation of Clinical Chemistry

10.4 URINALYSIS

The following commercial reference controls were used to monitor the performance of the methods:

- Liquichek Urinalysis Control – Levels 1 and 2 – (in the assay control of Osmolality, pH, Protein, Glucose, Ketone, Bilirubin, Blood and Urobilinogen)
  (Bio-Rad, ECS Division, Anaheim, California/USA)

The following methods/instrumentation were used to determine the parameters listed:

Parameter	Unit	Method/Instrumentation
Volume/17 hours	mL	Metabolism cage, Model K. Ehret & Co., Emmendingen, Germany. Collection of urine over approximately 17 hours in specimen vials. Measurement of urine volume using a graduated sampling tube.
Specific gravity (SPEC. GRAV.)	1	Atago Uricon Refractometer
Osmolality	mmol/kg	Freezing-point depression- Fiske 2400 Multi-Sample Osmometer
Color	-	Visual observation
Appearance	-	Visual observation

Instrumentation: Miditron M Semi-Automated Urine Chemistry Analyzer

Parameter	Score	Method
pH		Reagent-Test-Strip (Roche Diagnostics Combur[10] Test M)
Protein	0= negative 1= 0.25 g/L 2= 0.75 g/L 2= 1.50 g/L 3= ≥5.00 g/L	Reagent-Test-Strip (Roche Diagnostics Combur[10] Test M) Questionable results are confirmed with Albustix Reagent-Test-Strip (Bayer Diagnostics)
Glucose	0= normal 1= 3 mmol/L 1= 6 mmol/L 2= 17 mmol/L 3= ≥56 mmol/L	Reagent-Test-Strip (Roche Diagnostics Combur[10] Test M)
Ketone	0= negative 0= 0.5 mmol/L 1= 1.5 mmol/L 2= 5.0 mmol/L 3= ≥15.0 mmol/L	Reagent-Test-Strip (Roche Diagnostics Combur[10] Test M)

Parameter	Score	Method
Bilirubin	0= negative 1= 17 µmol/L 2= 50 µmol/L 3= ≥100 µmol/L	Reagent-Test-Strip (Roche Diagnostics Combur[10] Test M) Positive results are confirmed with Ictotest Reagent Tablets (Ames)
Blood	0= negative 1= 10 RBCs/µL 1= 25 RBCs/µL 2= 50 RBCs/µL 2= 150 RBCs/µL 3= ≥250 RBCs/µL	Reagent-Test-Strip (Roche Diagnostics Combur[10] Test M) Questionable results are confirmed with Hemastix Reagent-Test-Strip (Bayer Diagnostics)
Urobilinogen	0= normal 1= 17 µmol/L 2= 68 µmol/L 3= 135 µmol/L 3= ≥203 µmol/L	Reagent-Test-Strip (Roche Diagnostics Combur[10] Test M)

Key to characterization of urine test score ratings when used with the Roche Reagent-Test-Strip:

0 = negative or normal
1 = small amount
2 = moderate amount
3 = large amount

Instrumentation: Leitz Laborlux 12 light microscope

Parameter	Score	Method
Urine Sediment[1] (SED. MICRO.)	normal/abnormal (amount)	Specimen centrifugation, at 3000 r.p.m. (1500 x g) for 10 minutes
	0 = normal 1 = small 2 = moderate 3 = large	Microscopic examination of 10 high power fields (400x) in different parts of cover-slipped area for normal and abnormal constituents by light microscopy

Key to abbreviations of urinary constituents:

RBC = Red blood cells
WBC = White blood cells
CA.OXALATE = Calcium oxalate crystals
TRIP. PHOS. = Triple phosphate crystals
URIC ACID = Uric acid crystals

[1] Normal urine contains a minute amount of cellular and non-cellular constituents of the urinary tract including white cells, red cells, epithelial cells, hyaline or granular casts, and/or amorphous or crystalline forms of normal solids. The presence of abnormal amounts of urinary constituents is scored from 1 to 3.

The evaluation is based on findings relative to the control group, on dose-related changes, on the presence of a small amount of red blood cells, on increased amounts of white blood cells, and/or other urinary constituents.

11 PATHOLOGY

Necropsy

Necropsy was performed at the RCC facility in Itingen. All animals surviving until terminal sacrifice and all moribund animals were anaesthetised by an intraperitoneal injection of pentobarbital (approximately 300 mg/kg) and sacrificed by exsanguination. In all animals (including animals found dead) complete examination was performed by experienced prosectors. One of the responsible pathologists (Dr. H.J. Chevalier or Dr. K. Weber) were present in the necropsy room for the duration of all scheduled necropsies. Whenever feasible, Dr. H.J. Chevalier or Dr. K. Weber were also present at necropsy of moribund or previously dead animals.

All macroscopic abnormalities were described and reported. All gross masses and clinically observed growths were confirmed at necropsy.

Dates for interim necropsy after one year of exposure		
Subgroup A, chronic toxicity phase:	males/females	31-AUG to 04-SEP-1998
Dates for interim necropsy after one year of exposure and one year of recovery		
Subgroup B, recovery phase:	males/females	30-AUG to 03-SEP-1999
Dates for terminal necropsy after two years of exposure		
Subgroup C, oncogenicity phase:	males/females	02 to 14-SEP-1999 (excluding 4/5 and 11/12-SEP-1999)

Samples of the following tissues and organs were collected from the animals at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution (10% v/v formalin). Lungs and urinary bladder were infused with the fixative. The nasal cavities were instilled with the fixative. In addition, the oviducts were sampled, placed in a separate capsule and fixed further for histological processing and examination.

Adrenal glands
Aorta
Brain
Cecum
Colon
Duodenum
Epididymes
Esophagus
Extraorbital lacrimal glands
Eyes with optic nerves
Femur
Harderian glands
Heart
Ileum
Jejunum
Joint, femurotibial
Kidneys
Larynx
Liver
Lungs (infused with formalin)
Lymph nodes - mandibular, mesenteric, mediastinal
Mammary gland area (inguinal area)
Nasal cavities with paranasal sinuses
Nasopharynx
Ovaries
Oviducts
Pancreas
Pituitary gland
Prostate
Rectum
Salivary glands - mandibular, sublingual
Sciatic nerve
Seminal vesicles
Skeletal muscle (thigh region)
Skin (abdomen left side)
Spinal cord - cervical, midthoracic, lumbar
Spleen
Sternum with bone marrow
Stomach
Testes
Thymus
Thyroid gland with parathyroid glands
Tongue
Trachea
Urinary bladder (infused with formalin)
Uterus
Vagina
Zymbal glands
All gross lesions
Transponders and ears (check of animal's identity)
Tumors (where technically possible the weight and size of tumors were also recorded)

Organ Weights

The following organ weights were recorded on the scheduled dates of necropsy:

Adrenal glands	Liver	Spleen
Brain	Lungs	Testes
Heart	Ovaries	Thymus
Kidneys	Pituitary gland	

Organ to body weight and organ to brain weight ratios were calculated.

Histotechnology for Light Microscopy

The responsible laboratory for histological processing was Propath UK Ltd, Willow Court, Netherwood Road, Hereford, HR2 6JU (U.K.).

The organs listed below were processed, embedded in paraffin wax, cut at a nominal thickness of 4 micrometers and stained with hematoxylin and eosin.

- All organs and tissues listed under necropsy of all animals in the control and high dose groups and all animals that died or were killed during the study
- All gross lesions in all animals
- From the surviving rats of the intermediate groups 2 – 4, liver, lungs, kidneys, nasal cavities and testes were histologically processed.

Histopathology

Slides from all tissues/organs allocated to histotechnological processing for light microscopy (see above) were examined.

The pathologist responsible for evaluation was Dr. H.-J. Chevalier, EPS, Hauptstrasse 77a, CH-4132 Muttenz.

Where possible, gross observations were correlated with microscopic findings.

The histopathology was subjected to an external peer review. Dr. Peter Mann, EPL, Experimental Pathology Laboratories, Inc., P.O. Box 12766, Research Triangle Park, NC 27709, USA was nominated as reviewing pathologist.

Slides from the testes of subgroup A (one year of exposure) male animals, all dose levels, were shipped to the Sponsor for morphometric evaluation of Leydig cell neoplasms. Dr. James Crissman was the Principal Investigator and the pathologist responsible for generating this data and creating a Contributing Scientist Report (Attachment 7). Details of the morphometry methods were clarified in a subsequent protocol addendum. Slides were returned to RCC Ltd before the finalisation of the study.

12 STATISTICAL ANALYSIS

The computer-generated values which appear in the tables represent the rounded-off results of calculations which used the exact raw data values.

Analysis was two-tailed for significance levels of 5% and 1%. All means were presented with standard deviations. Analysis of body weight, food consumption as well as organ weights and clinical pathology (excluding differential white cell counts other than segmented neutrophils and lymphocytes) were analysed by a one way analysis of variance followed by comparison of the control group to each treated group by Dunnett's test[1]. The Steel-Test[2] (many-one rank test) was applied instead of the Dunnett-test for the other parameters in the clinical laboratory investigations.

The one sided exact Fisher-test[3] was applied for pathology data.

12.1 STATISTICAL ANALYSIS OF HISTOPATHOLOGY DATA

Histopathology data were statistically analysed by Environ Health Sciences Institute. Analysis of the histopathology data was done independently for each study group (Attachment 9). Management, analysis and report generation of the data were done using SAS/BASE and SAS/STAT® software[4]. The Thomas, Breslow and Gart program[5] was used for the survival analysis. Computer programs were written by Environ to calculate Fisher's Exact Tests[3], Peto Mortality Prevalence test[6] for trend, and the Poly3[7] trend test.

Survival

Survival analysis was performed on all subgroups of the study. The Thomas, Breslow and Gart program was used to do the survival analysis. This program provides the Kaplan-Meier product-limit procedure in graphic format as well as an assessment of treatment related effects on survival by the Cox method.

Neoplastic Lesions

Statistical analysis of data for neoplastic lesions was done in two tiers. In the first tier, Fisher's Exact and the Peto Mortality Prevalence test for trend were calculated for the data. The Peto analysis was done using the grades provided in the data. For each lesion where there was a combination of incidental, fatal and/or mortality independent grades, the calculations of the observed, expected and variance terms was calculated for the incidental, fatal and mortality independent grades separately and then combined to produce an overall test statistic and p-value. If the Peto analysis was not significant ($p>0.05$), then second tier-analysis of trend using the Poly3 test was done.

Non-Neoplastic Lesions

The Poly3 test was used to determine the overall incidence trend across all groups and for pair-wise comparisons between the control and the dosed groups. The Jonchkeere-Terpstra test[8] was used to determine significant shift in the non-neoplastic finding grades. This test was used to check both for an overall trend to increased or decreased severity grade or increased/decreased incidence with increased dose, and to do pair-wise comparisons between the control and the dosed groups.

13 SUMMARY OF DEVIATIONS

Summary of deviations to study plan can be found in Attachment 6.

14 RESULTS

14.1 EXPOSURE CONCENTRATIONS

Summary (weekly mean) pp. 108 to 123,
Daily data pp. 778 to 921.

The concentration of the test item (HMDS) in the exposure chambers of dose groups 2 – 5 (100 - 5000 ppm) was continuously monitored by GC analysis and recorded. The nominal concentration was determined daily for each group from knowledge of the quantity of test item used and the air flow rate through the chamber. The mean chamber concentrations (mg/L) over the entire exposure period (calculated as mean of the weekly means) are summarized in Table 1.

Targeted exposure concentrations were 100 ppm (0.7 mg//L) for Group 2, 400 ppm (2.7 mg//L) for Group 3, 1600 ppm (10.6 mg//L) for Group 4 and 5000 ppm (33.2 mg//L) for Group 5. The overall mean nominal chamber concentrations (calculated as mean of the weekly means) for males were 103.9, 409.9, 1639.4 and 5118.1ppm (0.727, 2.767, 10.861, and 33.984 mg/L HMDS, respectively). The GC analyses of chamber concentrations for males were 98.6, 396.1, 1588.1 and 4900.5 ppm (0.690, 2.674, 10.522, and 32.539 mg/L HMDS, respectively). The overall mean nominal chamber concentrations for females were 103.6, 411.0, 1630.8 and 5110.1 ppm (0.725, 2.774, 10.804, and 33.931 mg/L HMDS, respectively). The GC analysis of chamber concentrations for females yielded 98.6, 395.9, 1589.0 and 4908.9 ppm (0.690, 2.672, 10.528, and 32.595 mg/L HMDS, respectively).

The mean chamber concentrations determined by GC analysis (Week 1 to 107) were within 2% of target. For all groups the coefficient of variation (SD/mean × 100) of the weekly means was less than 1.7%. This demonstrates excellent control over the vapor generation and inhalation system throughout the duration of the study. The mean chamber concentration measured by gravimetry ranged from 2.6 % to 6.1 % higher than measurement by GC analysis.

In the air control group, the filtered air supplied to the chamber was sampled with a gas bag at least three times per exposure period: at the beginning, at an intermediate time and at the end of the exposure period and subsequently analysed by GC. All data recorded for this group were below the detection limit of 2-5 ppm.

14.2 EXPOSURE CONDITIONS

Temperature, air flow and humidity in the exposure chambers were recorded continuously, oxygen concentration was recorded once during each exposure period in the HMDS groups and continuously in the control. The daily range for temperature and humidity and the oxygen values recorded are given in Attachment 1. The Table 2. summarises the minimal and maximal values recorded in the different groups during the entire exposure period.

14.3 MORTALITY

See figures pp. 83 to 88 and individual data pp. 922 to 951.

A total of 270 of 1050 animals (25.7%) were killed *in extremis* or died before the scheduled one or two year necropsies.

During the first year only 12 animals (1.1% of total) died prematurely: two females in group 2 (100 ppm) - one of which, female 368, died shortly after the blood collection procedure - two females and one male in group 3 (400 ppm), two males and one female in group 4 (1600 ppm), and two males and two females in group 5 (5000 ppm). Of those twelve animals, one animal was from subgroup A, two animals were from subgroup B and nine animals were from subgroup C. Although there were no deaths in the control group and the number of deaths was marginally higher in the high dose group, the distribution was considered to be incidental. From the microscopic findings, six of the twelve deaths could be attributed to neoplasms distributed as three in group 3 (400 ppm), one in group 4 (1600 ppm) and two in group 5 (5000 ppm). Since the neoplasms were of varying types and without dose relationship the deaths were considered to be unrelated to treatment with HMDS.

Therefore from 839 animals scheduled for sacrifice after 2 years, 258 animals (30.8%) were killed *in extremis* or died before the scheduled two-year necropsy. The group incidence and distribution of the unscheduled mortality in subgroups B (one year of exposure and one year of recovery) and C (two years of exposure) are summarized in Table 3. There was no significant difference in the survival among the dosed groups and control in all of the subgroups. Complete results of the survival analysis are given in Attachment 9.

The incidence of mortality was higher in the males than in the females. However, for both sexes, there were no test item and/or dose-related differences between control animals and exposed animals. In addition, the difference in the mortality incidence between subgroup B (one year of exposure and one year of recovery) animals (29.3%) and subgroup C (two years of exposure) animals (31.2%) was minimal demonstrating that there was no adverse effect of the exposure procedure on the survival rate of the subgroup C (two years of exposure) animals.

14.4 CLINICAL SIGNS

Summary pp. 124 to 244, Individual data pp. 952 to 1287.

Up to and including the highest vapor concentration of 5000 ppm, there were no clinical signs which were considered to be caused by the exposure to HMDS.

Hair loss at different body regions and crusts on the eyelid (right, left or both sides) were the clinical signs mainly observed during this study. Both findings are common for this strain of rats. Neither the incidence nor the severity gave an indication of test item related effects.

In all dose groups, there were some incidental findings of temporary duration. These findings mainly regarded the external aspect of the animal: yellow skin towards the end of the study, which is normal in old Fisher rats, temporary emaciation, focal or maculate erythema, swelling, nodules, abscess, scars, crusts, sore spots or scabbed wounds on different parts of the body, open or crusted masses mainly on the abdomen or genital region, or findings to eye(s) (opacity, white, injured, scars, shrunk, scabbed wound, enlarged, half closed lids or swollen). The incidences of these findings mostly ranged between 5 and 15%, however in one occasion, i.e. crusted mass in the genital region in the subgroup B males of group 5 (5000 ppm) during Weeks 92 and 93, the incidence increased to about 20%.

In addition some individual events which occurred during the study are summarised below:

Decreased (spontaneous) activity was observed in male 270 (group 2, 100 ppm, subgroup C), male 521 and female 610 (group 3, 400 ppm, subgroup C), and male 715 (group 4, 1600 ppm, subgroup C) that had additionally exhibited a left lame (hindleg) and dragging of hindlimbs. These clinical signs were recorded before these animals were killed in extremis.

Uncoordinated movements were noted in male 280 (group 2, 100 ppm, subgroup C). Paresis (hindlegs) in male 27 (group 1, air control, subgroup B), paresis (hindlegs) and dragging of hindlimbs in male 302 (group 2, 100 ppm, subgroup C), dragging of hindlimbs in male 901 and female 1038 (group 5, 5000 ppm, subgroup C) were also observed. Decreased activity (movement of hindleg right) was observed in male 494 (group 3, 400 ppm, subgroup C). Hunched posture in female 1012 (group 5, 5000 ppm, subgroup C) was noted shortly before the animal was killed in extremis.

Head tilt (in a few animals together with uncoordinated movements or decreased spontaneous activity) was seen in males 31 (group 1, air control, subgroup B) and 44 (group 1, air control, subgroup C), females 365 and 400 (group 2, 100 ppm, subgroup C), male 465 and female 614 (group 3, 400 ppm, subgroup C), female 792 (group 4, 1600 ppm, subgroup C) and males 878 (group 5, 5000 ppm, subgroup B) and 911 (group 5, 5000 ppm, subgroup C).

Labored respiration was observed in male 280 (group 2, 100 ppm, subgroup C), males 468, 477 and 501 (group 3, 400 ppm, subgroup C) and female 564 (group 3, 400 ppm, subgroup B), also in male 943 (group 5, 5000 ppm, subgroup C).

Tachypnea was seen in male 36 (group 1, air control, subgroup B), male 472 (group 3, 400 ppm, subgroup C), male 728 (group 4, 1600 ppm, subgroup C), male 937 (group 5, 5000 ppm, subgroup C). Dyspnea was noted in female 804 (group 4, 1600 ppm, subgroup C).

Lacrimation was observed in females 123 (group 1, air control, subgroup A) and 170 (group 1, air control, subgroup C) and in male 941 (group 5, 5000 ppm, subgroup C). Bleeding from the right cheek was noted in male 462 (group 3, 400 ppm, subgroup C). Bleeding from the vagina (vaginal opening) and prolapse of vagina was noted in females 136 (group 1, air control, subgroup B) and 194 (group 1, air control, subgroup C), females 548 (group 3, 400 ppm, subgroup B) and 575 (group 3, 400 ppm, subgroup C), and female 1014 (group 5, 5000 ppm, subgroup C). Chromodacryorrhea was observed in male 267 (group 2, 100 ppm, subgroup C) and male 667 (group 4, 1600 ppm, subgroup B), which also had opaque lacrimation.

Black skin was noted in female 159 (group 1, air control, subgroup C). Male 260 (group 2, 100 ppm, subgroup C) collapsed and spontaneously died on the same day. Female 180 (group 1, air control, subgroup C) and male 520 (group 3, 400 ppm, subgroup C) had a missing or necrotic apex of the tail, respectively. Purple skin was noted in male 442 (group 3, 400 ppm, subgroup B). Purple discoloration of the skin in the testicular region was observed in male 240 (group 2, 100 ppm, subgroup B), male 298 (group 2, 100 ppm, subgroup C) and male 486 (group 3, 400 ppm, subgroup C). Female 388 (group 2, 100 ppm, subgroup C) and male 454 (group 3, 400 ppm, subgroup B) had ulcers on skin or tail, respectively. Weakness or poor condition shortly before death was observed in male 687 (group 4, 400 ppm, subgroup C), males 921 and 938, and females 1006 and 1018 (group 5, 5000 ppm, subgroup C).

14.5 MASSES

Summary pp. 245 to 304, Individual data pp. 1288 to 1462.

Up to and including the highest vapor concentration of 5000 ppm, there were no masses which were considered to be related to HMDS treatment.

In subgroup A (sacrifice after one year exposure), no masses were noted in the females of any group (air control, 100, 400, 1600 or 5000 ppm). Males in each group showed between 5 and 15% occurrences of small mass in the genital region. This finding was mainly recorded toward the end of the year exposure. Masses in the abdominal region (5-15%) were noted in two males in each of groups 1 (air control) and 3 (400 ppm) and a mass in the left flank region was noted in one male of group 4 (1600 ppm).

In subgroup B (one year exposure / one year recovery period) females, only a few animals developed masses, all of which were recorded during the second year: one female with mass in the genital region in each of groups 1 (air control) and 4 (1600 ppm), 5 females with masses on different parts of the body in group 2 (100 ppm), no females with masses in group 3 (400 ppm) and two females with masses in the genital or right flank region in group 5 (5000 ppm). In male animals, masses were seen in particular in the genital region and in the abdomen. Except for a mass in 1 male of group 5 (5000 ppm) and small transient masses in 3 males of group 2 (100 ppm) which were observed during the first year, all other masses were recorded during the second year (the post-exposure recovery period). The incidence or size of the developed masses did not give any indication of a test-item related effect. The highest frequency (70.0%) was noted in groups 1 (air control) and 2 (100 ppm) whereas the lowest frequency (26.3%) was recorded in group 5 (5000 ppm).

In subgroup C (two years exposure), the number of females which developed mass(es) was lower than in male animals. In females the percentage of animals with one or more masses ranged between 9.2% (groups 3 and 4, 400 and 1600 ppm, respectively) and 18.5% in group 1 (air control). In males the values ranged between 34.4% in group 5 (5000 ppm) and 63.1% in group 2 (100 ppm) with a control value of 53.8%. Masses were seen in particular in the genital region and in the abdomen. Except for a few males and females distributed over most dose groups the masses developed during the second year of the exposure period. The incidences or size of the developed masses did not give any indication of a test-item related effect.

14.6 OPHTHALMOSCOPIC EXAMINATION

Summary pp. 305 to 310, Individual data pp. 1462 to 1474.

None of the abnormal findings noted at ophthalmoscopic examination gave any indication of test item related effects.

After one year and towards the end of the two year exposure period 100% of the animals (except one female in group 4 after one year) had corneal opacity which is very common in Fischer rats. Other findings of lenticular opacity and persistent pupillary membrane were noted in some animals equally distributed among the groups or (for the lenticular opacity in males) no dose-relationship was evident.

14.7 FOOD CONSUMPTION / RELATIVE FOOD CONSUMPTION

Figures pp. 95 to 106, Summary pp. 442 to 711, Individual data pp. 1625 to 1744.

The mean of the weekly mean food consumption (grams/animal/day) during the respective period with the percent difference from the corresponding control is summarised in the Table 4.

In addition to the summary data of all the weekly mean food consumption measurements for each subgroup, assessment of the food consumption at approximately 13/26 week intervals during the course of the study (Weeks 1/2, 13/14, 26/27, 52/53, 79/80 and 103/104/105) together with the percent difference from control is also presented in the summary data section.

In males of all exposure groups there were a few statistically significant differences (Dunnett-Test based on pooled variance, significant at 5% or 1% levels) noted during the study, particularly for group 3 (400 ppm) of subgroup C (two years of exposure), but these differences were also apparent during the acclimatisation phase. For the 13/26 week intervals stated above, the food consumption values for males ranged from -11 to +9 % from control. Of these, none of the high dose values was statistically significantly different from control. Analysis of food consumption at these set intervals only represents isolated weeks of the study and the mean of the weekly means throughout the study is considered the more appropriate assessment. Data showed that differences in food consumption for all exposed male animals from the respective control animals were negligible (ranging from -2 to +5%), lacked dose relationship and were inconsistent between the subgroups. Therefore all differences were considered to be incidental and unrelated to exposure to the test item.

In the groups 4 (1600 ppm) and 5 (5000 ppm) of subgroups A (one year of exposure) and C (two years of exposure) females, the slight reduction in food consumption (mean of means: -3.5 to -8.8% of control) was considered to be caused by exposure to the test item. In these two subgroup females the difference from the corresponding controls attained statistical significance on 16 and 21 occasions (N=33 for subgroup A) and 23 and 40 occasions (N=60 for subgroup C) for groups 4 (1600 ppm) and 5 (5000 ppm) respectively.

In the subgroup B (one year of exposure and one year of recovery) females this finding was not so clearly evident. The calculation of the mean food consumption during the exposure year (mean of means) gives: 11.0 ± 0.7 (N=33) grams / animal / day in group 1 (air control) and 10.5 ± 0.8 (N=33) in group 5 (5000 ppm). This slight difference (-4.5 % compared with control) is indicative of the effect recorded for subgroup A (one year of exposure) and C (two years of exposure) females but was statistically significant on only 8 out of the 33 measurements during the exposure year.

14.8 BODY WEIGHTS

Figures p. 89 to 94, Summary pp. 311 to 441, Individual data pp. 1475 to 1624.

Occurrences of significant change in body weights are summarised in Table 5. In addition to the summary data of all group mean body weight measurements for each subgroup, assessment of the body weight at approximately 13/26 week intervals during the course of the study (Weeks 13, 26, 52, 78 and 104/105) together with the percent difference from control is also presented in the summary data.

In all male groups, the differences in mean body weights noted between the exposed animals and the respective control males were considered not to be caused by the exposure of the animals to the test item. The differences in females were equivocal with a possible minimal effect at 1600 and 5000 ppm associated with a slight reduction in food consumption at these dose levels. However, there were several weeks during which the differences from the respective control value attained statistical significance.

Subgroup A (one year exposure)

For Group 3 (400 ppm) males, a decrease in body weights (compared to control) occurred at Week 1 (-7%) and Week 2 (-6%). Decreases in body weights occurred at Week 1 (-6%), Week 2 (-5%), Week 6 (-5%), Week 8 (-4%) and Week 9 (-5%) in group 5 (5000 ppm) males.

For Group 2 females, a decrease in body weights (compared to control) occurred at Week 4 (-4%) while an increase in body weight (compared to control) at Week 36 (6%). Decreases in body weights for group 3 females occurred at Week 1 (-4%), Week 46 (-4%), and Week 50 (-4%). For group 4 (1600 ppm) females, a decrease in body weight (compared to control) occurred at weeks 2-12 (-4 to -6%), weeks 14-34 (-4 to -7%) and weeks 38-52 (-6 to -8%). Decreases in body weights for group 5 (5000 ppm) females occurred at weeks 5-8 (-4 to -5%), weeks 10-34 (-4 to -8%), and weeks 38-52 (-7 to -8%).

Subgroup B (one year exposure and one year recovery)

Group 2 (100 ppm) males displayed an increase in body weights (compared to control) at weeks 44-48 (4 to 5%). Increases in body weights for group 3 (400 ppm) males occurred at weeks 60 and 62 (4%). For group 4 (1600 ppm) males, a decrease in body weight occurred at weeks 88 (-5%), 92 (-6%), 96 (-6%), and 104 (-9%). Decreases in body weights for group 5 (5000 ppm) males occurred at Week 90 (-6%), Week 96 (-6%), Week 98 (-8%), and 100 (-11%).

Group 2 (100 ppm) females displayed an increase in body weights (compared to control) at Week 4 (4%), weeks 6 and 7 (6%), weeks 9-90 (5 to 9%), and weeks 96-102 (8 to 9%). Increases in body weights for group 3 (400 ppm) females occurred at Week 2 (5%), Week 7 (4%), weeks 9-22 (4 to 6%) and weeks 30 and 32 (4%). A decrease in body weight was observed in group 5 (5000 ppm) females at Week 26 (-4%).

Subgroup C (two years exposure)

Group 3 (400 ppm) males displayed an increase in body weights (compared to control) at Week 3 (3%), weeks 22 to 64 (2 to 4%), and Week 104 (6%). A decrease in body weights was observed in group 4 (1600 ppm) males during weeks 66-74 (-3%). For group 5 (5000 ppm) males, a decrease in body weight occurred at weeks 64-76 (-3 to -4%) and Week 82 (-3%).

Group 2 (100 ppm) females displayed a decrease in body weight (compared to control) at Week 1 (-3%), Week 5 (-2%) and an increase in body weight at Week 32 (2%) and weeks 36-40 (2 to 3%). A decrease in body weight was observed in group 3 (400 ppm) females during Week 1 (-4%), and an increase in body weight occurred at Week 16 (3%) and weeks 18, 36, and 42 (2%). For group 4 (1600 ppm) females, a decrease in body weight was recorded for weeks 3-13 (-3 to -4%), weeks 16 and 18 (-2%), weeks 26-34 (-3%), weeks 60-92 (-3 to -5%), and weeks 96 and 105 (-4%). Body weight decreases occurred in group 5 (5000 ppm) females at Week 6 (-2%), Week 26 (-3%), weeks 28 and 32 (-2%), and weeks 58-105 (-2 to -7%).

Assessment of the male body weights at approximately 13/26 week intervals during the course of the study (Weeks 13, 26, 52, 78 and 104/105) together with the percent difference from control showed few statistically significant differences from control:

Week 1:	Subgroup A, decreased for group 3, 400 ppm, males ($p<0.01$), -7% from control
	Subgroup A, decreased for group 5, 5000 ppm males ($p<0.05$), -6% from control
Week 26:	Subgroup C, increased for group 3, 400 ppm males ($p<0.01$), +3% from control
Week 52:	Subgroup C, increased for group 3, 400 ppm males ($p<0.05$), +3% from control
Week 104:	Subgroup B, decreased for group 4, 1600 ppm males ($p<0.05$), -9% from control

Apart from three of the values above (of which two values [Week 1] were before the start of treatment) and a subgroup B, group 5 (5000 ppm) value at Week 105 (-8% from control, not-significant) the body weight measurements recorded at the set intervals examined were ± 4% from control and therefore it is considered that there was no exposure-related effect on male body weight.

In females, statistically significant differences from controls at approximately 13/26 week intervals were:

Week 1:	Subgroup A, decreased for group 3, 400 ppm females ($p<0.05$), -4% from control
	Subgroup C, decreased for group 2, 100 ppm females ($p<0.05$), -3% from control
	Subgroup C, decreased for group 3, 400 ppm females ($p<0.01$), -4% from control
Week 13:	Subgroup A, decreased for group 5, 5000 ppm females ($p<0.05$), -4% from control
	Subgroup B, increased for group 2, 100 ppm females ($p<0.01$), +7% from control
	Subgroup B, increased for group 3, 400 ppm females ($p<0.05$), +5% from control
	Subgroup C, decreased for group 4, 1600 ppm females ($p<0.01$), -3% from control
Week 26:	Subgroup A, decreased for group 4, 1600 ppm females ($p<0.01$), -7% from control
	Subgroup A, decreased for group 5, 5000 ppm females ($p<0.01$), -8% from control
	Subgroup B, increased for group 2, 100 ppm females ($p<0.01$), +5% from control
	Subgroup B, decreased for group 5, 5000 ppm females ($p<0.05$), -4% from control
	Subgroup C, decreased for group 4, 1600 ppm females ($p<0.01$), -3% from control
	Subgroup C, decreased for group 5, 5000 ppm females ($p<0.01$), -3% from control
Week 52:	Subgroup A, decreased for group 4, 1600 ppm females ($p<0.01$), -7% from control
	Subgroup A, decreased for group 5, 5000 ppm females ($p<0.01$), -8% from control
	Subgroup B, increased for group 2, 100 ppm females ($p<0.01$), +7% from control
Week 78:	Subgroup B, increased for group 2, 100 ppm females ($p<0.01$), +9% from control
	Subgroup C, decreased for group 4, 1600 ppm females ($p<0.01$), -4% from control
	Subgroup C, decreased for group 5, 5000 ppm females ($p<0.01$), -6% from control

Week 104/105: Subgroup C, decreased for group 4, 1600 ppm females ($p<0.05$), -4% from control
Subgroup C, decreased for group 5, 5000 ppm females ($p<0.01$), -7% from control

In females of subgroup A (one year of exposure), the mean body weights in groups 4 (1600 ppm) and 5 (5000 ppm) were statistically significantly reduced from the respective control values on 30 and 27 occasions (N=33) respectively. In females of subgroup C (two years of exposure) the mean body weights in groups 4 (1600 ppm) and 5 (5000 ppm) were statistically significantly reduced from the respective control values on 37 and 29 occasions (N=60) respectively, mainly during the second year for group 5 (5000 ppm).

However, in spite of the statistical significance, the difference remained marginal (-2 to -8% from control for the weeks of measurement quoted above) and there was up to -4% difference in body weight from control before the start of exposures at Week 1. In addition, there was no dose-relationship in body weight between the groups, and there was no confirmation of a similar response in the subgroup B (one year of exposure and one year of recovery) in all groups. This finding can also be correlated to the slight reduction in food consumption noted in these two subgroups.

14.9 CLINICAL LABORATORY INVESTIGATIONS

Summary pp. 712 to 727, Individual data pp. 1745 to 1990.

There were no major effects of toxicological significance on hematology, clinical biochemistry and urinalysis data. However, for some parameters minor inter-group differences were recorded between treated animals and controls at different time points of the treatment. The findings which attained statistical significance were as follows:

Hematology

- Erythrocyte count (RBC): reduced in the males at 1600 ppm after 6 months (-3%) and in the males at 5000 ppm after 12 months (-5%).
- Hemoglobin (HB) concentration: increased in the females at 5000 ppm after 3 months (6%), reduced in the males at 1600 and 5000 ppm after 6 months (-2%), after 12 months at 1600 ppm (-3%), and at 5000 ppm (-5%), and in the females at 400 (-2%), 1600 and 5000 ppm after 6 months (-3%).
- Hematocrit (HCT): reduced in the males at 1600 ppm after 6 months (-2%) and in the males at 5000 ppm after 12 months (-2%).
- Mean corpuscular volume (MCV): increased in the males at 5000 ppm after 3 and 12 months (2%) and in the males at 1600 ppm after 6 months (1%) and 12 months (2%), increased in the females at 5000 ppm after 6 months (2%) and 12 months (1%) and in the females at 1600 ppm after 6 months (1%).
- Mean corpuscular hemoglobin (MCH): reduced in the males at 400 and 1600 ppm after 3 months (-2%), and in the females at 400, 1600 and 5000 ppm after 6 months (-2%).
- Mean corpuscular hemoglobin concentration (MCHC): reduced in the males at 5000 ppm after 3 and 12 months (-2%) and 6 months (-1%) and in the males at 1600 ppm after 3 and 12 months (-2%), increased in the females at 5000 ppm after 3 months (5%), and reduced in the females at 400, 1600 (-2%) and 5000 ppm (-4%) after 6 months.
- Platelet count: increased in the males at 1600 (9%) and 5000 ppm (9%) after 3 months and after 6 months (9% and 15%, respectively).

- Reticulocyte count: reduced in males at 5000 ppm after 3 months (absolute, -9%), increased in the females at 400 ppm after 3 months (relative, 14%; absolute, 13%) and in females at 100 ppm after 12 months (absolute, 17%).
- High Fluorescence Reticulocyte Ratio (HFR): after 3 months increased in the males at 400 ppm (21%), and reduced in the females at 100 (-22%) and 1600 ppm (-24%), increased in the males at 100 ppm (18%) and in the females at 400 (46%) and 5000 ppm (40%) after 6 months and in the males at 400 ppm (30%), 1600 ppm (37%) and 5000 ppm (38%) and the females at 1600 ppm (40%) after 12 months.
- Middle Fluorescence Reticulocyte Ratio (MFR): after 3 months increased in the females at 400 ppm (8%) and reduced in the males 5000 ppm (-6%), and increased in the males at 400 ppm (5%) after 12 months.
- Low Fluorescence Reticulocyte Ratio (LFR): after 3 months increased in the females at 100 (3%) and 1600 ppm (4%) and reduced in the females at 400 ppm (-6%), reduced in the males at 100 ppm (-4%) and in the females at 400 ppm (-5%) after 6 months and in the males at 400 ppm (-6%), 1600 ppm (-6%) and 5000 ppm (-5%) after 12 months.
- Total Leukocyte count (WBC): increased in the females at 100 ppm and 400 ppm (15%) after 6 months.
- Differential Leukocyte count relative (WBC): reduced segmented neutrophils (-32%) and increased lymphocytes in the females at 400 ppm (7%) after 3 months.
- Differential Leukocyte count absolute (WBC): increased lymphocytes in the females at 400 ppm after 3 (18%) and 6 months (12%), in the females at 1600 ppm after 3 months (15%), increased segmented neutrophils in the females at 1600 ppm after 6 months (43%).
- Prothrombin time (PT): reduced in the males at 400 ppm (-2%), 1600 ppm (-4%) and 5000 ppm (-5%) after 3 months, reduced in the males at 400 ppm and 1600 ppm (-3%) after 6 months, in the males at 400 ppm, 1600 and 5000 ppm (-3%) at 12 months and females at 400 ppm and 5000 ppm (-4%) and at 1600 ppm (-3%) at 12 months.
- Activated Partial Prothrombin time (APTT): reduced in the males at 1600 ppm (-3%) and 5000 ppm (-5%) after 3 months, reduced in the males at 400 ppm (-7%), 1600 ppm (-6%) and 5000 ppm (-5%) after 6 months, and increased in the females at 5000 ppm (5%) after 6 months and in the males at 1600 ppm at 12 months (4%).

Clinical biochemistry

- Glucose concentration: increased in the males at 100 ppm (9%), 400 (17%) and 1600 ppm (8%) and in the females at 400 ppm (12%), and decreased in females at 5000 ppm (-12%) after 3 months, increased in the males at 400 ppm (10%) and decreased in the females at 5000 ppm (-7%) after 6 months, increased in the males at 400 ppm (13%) and 1600 ppm (9%) and decreased in the females at 5000 ppm (-14%) after 12 months.
- Urea concentration: increased in the males at 100 ppm (7%), 400 ppm (11%), 1600 ppm (16%) and 5000 ppm (30%) after 3 months, increased in the males at 400 ppm (12%), 1600 ppm (13%) and 5000 ppm (29%) after 6 months, increased in the males at 1600 ppm (7%) and 5000 ppm (8%) after 12 months, and decreased in the females at 400 ppm (-9%) after 3 months and in the females at 5000 ppm after 3 and 6 months (-8%).
- Creatinine concentration: increased in the males at 100 ppm (3%), 400 ppm (7%), 1600 ppm (13%) and 5000 ppm (21%) after 3 months, increased in the males at 400 ppm (5%, 4%), at 1600 ppm (13%, 16%), and 5000 ppm (26%, 22%) after 6 and 12 months, respectively.

- Bilirubin concentration: increased in the females at 1600 ppm (13%) and reduced in the males at 100 ppm (-18%) and 400 ppm (-21%) after 3 months, reduced in the females at 100 ppm (-18%) and 400 ppm (-19%) and increased in the males at 5000 ppm (19%) after 6 months, reduced in the females at 400 ppm after 12 months (-20%).
- Total cholesterol concentration: increased in the males 1600 ppm (9%) and 5000 ppm (29%) after 3 months and the females 1600 ppm (7%) and 5000 ppm (11%), increased in the males at 1600 ppm (12%) and 5000 ppm (26%) after 6 months, increased in the females at 5000 ppm after 6 (13%) and 12 months (12%).
- Triglyceride concentration: increased in the males at 5000 ppm after 3 months (29%) and decreased in the females at 1600 ppm (-19%) after 6 months.
- Phospholipid concentration: increased in the males at 1600 ppm (7%) and 5000 ppm (20%) and females at 1600 ppm (5%) and 5000 ppm (8%) after 3 months, increased in the males at 1600 ppm (8%) and 5000 ppm (17%) after 6 months, increased in the females at 5000 ppm after 6 (10%) and 12 months (9%).
- Aspartate aminotransferase (ASAT/GOT) activity: reduced in the males at 1600 ppm (-17%) after 6 months, and in the males at 400 ppm (-22%), 1600 ppm (-22%) and 5000 ppm (-37%) after 12 months. Reduced in the females at 100 ppm (-6%, -13%), 400 ppm (-12%, -15%), 1600 ppm (-11%, -22%) and 5000 ppm (-12%, -21%) after 3 and 6 months, respectively.
- Alanine aminotransferase (ALAT/GPT) activity: reduced in the females at 400 ppm (-12%) and in the males (-8%) and females (-12%) at 5000 ppm after 3 months, reduced in the males at 1600 ppm and 5000 ppm after 6 months (-21%), and in the males at 400 ppm (-31%), 1600 ppm (-25%) and 5000 ppm (-45%) after 12 months.
- Lactate dehydrogenase (LDH) activity: reduced in the females at 400 ppm after 3 months (-37%) and increased in the males at 5000 ppm after 6 months (29%).
- Creatine kinase (CK) activity: reduced in the females at 400 ppm after 3 months (-28%).
- Alkaline phosphatase (ALP) activity: reduced in the males at 5000 ppm after 3 months (-6%), reduced in the males at 1600 ppm (-10%) and 5000 ppm (-9%) after 6 months, and in the males at 400 ppm (-13%) and 5000 ppm (-19%) and in the females at 1600 ppm after 12 months (-22%).
- Gamma-glutamyl-transferase (G-GT) activity: increased in the males at 400 (26%), 1600 (33%) and 5000 ppm (56%) after 3 months, at 5000 ppm after 6 months (86%), and at 1600 ppm (45%) and 5000 ppm (73%) after 12 months and increased in the females at 5000 ppm after 3 (32%), 6 (77%) and 12 months (156%).
- Calcium concentration increased in the males at 400 ppm (2%, 4%), 1600 ppm (2%, 5%) and 5000 ppm (3%, 6%) after 3 and 6 months, respectively, in the males at 1600 ppm (2%) and 5000 ppm (4%) after 12 months, and in the females at 1600 ppm (2%) and 5000 ppm (3%) after 6 months.
- Phosphorus concentration: increased in males at 400 ppm after 3 months (6%) and after 6 months (7%).
- Sodium concentration: increased in the males at 400 ppm (1%), 1600 ppm (<1%) and 5000 ppm (1%) after 3 months, in the males at 1600 ppm (1%) and 5000 ppm (1%) after 6 and 12 months, in the females at 400 ppm (1%), 1600 ppm (1%, 2%) and 5000 ppm (2%) after 3 and 6 months, respectively and in the females at 1600 ppm and 5000 ppm after 12 months (1%).

- Potassium concentration: increased in the males at 400 ppm after 3 and 12 months (7%), increased in the females at 1600 ppm (6%) and 5000 ppm (8%) after 3 months and decreased in the females at 1600 ppm after 6 months (-5%).
- Chloride concentration: increased in males at 400 ppm, 1600 ppm and 5000 ppm (1%) after 3 months, and in the males at 100 ppm and 1600 ppm (1%) after 12 months. Increased in the females at 100 ppm (1%), 400 ppm (2%), 1600 ppm (2%) and 5000 ppm (3%) after 3 months, increased in females at 400 ppm, 1600 ppm and 5000 ppm after 6 months (1%) and in the females at 1600 ppm and 5000 ppm (1%) after 12 months.
- Albumin concentration: reduced in the males at 100 ppm after 3 months (-1%) and increased in the males at 1600 ppm (1%, 5%) and 5000 ppm (3%, 6%) after 3 and 12 months, respectively.
- Total protein concentration: increased in the males at 1600 ppm (3%) and 5000 ppm (6%) after 3 months, in the males (6%) and females (2%) at 5000 ppm after 6 months, and in the males at 400 ppm (4%), 1600 ppm (6%) and 5000 ppm (8%) after 12 months.
- Globulin concentration: increased in the males at 1600 ppm (5%) and 5000 ppm (8%) after 3 months, in the males at 5000 ppm after 6 months (10%), and in the males at 400 ppm (5%), 1600 ppm (7%) and 5000 ppm (9%) and in the females at 100 ppm (6%) and 5000 ppm (5%) after 12 months.
- Albumin/Globulin ratio: reduced in the males (-4%) and females (-3%) at 1600 ppm and in the males at 5000 ppm (-5%) after 3 months, increased in the females at 1600 ppm (3%) and decreased in the males at 5000 ppm (-6%) after 6 months, and in the males at 1600 and 5000 ppm (-3%) and in the females at 100 (-6%), 400 (-4%) and 5000 ppm (-6%) after 12 months.

Urinalysis

- Urine output (Volume/17hour) increased in the males at 1600 ppm (19%) and at 5000 ppm (33%) after 6 months and in males at 5000 ppm after 12 months (20%).
- Specific gravity reduced in the males at 1600 ppm and 5000 ppm after 6 months, and increased in the males at 400 ppm after 12 months.
- Osmolality increased in the males at 400 ppm after 3 (24%) and 12 months (33%), reduced in the males at 1600 ppm (-11%) and 5000 ppm (-12%) after 6 months, reduced in the females at 400 ppm after 12 months (-30%).
- Protein (score) increased in the males at 400 ppm, 1600 ppm and 5000 ppm after 3 months (100%) and in the females at 400 ppm after 6 months (100%).
- Blood (score) increased in the males at 1600 ppm and 5000 ppm after 3 months (100%).

Assessment of the significance of the clinical laboratory measurements

The slight changes noted in the red cell parameters consisting of occasionally lower HB, HCT and MCHC values and higher MCV values may possibly reflect an increased hemoglobin turnover. Little toxicological relevance is associated with these findings since the changes were minimal in degree and unaccompanied by any observable increase in hematopoietic activity or other morphological findings (i.e. no test item related hyperplasia in bone marrow and spleen).

The increase in urea and creatinine and imbalances of electrolytes, which were generally dose related, may be related to the morphological tubular changes noted in the kidneys. All other findings in the clinical laboratory investigations, including the increase in lipids and proteins and changes in enzyme activity (particularly the increases in gamma glutamyl transferase), suggest metabolic adaptive changes, primarily related to the liver.

All other statistical differences observed in the results of the hematology, clinical biochemistry and urinalysis data were considered to be incidental and unrelated to treatment and within or near the limits of the normal background data encountered in Fischer rats of this strain and age (for reference, refer to historical control data for untreated Fischer 344 (SPF) rats, see Attachment 4). In addition, the variations were slight in magnitude and/or displayed no dose-relationship and/or were not consistently present for both sexes or at the different occasions of measurement.

14.10 ORGAN WEIGHTS

Summary pp. 728 to 757, Individual data pp. 1991 to 2098.

At terminal necropsy the weight of adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes and thymus were recorded. The following statistically significant differences from control were noted (Dunnett-Test based on pooled variance significant at 5% or 1%):

After one year of exposure (Subgroup A animals)

Statistically significant findings in the absolute organ weights were observed in reduced brain weight (-2%) in group 5 (5000 ppm) males, increased liver weight (13%) in group 5 (5000 ppm) males, increased lung weight (10%) in group 2 females, reduced kidneys (-7%), adrenals (-10%) and spleen weight (-10%) in group 5 (5000 ppm) females.

Statistically significant findings in the organ/body weight ratio include increased liver weight in groups 4 (1600 ppm) (7%) and 5 (5000 ppm) (15%) males, increased kidneys weight (6%) in group 5 (5000 ppm) males, increased testes weight (5%) in group 5 (5000 ppm) males, increased brain weight in groups 4 (1600 ppm) (7%) and 5 (5000 ppm) (7%) females, increased pituitary weight (20%) in group 4 (1600 ppm) females and increased lung weight (9%) in group 2 (100 ppm) females.

Organ/brain weight ratios findings include dose-related increased liver weight in all treated males, statistically significant in groups 4 (1600 ppm) (7%) and 5 (5000 ppm) (15%) and increased testes weight in all treated males, statistically significant in group 5 (5000 ppm) (6%).

Other statistically significant findings in organ/brain weight ratio include increased kidneys weight in groups 2 (100 ppm) (5%) and 5 (5000 ppm) (6%) males and increased lung weight (14%) in group 2 (100 ppm) females.

After one year of exposure and one year of recovery (Subgroup B animals)

There was a statistically significant increase in pituitary weight (180%) in group 2 (100 ppm) females in absolute organ weight (grams).

Statistically significant findings in the organ/body weight ratios include increased brain weight (14%) in group 4 (1600 ppm) males, reduced brain weight in groups 2 (100 ppm) (-10%), 4 (1600 ppm) (-10%) and 5 (5000 ppm) (-8%) females, reduced heart weight in all female groups 2 (100 ppm) (-16%), 3 (400 ppm) (-15%), 4 (1600 ppm) (-17%) and 5 (5000 ppm) (-18%), reduced lung weight in groups 2 (100 ppm) and 5 (5000 ppm) females (-24%), reduced liver weight (-11%) in group 5 (5000 ppm) females and reduced kidneys weight in groups 3 (400 ppm) (-12%), 4 (1600 ppm) (-15%) and 5 (5000 ppm) (-14%) females.

Statistically significant findings in organ/brain weight ratio include increased pituitary weight (167%) in group 2 (100 ppm) females and reduced heart weight (-9%) in groups 3 (400 ppm) and 5 (5000 ppm) females.

After two years of exposure (Subgroup C animals)

Findings in the absolute organ weights in grams in subgroup C include a dose-related increase in testes weight in all treated groups, statistically significant in the groups 4 (1600 ppm) (36%) and 5 (5000 ppm) (46%), reduced heart weight (-4%) and adrenal weight (-29%) in group 5 (5000 ppm) females.

Findings in organ/body weight ratios include a dose-related increase in testes weight in all male treated groups, statistically significant in the groups 4 (1600 ppm) (40%) and 5 (5000 ppm) (47%), statistically significant increased brain weight (8%) in group 5 (5000 ppm) females and kidneys weight in groups 3 (400 ppm) (6%) and 4 (1600 ppm) (8%) females.

Findings in organ/brain weight ratios include a dose-related increase in testes weight in all male treated groups, statistically significant in groups 4 (1600 ppm) (36%) and 5 (5000 ppm) (46%). Other statistically significant findings in organ to brain weight ratio include reduced heart weight (-5%) and adrenal weight (-31%) in group 5 (5000 ppm) females.

Assessment of the significance of the organ weight changes

The increased relative testes weights in Subgroup A (one year exposure) males of the high dose level (5000 ppm) and of the absolute and relative testes weights in Subgroup C (two years exposure) males of the high-mid (1600 ppm) and high dose levels (5000 ppm) were considered to be test item related. These findings also correlated with the results of the histopathological examination and incidence of multifocal Leydig cell hyperplasia observed in the testes of the male rats for these groups. Increased absolute and relative testes weights in Subgroup C (two years of exposure) males were also recorded in groups 2 (100 ppm) and 3 (400 ppm) such that a dose-relationship was evident across all groups but none of the differences at the lower the dose levels achieved statistical significance.

In the Subgroup A (one year of exposure) males, a test item related effect on the increase in absolute and relative liver weight at 5000 ppm and increase in the relative liver weight at 1600 ppm cannot be ruled out. However, there were no correlated findings at histopathological examination. Therefore, the increased liver weights in males but not in females after one year of exposure suggested transient metabolic adaptive changes without any toxicological relevance. This finding was not diagnosed in subgroup C (two years exposure) animals of both sexes.

All other organ weight changes were considered to be unrelated to treatment with HMDS. They were considered to be isolated incidental findings, or they were (for example the kidneys) without any consistency between the different dose levels or sacrifice time points. In addition they may have been confounded by the lower terminal (necropsy) body weight noted in some occasions.

14.11 MACROSCOPIC EXAMINATION

Summary pp. 758 to 776, Individual data, see Pathology Report, Attachment 8, pp. 2528 to 5055.

In subgroup A (one year of exposure) between 20% and 45% of the animals per group were without any findings, whereas in the two year old animals no males were without findings and 5-15% of females per group were without findings.

Liver

In subgroup A (one year of exposure) an enlarged liver, correlating with liver weight, was recorded in 6 of 20 group 5 (5000 ppm) males. This finding was statistically significantly ($p<0.05$) higher than control for which no enlargement was recorded.

Kidneys

In subgroup C (two years of exposure), nodules in the kidneys were present in 7 of 65 group 5 (5000 ppm) males. This finding was statistically significantly ($p<0.05$) higher than control for which no nodules were found. The finding was also present in 3 males from group 4 (1600 ppm).

Testes

In subgroup C (two years of exposure), the incidence of enlarged testes was higher in all treated male groups than the control group and the finding was statistically significant ($p<0.05$) for group 2 (100 ppm) and $p<0.01$ for groups 4 (1600 ppm) and 5 (5000 ppm). The observation in groups 4 and 5 correlated with the recorded increase in testicular weight for these groups.

Neither the type nor the incidences of all other abnormal findings noted at terminal necropsy gave indications of test item related effects.

14.12 MICROSCOPIC EXAMINATION

Morphometric Testes Evaluation Report, Attachment 7, pp. 2512 to 2526
Pathology Report, Attachment 8, pp. 2528 to 5055.
Statistical Analysis of Histopathology Data, Attachment 9, pp. 5057 to 5254

ONE YEAR INTERIM SACRIFICE

NON-NEOPLASTIC FINDINGS

Statistical significance in the Poly3 trend test of increasing trend over increasing dose and one or more of the Poly3 pair-wise comparisons of the dosed groups to control in the combined grades, or a significant Jonckheere-Terpstra trend test and one or more of the Jonckheere-Terpstra pair-wise comparisons of the dosed groups to control was seen in the endpoints listed in Table 6. Those endpoints that showed a statistically significant negative trend by either the Poly3 or the Jonckheere-Terpstra trend tests and statistically significant pair-wise decreases between the control and the dosed groups are listed in Table 7.

Kidneys

Calcified cylinders in the papillary tubules were noted in 19 male animals each of groups 4 (1600 ppm) and 5 (5000 ppm). The incidence of this finding was statistically significant and the severity of this alteration showed a dose-dependent pattern.

Tubular granular casts were noted in 2 male animals of group 5 (5000 ppm). Tubular hyaline casts were noted in male animals of all groups, including controls. The incidence of these casts was statistically significantly increased in males of group 5 (5000 ppm). Hyaline casts were also noted in 3 females of group 4 (1600 ppm) (statistically significant) and 1 female of group 5 (5000 ppm).

Cortical tubular mineralization was noted in male animals of all groups, including controls, and in 1 female each of groups 3 (400 ppm) and 5 (5000 ppm). The severity of this finding was increased in males of groups 3-5 (400 - 5000 ppm).

A number of other findings, such as chronic progressive nephropathy, tubular regeneration, papillary mineralization, calceal mineralization, corticomedullary mineralization in female animals, and cortical cyst, were noted in the kidneys. The incidence and severity of these findings did not distinguish exposed animals from controls.

Testes

Using morphometric analysis, multifocal Leydig cell hyperplasia was found to be present in all male animals after one year, regardless of treatment (Attachment 7). The severity of Leydig cell hyperplasia was increased in the animals of groups 2 - 5 (100 - 5000 ppm).

Nasal cavity

Eosinophilic inclusions, mainly in olfactory and respiratory epithelium, were statistically significantly increased in males of all exposure groups.

Sternum

The incidence of enchondral hyperostosis was statistically significantly increased in male animals of group 5 (5000 ppm).

Other organs/tissues

A number of non-neoplastic findings were noted in the other organs and tissues examined. The type, incidence, and severity of these findings did not distinguish exposed animals from controls.

NEOPLASTIC FINDINGS

Statistical significance in either the Peto Mortality Prevalence or Poly3 trend tests and one or more of the Fisher's Exact pair-wise comparisons of the dosed groups to control was seen only in the male animals of the one year exposure groups as shown in Table 8.

Testes

Benign Leydig cell tumors were noted in 4 of 20 animals of group 1 (air control), 13 animals of group 2 (100 ppm), 13 animals of group 3 (400 ppm), 15 animals of group 4 (1600 ppm) and 18 animals of group 5 (5000ppm). The incidence of Leydig cell tumors was statistically significantly increased ($p<0.01$) over controls at all HMDS exposure levels (Table 8.).

Other organs/tissues

A number of neoplastic findings were noted in the other organs and tissues examined. The type and organ distribution of these findings did not distinguish exposed animals from controls.

TWO YEAR TERMINAL SACRIFICE

NON-NEOPLASTIC FINDINGS

Statistical significance in the Poly3 trend test of increasing trend over increasing dose and one or more of the Poly-3 pair-wise comparisons of the dosed groups to control in the combined grades, or a significant Jonckheere-Terpstra trend test and one or more of the Jonckheere-Terpstra pair-wise comparisons of the dosed groups to control was seen in the endpoints listed in Table 6. Those endpoints that showed a statistically significant negative trend by either the Poly-3 or the Jonckheere-Terpstra trend tests and statistically significant pair-wise decreases between the control and the dosed groups are listed in Table 7.

Kidneys

Calcified cylinders in the papillary tubules of male animals were noted in 24 animals of group 3 (400 ppm), 63 animals of group 4 (1600 ppm), and all 65 animals of group 5 (5000 ppm). The incidence of this finding was statistically significantly increased and the severity of this alteration was dose-dependent.

The incidence of transitional cell hyperplasia on the renal papilla was statistically significantly increased in males of group 4 (1600 ppm) and 5 (5000 ppm). Transitional cell hyperplasia on the renal papilla was noted in 32 male animals of group 4 (1600 ppm) and 49 male animals of group 5 (5000 ppm). This change was also noted in 1 male animal of group 1 (air control) and 1 female animal of group 3 (400 ppm).

Multifocal cortical mineralization was noted in male and female animals of all groups. The incidence of this finding was statistically significantly increased in male animals of groups 4 (1600 ppm) and 5 (5000 ppm), while it was statistically significantly decreased in female animals of all exposed groups.

Yellow-brown pigment deposits in the proximal tubular epithelium were noted in male and female animals of all groups. The incidence of these deposits was statistically significantly increased in males of groups 4 (1600 ppm) and 5 (5000 ppm), while it was statistically significantly decreased in females of groups 2 (100 ppm) and 5 (5000 ppm).

A large number of other findings were noted in the kidneys. The incidence and severity of these findings did not distinguish exposed animals from controls.

Lungs

A number of findings were noted in the lungs. Except for alveolar histiocytosis, focal alveolitis and foreign body granuloma, these findings did not distinguish exposed animals from controls. A statistically significant increase of alveolar histiocytosis was noted in females of groups 4 (1600 ppm) and 5 (5000 ppm), and focal alveolitis was statistically significantly increased in males and females of group 5 (5000 ppm), while foreign body granuloma was statistically significantly increased in males of group 5 (5000 ppm). A number of other findings were noted in the lungs. The incidence and severity of these findings did not distinguish exposed animals from controls.

Nasal cavity

The incidence and severity of eosinophilic inclusions, mainly in olfactory and respiratory epithelium, were statistically significantly increased in males of groups 4 (1600 ppm) and 5 (5000 ppm).

Sternum

Enchondral hyperostosis was noted in animals of all groups. The incidence and severity of this change was statistically significantly increased in male animals of group 5 (5000 ppm) whereas a decreased incidence of enchondral hyperostosis was noted females of groups 2 - 5 (100 to 5000 ppm).

Femur

The incidence of enchondral hyperostosis was statistically significantly decreased in female animals of groups 2 - 5 (100 - 5000 ppm) and in males of groups 2 to 4 (100 to 1600 ppm).

Bone Marrow

Atrophy was noted in males and females of all groups. The incidence of this atrophy was statistically significantly increased in females of group 5 (5000 ppm). There was no clinically significant decrease in erythroid parameters.

Liver

The incidence of microgranuloma was statistically significantly increased in female animals of group 3 (400 ppm) and group 5 (5000 ppm). The effect was not seen in males and there was no clear dose response.

Tongue

The incidence of arteritis/periarteritis and vascular mineralization was statistically significantly increased in female animals of group 5 (5000 ppm).

Aorta

The incidence and severity of mineralization was statistically significantly increased in males of group 5 (5000 ppm).

Eyes

The incidence of uveitis was statistically significantly increased in males of group 5 (5000 ppm).

Other organs/tissues

A number of non-neoplastic findings were noted in the other organs and tissues examined. The type, incidence, and severity of these findings did not distinguish exposed animals from controls.

NEOPLASTIC FINDINGS

Statistical significance in either the Peto or Poly3 trend tests and one or more of the Fisher's Exact pair-wise comparisons of the dosed groups to control was seen only in the male animals of the 104-Week exposure groups (Table 8.).

Kidneys

The incidence of neoplastic findings in kidneys is presented in Table 8. Tubular cell adenomas were noted in 2 males of group 4 (1600 ppm) and 4 males of group 5 (5000 ppm). Tubular carcinomas were noted in 1 male of group 4 (1600 ppm) and 2 males of group 5 (5000 ppm).

Other organs/tissues

A number of neoplastic findings were noted in the other organs and tissues examined. The type and organ distribution of these findings did not statistically significantly distinguish exposed animals from controls.

SACRIFICE AFTER ONE YEAR OF EXPOSURE AND ONE YEAR OF RECOVERY

NON-NEOPLASTIC FINDINGS

Statistical significance in the Poly3 trend test of increasing trend over increasing dose and one or more of the Poly-3 pair-wise comparisons of the dosed groups to control in the combined grades, or a significant Jonckheere-Terpstra trend test and one or more of the Jonckheere-Terpstra pair-wise comparisons of the dosed groups to control was seen in the endpoints listed in Table 6. Those endpoints that showed a statistically significant negative trend by either the Poly-3 or the Jonckheere-Terpstra trend tests and statistically significant pair-wise decreases between the control and the dosed groups are listed in Table 7.

Kidneys

Calcified cylinders in the papillary tubules of male animals were noted in 4 animals of group 3 (400 ppm), 19 animals of group 4 (1600 ppm), and 19 animals of group 5 (5000 ppm). The severity of this alteration showed a dose-dependent pattern. The incidence of this finding was statistically significantly increased in males of groups 3 (400 ppm), 4 (1600 ppm) and 5 (5000 ppm).

Transitional cell hyperplasia on the renal papilla was noted in 7 male animals of group 4 (1600 ppm) and 12 male animals of group 5 (5000 ppm). This change was also noted in 2 male animals of group 1 (air control) and 1 male of group 2 (100 ppm). The incidence of this finding was statistically significantly increased in males of groups 4 (1600 ppm) and 5 (5000 ppm).

The incidence of hyaline casts was statistically significantly increased in males of groups 5 (5000 ppm).

A number of other findings were noted in the kidneys. The incidence and severity of these findings did not distinguish exposed animals from controls.

Sternum

Enchondral hyperostosis was noted in animals of all groups. The incidence of this change was statistically significantly increased in male animals of group 5 (5000 ppm) and statistically significantly decreased in females of all groups exposed to the test item.

Nasal cavity

Eosinophilic inclusions, mainly in olfactory and respiratory epithelium, were statistically significantly increased in males of groups 4 (1600 ppm) and 5 (5000 ppm) and females of groups 2 to 5 (100 to 5000 ppm).

Aorta / Heart / Tongue

The incidence and severity of mineralization in the aorta was statistically significantly increased in male animals of group 5 (5000 ppm). Vascular mineralization of the tongue was statistically significantly increased in females of groups 2-5 (100 to 5000 ppm). Vascular mineralization of the heart was statistically significantly increased in males of group 5 (5000 ppm). These findings are possibly due to renal effects.

Other organs/tissues

A number of non-neoplastic findings were noted in the other organs and tissues examined. The type, incidence, and severity of these findings did not distinguish exposed animals from controls.

NEOPLASTIC FINDINGS

A number of neoplastic findings were noted in the organs and tissues examined. The type and organ distribution of these findings did not distinguish exposed animals from controls.

Kidneys
A carcinosarcoma was noted in 1 male of group 5 (5000 ppm).

IN CONCLUSION, the following findings are considered to be related to the exposure to the test item:

- Kidneys: calcified cylinders in papillary tubules in male group 3 (400 ppm), and/or groups 4 (1600 ppm) and 5 (5000 ppm) at all sacrifice time points, transitional cell hyperplasia in male groups 4 (1600 ppm) and 5 (5000 ppm) at terminal sacrifice and after recovery.
- Testes: Increased incidence of Leydig cell tumors at all doses after one year of exposure. The majority of animals in subgroups B (one year of exposure and one year of recovery) and C (two years of exposure) developed Leydig cell tumors regardless of dose.
- Nasal cavity: increased incidence of eosinophilic inclusions in olfactory/respiratory epithelium of males in groups 4 (1600 ppm) and 5 (5000 ppm) at all sacrifice time points.

From the neoplastic findings diagnosed in this study, the occurrence of renal tubular adenomas and carcinomas, and a carcinosarcoma in males of groups 4 (1600 ppm) and 5 (5000 ppm) were not excluded as possibly related to the exposure to the test item, since these findings did not occur in groups 1 - 3 after two years of exposure.

All other findings diagnosed in this study are considered to be incidental findings commonly observed in animals of this age and strain.

15 DISCUSSION AND CONCLUSIONS

This combined toxicity/oncogenicity study comprised five groups of 105 male and 105 female Fischer 344 rats each, giving a total of 1050 animals. The animals were treated with hexamethyldisiloxane (HMDS) by whole body exposure. The target exposure levels were: 0 ppm (0 mg/L, control Group 1), 100 ppm (= 0.7 mg/L Group 2), 400 ppm (= 2.7 mg/L, Group 3), 1600 ppm (= 10.6 mg/L, Group 4), and 5000 ppm (= 33.2 mg/L, Group 5). The animals were exposed for 6 hours/day, 5 days/week. The animals in subgroup A (20 males and 20 females at each exposure concentration) were sacrificed after one year of exposure. The subgroup B animals (20 males and 20 females at each exposure concentration) were sacrificed after 1 year of exposure and 1 year of recovery period. Finally, the subgroup C animals (65 males and 65 females at each exposure concentration) were sacrificed after two years of exposure.

At all exposure levels (100, 400, 1600 and 5000 ppm), there were no test item related mortalities or clinical signs. No test item related abnormal findings were noted at ophthalmoscopic examination.

In the females at 1600 and 5000 ppm, a slight reduction in food consumption and possibly a correlated slightly reduced body weight (without any dose-relationship) could be a minimal effect of exposure to the test item. In the males of all groups, and in the females exposed to 100 or 400 ppm test item, no effects on food consumption or body weight gain were noted.

The changes noted in the hematological parameters may possibly reflect an increased hemoglobin turnover. Observed occasional small decreases in hematological parameters (RBC, HB, HCT) in males, slight increases in mean corpuscular volume (MCV) and decreases in mean corpuscular hemoglobin (MCH) concentration are considered mild and of little toxicological relevance.

The increase in urea, creatinine and electrolytes may be related to the morphological tubular changes noted in the kidneys as discussed below. All other findings in the clinical laboratory investigations, including the increase in lipids and changes in enzyme activity, suggest metabolic adaptive changes, primarily related to the liver. Increased absolute and relative liver weight in subgroup A (one year of exposure) males at 5000 ppm and increased relative liver weight at 1600 ppm together with the macroscopic finding of an enlarged liver in a proportion of subgroup A (one year of exposure) males at 5000 ppm support this suggestion. However, there were no correlated findings at histopathological examination. Therefore the changes noted in the liver and associated changes in the clinical biochemistry are considered to be the consequence of a temporary metabolic adaptation without any toxicological relevance.

Microscopic observation identified the kidneys, the testes and the nasal cavities as target organs.

In the kidneys, calcified cylinders in tubules of the papilla were noted in the males of groups 3 (400 ppm), 4 (1600 ppm) and 5 (5000 ppm) sacrificed after two years. Renal toxicity in rats is strain, sex and age dependent and may be related to physiological parameters. Similar findings were noted at 1600 and 5000 ppm at the one year interim sacrifice. Renal toxicity in the males of these two dose levels developed to transitional cell hyperplasia at terminal sacrifice after two years exposure as well as after the one year post-exposure recovery period. In addition, it should not be excluded that the occurrence of renal tubular adenomas and carcinomas and a carcinosarcoma in some males at 1600 and 5000 ppm is related to the exposure with the test item since these findings did not occur in group 1 (air control) or at 100 and 400 ppm. However, these effects on the kidneys do not adversely affect the survival rate of the animals. Nevertheless, the calcified cylinders in tubules of the papilla may be considered as a starting change of renal toxicity. A follow-up mechanistic study showed that an alpha 2u-globulin mediated mechanism was responsible for the observed nephropathy and kidney neoplasia in males[9].

Test item related increase of the testes weight was noted in the subgroup A (one year of exposure) and subgroup C (two years of exposure) males. This finding also correlated with the macroscopic finding of increased incidence of enlarged testes in all treated male groups of subgroup C (two years of exposure) and the following microscopic findings: Increased severity in Leydig cell hyperplasia and increased incidence in Leydig cell tumors at all dose levels was noted in the testes of the males exposed and sacrificed after one year. These testicular tumors are spontaneous and very common in Fischer 344 rats. In fact at the two years sacrifice time point only a few male animals did not show this finding. The one-year data suggest that HMDS exposure accelerated progression of this common tumor.

It was also noticed that hyperostosis was generally increased in males and decreased in females. Overall, the inconsistency of the effect between sexes and between different bones (sternum and femur) in the males, as well as the lack of dose-response in females, suggests that the finding is of little toxicological significance.

At 1600 and 5000 ppm, increased incidence of eosinophilic inclusions in olfactory epithelium was noted in the males at all sacrifice points. Eosinophilic inclusions are increased in numbers in response to some mildly irritant chemicals but are also commonly seen in ageing rats[10]. Since there were no other changes associated with a response to an irritant, such as an inflammatory cell infiltration or degenerative changes to the epithelium, the finding was considered to be non-specific and of low toxicological importance.

No microscopic findings, considered to be exposure-related, were noted in the female animals at all exposure levels. However, since the incidence of Leydig cell tumors was increased at all exposure levels a No Observable Effect Level (NOEL) was not determined from this study.

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