

TSCA HEALTH & SAFETY STUDY COVER SHEET

13016

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1.0 SUBMISSION TYPE --Contains CBI <input type="checkbox"/> 8(d) <input checked="" type="checkbox"/> 8(e) <input type="checkbox"/> FYI <input type="checkbox"/> 4 <input type="checkbox"/> OTHER: Specify <u>8EHQ-1298-14328</u> XX- Initial Submission -Follow-up Submission -Final Report Submission Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any: # _____ <input type="checkbox"/> continuation sheet attached		
2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e): optional for §4, 8(d) & FYI) X - YES <input type="checkbox"/> NO	2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID Cert# P 917006753 98-2-25	2.3 FOR EPA USE ONLY
3.0 CHEMICAL/TEST SUBSTANCE IDENTITY --Contains CBI <i>Reported Chemical Name (specify nomenclature if other than CAS name):</i> CAS#: 111988-49-9 (Cyanamide, [3-(6-chloro-3-pyridinyl)methyl]-2]thiazolidinylidene)-, Purity _____ % X - Single Ingredient <input type="checkbox"/> Commercial/Tech Grade <input type="checkbox"/> Mixture Trade Name: <u>YRC 2894</u> Common Name: <u>Chlornicotinyl</u>		
4.0 REPORT/STUDY TITLE -- Contains CBI Study on acute Inhalation Toxicity in Rats According to OECD # 403, Report # 28115 <input type="checkbox"/> Continuation sheet attached		
5.1 STUDY/TSCATS INDEXING TERMS [CHECK ONE] HEALTH EFFECTS (HE): <input checked="" type="checkbox"/> ENVIRONMENTAL EFFECTS (EE): _____ ENVIRONMENTAL FATE (EF): _____		
5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes) STUDY SUBJECT ROUTE OF VEHICLE OF TYPE: <u>ATOX</u> ORGANISM (HE, EE only): <u>RATS</u> EXPOSURE (HE only): _____ EXPOSURE (HEonly) _____ Other: _____ Other: _____ Other: _____		
6.0 REPORT/STUDY INFORMATION <input type="checkbox"/> Contains CBI X- Study is GLP Laboratory <u>Bayer Toxicology, Wuppertal, Germany</u> Report/Study Date: <u>11/4/98</u> Source of Data/Study Sponsor (if different than submitter) _____ Number of pages : <u>101</u> <input type="checkbox"/> continuation sheet attached		
7.0 SUBMITTER INFORMATION <input type="checkbox"/> Contains CBI Submitter: <u>Donald W. Lamb, Ph.D</u> Title: <u>V. P., Prod. Safety & Reg. Affrs</u> Phone: <u>412-777-7431</u> Company Name: <u>Bayer Corporation</u> Company Address: <u>100 Bayer Road</u> <u>Pittsburgh, PA 15205-9741</u> Submitter Address (if different): _____ Technical Contact: <u>Donald W. Lamb, Ph.D</u> Phone: <u>(412)777-7431</u> <input type="checkbox"/> continuation sheet attached		
8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS <input type="checkbox"/> Contains CBI This compound is an experimental pesticide. <input type="checkbox"/> continuation sheet attached		

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9.0 CONTINUATION SHEET

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CONTINUED FROM COVER SHEET SECTION # 2.1

An LC50 value less than or equal to 2 mg/l (2000 mg/m3) is a trigger for reporting acute inhalation studies. The 4-hour LC50 value was less than 2 mg/l for females (LC50 for females >690 <1535 mg/m3).

Abstract

A study to evaluate the acute inhalation toxicity of YRC 2894 480 SC 05776/0096 in male and female Wistar rats was conducted. Groups of rats were exposed nose-only to average liquid aerosol concentrations of 690 mg/m3 and 1535 mg/m3 air. The results are summarized as follows:

Rat LC50 : male >1535 mg/m3 female >690<1535 mg/m3

male & female NOAEL: <690 mg/m3 air

Exposure to the maximum tested concentration of 1535 mg/m3 was accompanied with mortality in female animals only. Clinical signs were observed in both sexes: respiratory tract irritation (bradypnea, labored breathing pattern, dyspnea, nostrils reddened and encrusted), hypothermia, piloerection, salivation, pallor, stilted gait, increased muscle tone, retardation in body weight gain, and central nervous effects (tremor, reduced motility, atony, and dilated pupils). The duration of signs appeared to be governed by respiratory signs such as labored breathing (until day 13) and unspecific signs such as piloerection (until day 7).

A concentration of 690 mg/m3 was tolerated without mortality in both sexes, but there were symptoms of toxicity: respiratory tract irritation (bradypnea and labored breathing pattern), retardation in body weight gain, and central nervous effects (reduced motility and tremor).

No treatment-related findings were detected in the rats sacrificed at the end of the 2-week post-exposure period or found dead.

In summary, the aerosolized test substance proved to have a moderate acute inhalation toxicity to female rats and a low acute inhalation toxicity to male rats. Specific pathognomonic findings indicate a causal relationship between exposure concentration and signs related to respiratory tract irritation.

BAYER AG

DEPARTMENT OF TOXICOLOGY
Friedrich-Ebert-Straße 217-333
D-42096 Wuppertal

Report No: 28115

Date: 04.11.1998

YRC 2894 480 SC 05776/0096
(c.n.: Thiacloprid)

**STUDY ON ACUTE INHALATION TOXICITY
IN RATS ACCORDING TO OECD No. 403**

by

Priv.- Doz. Dr. J. Pauluhn

Report Author

Dr. M. Temerowski

Study No.: T 6067418

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GOOD LABORATORY PRACTICE STATEMENT

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (GLP) and with the Principles of Good Laboratory Practice (GLP) according to Annex 1 ChemG (1994; cf. references) and meets the FIFRA Good Laboratory Practice Standards (40 CFR Part 160), with the exception that recognized differences exist between the GLP principles/standards of OECD and FIFRA (for instance, authority granted Agency inspectors and certain record retention requirements).

J. V. Pauluhn

Date: Nov. 4, 1998

Dr. J. Pauluhn D.A.B.T.
Board Approved Toxicologist (DGPT)
Study Director

SPONSOR:

BAYER AG

L. Macheimer

Date: Nov. 4, 1998

Dir. Dr. L. Macheimer
PH-PD/TOX LW

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Quality Assurance Statement**Test Item :** YRC 2894 480 SC 05776/0096**Study No.:** T6067418

Study-based inspections/audits were conducted by the Quality Assurance on the dates given below. Audit reports have been submitted in writing to the study director and, if necessary, also to the laboratory management, or other persons affected.

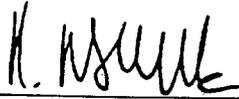
Date of audit**Date of report to study
director and/or management**

Sept. 10, 1998	(study plan)	Sept. 10, 1998
Sept. 14, 1998	(study conduct)	Sept. 14, 1998
Oct. 22, 1998 - Oct. 23, 1998	(first draft)	Oct. 23, 1998
Nov. 04, 1998	(final draft)	Nov. 04, 1998

The results of the study and the methods used have been correctly reported.

Quality Assurance Unit
PH-QA-C/GLP, Bayer AG

Date: Nov. 04, 1998

Responsible: 

Dr. R. Rauchschalbe

3 Signatures

Study director:

J. Pauluhn
(PD Dr. J. Pauluhn)

Date: Nov. 4, 1998

Report author:

M. Temerowski
(Dr. M. Temerowski)

Date: OCT 23, 1998

Institute
Head:

G. Schlüter
Dr. L. Machemer
(Prof. Dr. G. Schlüter)

Date: Nov. 4, 1998

4 Summary and Assessment

A study on the acute inhalation toxicity of YRC 2894 480 SC 05776/0096 (hereafter referred to as *test substance*) on rats has been conducted in accordance with OECD Guideline No. 403. Groups of rats (male and females) were nose-only exposed to an average liquid aerosol concentration of 690 mg/m³ and 1535 mg/m³ air. Attempts were made so that aerosol generated was respirable to rats. The results can be summarized as follows:

<p><u>LC₅₀ inhalation (aerosol, 4 hr)</u></p> <p>Males > 1535 mg/m³ ¹⁾</p> <p>Females: > 690 mg/m³ - < 1535 mg/m³ ¹⁾</p>	<p><u>NO(A)EL</u></p> <p>Males & females: < 690 mg/m³ air¹⁾.</p>
--	--

Observations and Measurements: Exposure to the maximum tested concentration of 1535 mg/m³ was accompanied with mortality in female animals only and - in both sexes - with clinical signs indicative of respiratory tract irritation (bradypnea, labored breathing pattern, dyspnoea, nostrils reddened and encrusted), hypothermia, piloerection, salivation, pallor, stilted gait, increased muscle tone, retardation in body weight gain and central nervous effects (tremor, reduced motility, atony, dilated pupils). The duration of signs appeared to be governed by respiratory signs such as laboured breathing (until day 13) and unspecific signs such as piloerection (until day 7). A concentration of 690 mg/m³ was tolerated without mortality in both sexes but with few symptoms, mainly due to respiratory tract irritation (bradypnea, labored breathing pattern), retardation in body weight gain and central nervous effects (reduced motility and tremor). No treatment-related findings were detected in the rats sacrificed at the end of the 2-week postexposure period or found dead.

¹ This concentration represent gravimetrically determined concentrations of the test substance in the rats' breathing zone.

With regard to the respirability of the aerosol generated internationally recognized recommendations such as of SOT (1992) were almost fulfilled, i.e. the MMAD was less than 4 μm in the low-dose groups (MMAD \approx 2,88 μm , GSD \approx 1.66) and slightly above 4 μm in the high-dose group (MMAD \approx 4,18 μm , GSD \approx 2,06).

In summary, the aerosolized test substance (liquid aerosol) proved to have a moderate acute inhalation toxicity to female rats and a low acute inhalation toxicity to male rats. Specific pathognomonic findings indicate a causal relationship between exposure concentration and signs related to respiratory tract irritation.

5 Introduction

This acute inhalation toxicity study was conducted in accordance with OECD Guideline No. 403 and the respective EU-Guideline using YRC 2894 480 SC 05776/0096 as a test substance. The study was performed on rats (nose-only exposure over 4 hours to a liquid aerosol, dynamic exposure conditions, 2 week observation period). This study served the purpose of product classification and to estimate the potential acute health hazard resulting from handling this product.

Testing facility:

Institute of Toxicology - Industrial Chemicals/Department of Occupational Toxicology, Bayer AG, D-42096 Wuppertal, Friedrich-Ebert-Straße 217 - 333, Germany.

Study/project identification:

Study no.: T 6067418

Study period:

September 14, 1998 - September 30, 1998

Experimental starting date: September 11, 1998 (technical pre-trials)

Study completion date: see signature of study director (page 7)

Sponsor:

BAYER AG, Agriculture Division

6 Responsibilities

Air conditioning/air make-up:	Dipl. Ing. G. Strietholt
Archiving of study data:	Prof. Dr. G. Schlüter
Biometric evaluation:	Dr. J. Pauluhn
Gross pathology:	Dr. Rosenbruch
Head of Department:	Prof. Dr. G. Schlüter
Laboratory Animal Services:	Dr. Petersen v. Gehr
Quality Assurance:	Dr. H. Lehn
Study Director:	Dr. J. Pauluhn
Report Author:	Dr. M. Temerowski
Analytical Chemistry:	Dr. M.G. Teller

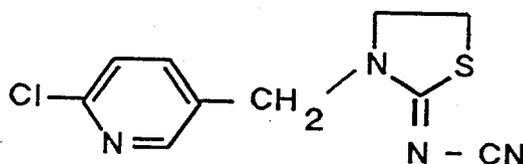
7 Materials and Methods

7.1 Test Substance

Test substance:	YRC 2894 480 SC05776/0096
Formulation no. (batch no.):	0100 based on formulation no. 05776/0096
Indication:	insecticide
Purity:	41.3 % YRC 2894
Specific density:	1,188 g/cm ³ at 20 °C
Manufacturer:	Bayer AG
Approval:	until November 05, 1998
Appearance:	white to pale-beige
Storage:	room temperature

Active ingredient

Common name:	Thiacloprid
Chemical name (C.A.):	Cyanamide, [3-[(6-chloro-3-Pyridinyl)Methyl]-2-Thiazolidinylidene]-
CAS Registry no.:	111988-49-9
Molecular weight:	252.8 g/mol
Molecular formula:	C ₁₀ H ₉ ClN ₄ S
Structural formula:	



7.2 Test System and Housing of Animals

Species and species justification: The study was carried out in rats, a rodent species recommended in the test guidelines.

Healthy young adult SPF bred Wistar rats, strain Hsd Cpb:WU (SPF), from the experimental animal breeder Harlan, Winkelmann, Borchon (Germany), were used. Animals of this strain have been used at Bayer AG in toxicological studies for years. Historical data on their physiology, diseases and spontaneous alterations are available. The state of health of the strain is randomly checked regularly at the instance of the Laboratory Animal Services, Bayer AG, for the most important specific infectious pathogens. The results of these examinations are archived.

Acclimatization: The animals were acclimatized to the animal room conditions for at least 5 days before use.

Identification: Animals were identified by both individual color-marking and cage-labels. All animals from this study were located on one cage-rack.

Randomization: Before the start of the study the health status of each animal was assessed. Animals were subsequently assigned to exposure groups at random (randomization procedure is described in section 7.17).

Health status: Only healthy rats free of signs were used for this study. The animals were not vaccinated or treated with anti-infective agents either before their arrival or during the acclimatization or study periods. The females were nulliparous and not pregnant.

Age and weight: At the study start the variation of individual weights did not exceed ± 10 per cent of the mean for each sex (see Appendix). Animals of the weight class used are about 7 (males) to 8-9 (females) weeks old and hence fulfill the criterion for young adults.

Animal housing: During the acclimatization and study periods the animals were housed singly in conventional Makrolon[®] Type II cages (based on A. Spiegel and R. Gönner, Zschr. Versuchstierkunde, 1, 38 (1961) and G. Meister, Zschr. Versuchstierkunde, 7, 144-153 (1965)). Cages and water bottles were changed twice

a week while unconsumed feed was changed once per week. The legal requirements for housing experimental animals (86/609 EEC) were followed.

Bedding: Bedding consisted of type S 8/15 low-dust wood granulate from Ssniff, Soest/Westfalen, Germany. The wood granulate was randomly checked for harmful constituents at the request of the Laboratory Animal Services, Bayer AG.

Animal rooms: All animals were housed in a single room. For reasons of space availability rats from other acute toxicity studies were housed in the same room, however mistakes in animal assignments were excluded by adequate spatial separation, clear cage labeling, and appropriate organization of all work procedures. The housing of several studies in one animal room is not considered to be a deviation from current GLP-requirements since many acute studies comprise of 10 animals only (as required to perform a limit test).

Environmental Conditions in the Animal Room

The animal room environment was as follows:

Room temperature:	22 ± 2 °C
Relative humidity:	approximately 50 %
Dark/light cycle:	12 h/12 h; artificial light from 6.00 a.m. to 6.00 p.m. Central European Time
Light intensity:	approximately 14 watt/m ² floor area
Ventilation:	approximately 10 air changes per hour

The room humidity and temperature were continuously monitored and documented using a calibrated thermohygrograph. Occasional deviations from these conditions occurred, e.g. as a result of animal room cleaning, but these had no detectable influence on the outcome of this study.

Cleaning, disinfection, and pest control: The animal room was regularly cleaned and disinfected once a week with an aqueous solution of TEGO[®] 2000. Contamination of the feed and contact with the test system were excluded. Pest control was not conducted in the animal room.

Feeding: Ration consisted of a standard fixed-formula diet (Altromin[®] 1324 pellets maintenance diet for rats and mice, Altromin GmbH, Lage) and tap water (drinking bottles). Both food and water were available *ad libitum*. The pelletized feed was contained in a rack in the stainless-steel wire cage cover. The nutritive composition and contaminant content of the standard diet was checked regularly by random sampling by the Laboratory Animal Services, Bayer AG. Details concerning general feed and water specifications are provided in the Appendix.

Water: Drinking quality tap-water (Drinking Water Decree of 05.12.1990, Bundesgesetzblatt [federal law gazette] part I, page 2612) was provided *ad libitum* in polycarbonate bottles containing approximately 300 ml (based on A. Spiegel and R. Gönner, Zschr. Versuchstierkunde, **1**, 38 (1961) and G. Meister, Zschr. Versuchstierkunde, **7**, 144-153 (1965)). The results of feed and water analyses are retained by Bayer AG. The available data provided no evidence of an impact on the study objective.

7.3 Test Guidelines

The study described below was carried out in accordance with OECD Guideline No. 403. The study conditions were adjusted so as to fulfill both the EC Guideline 92/69/EEC and the FIFRA § 81-3 (US EPA, 1984) guideline. Other published recommendations (US EPA, 1988; SOT, 1992; Pauluhn *et al.*, 1996) were also taken into account so as to comply with internationally recognized procedures.

7.4 Exposure Conditions

Mode of exposure: Animals were exposed to the aerosolized test substance in Plexiglas exposure tubes applying a *directed-flow* nose-only exposure principle. Tubes were chosen that accommodated the animals size. These tubes were designed so that the rat's tail remained outside the tube, thus restrained-induced hyperthermia can be avoided. This type of exposure is preferable to whole-body exposure on scientific (Pauluhn, 1984) and technical reasons (rapid attainment of steady-state concentrations, no problems with regard to test atmosphere inhomogeneities, better capabilities to control all inhalation chamber parameters, easier cleaning of exhaust air, and lower consumption of test substance). Moreover,

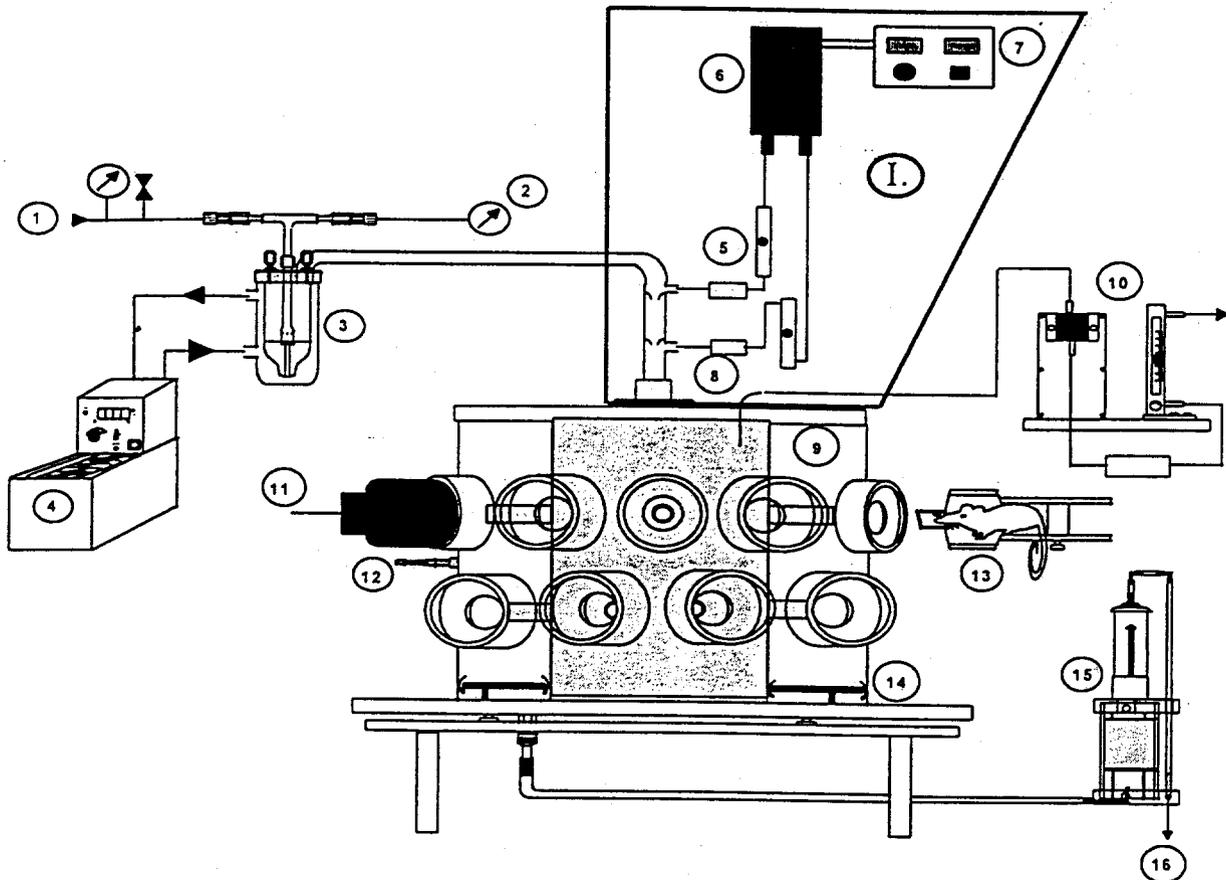
contamination of the fur can largely be avoided. The chambers used are commercially available (TSE, 61348 Bad Homburg) and the performance of this type of chamber has been published (Pauluhn, 1984; Pauluhn, 1988; Pauluhn, 1994).

Vehicle: The test substance was aerosolized as a 50% aqueous solution containing Levatit as vehicle.

7.5 Aerosol Generation and Exposure Technique

Aerosol generation/aqueous solution: The liquid was nebulized using a *Collison nebulizer* with conditioned compressed air (15 litres of air/min; dispersion pressure approximately ≈ 600 kPa; for details see Table 1, result section). The temperature of the solution was adjusted to 20 °C via a Julabo waterbath prior to nebulization. For generation of low-dose test atmospheres, the test substance concentration was reduced via by-passing of 50% of the primary air flow, purified by a cotton-wool-filter and re-delivered to the primary air stream (for details see figure 1).

Figure 1: Inhalation Chamber (schematic)



1. Compressed conditioned air supply	9. Exposure zone
2. Manometer	10. Real-time aerosol photometer
3. Collision-Nebulizer	11. Sensor for temperature/humidity measurements
4. Julabo-Thermostat	12. Sampling location ("breathing zone sampling")
I. No. 5.-8. for 750 mg/m³ only	13. Directed-flow nose-only exposure of rats in restrainer
5. Extraction of air with cotton-wool filter and Rotameter	14. Extraction of atmosphere from the chamber
6. Pump	15. Cotton-wool aerosol filter + HEPA filter
7. Digital control unit	16. Exhaust air
8. Dilution air supply with cotton-wool filter and Rotameter	

Inhalation Chamber: The aluminum inhalation chamber has the following dimensions: inner diameter = 14 cm, outer diameter = 35 cm (two-chamber system), height = 25 cm (internal volume = about 3.8 l). Control rats were exposed in a chamber consisting of 2 segments. All technical settings were adjusted accordingly and proportionally. The construction of the inhalation chamber is shown schematically in Figure 1. Details of this modular chamber and its validation with regard to spatial homogeneity of material distribution have been published (Pauluhn, 1994).

Conditioning the compressed air: Compressed air was supplied by Boge compressors and was conditioned (i.e. freed from water, dust, and oil) automatically by a VIA compressed air dryer. Adequate control devices were employed to control supply pressure.

Inhalation chamber steady-state concentration: The test atmosphere generation conditions provide an adequate number of air exchanges per hour (> 200 x, continuous generation of test atmosphere). Under such test conditions steady state is attained within the first minute of exposure ($t_{99\%} = 4.6 \times \text{chamber volume/flow rate}$; McFarland, 1976). The ratio between the air supplied and exhausted was chosen so that approximately 80-90% of the supplied air is removed via the exhaust system. The remainder provides adequate dead-space ventilation for the exposure tubes. At each exposure port a minimal air flow rate of 0.75 l/min was provided. The test atmosphere can by no means be diluted by bias-air-flows. The inhalation chamber was operated in a well ventilated chemical fume hood.

Air flows: During the exposure period air flows were monitored continuously and, if necessary, readjusted to the conditions required. Air flows were measured with calibrated flowmeters and/or soap bubble meter (Gilibrator, Ströhlein Instruments, Kaarst) and were checked for correct performance at regular intervals.

Treatment of exhaust air: The exhaust air was purified via cotton-wool and HEPA filters. These filters were disposed of by Bayer AG.

7.6 Inhalation Chamber Temperature and Humidity

The temperature and humidity measurements were made using a computerized system (Leybold Heraeus). These temperature and humidity sensors were located in the breathing zone area of the rats and in the exhaust location, respectively. The values were recorded at intervals of 10 min. The humidity sensors were calibrated using saturated salt solutions according to Greenspan (1977). The temperature sensors were calibrated with a calibration thermometer. Details of this type of monitoring have been reported elsewhere (Pauluhn, 1986).

7.7 Analysis of the Test Atmosphere

Nominal concentration: The nominal concentration was calculated from the ratio of the weight reduction of the collision nebulizer after cessation of the aerosol generation and the total throughput of air through the inhalation chamber. Specific information concerning air-flows and test atmosphere concentrations are provided in Table 1.

Gravimetric evaluation: The test-substance concentration was determined by gravimetric analysis (filter: Glass-Fibre-Filter, Sartorius, Göttingen, Germany; digital balance).

Samples were taken to allow direct comparison with cascade impactor analyses. All analytical concentrations reported refer to mg of test substance/m³ air.

7.8 Characterization of Aerodynamic Particle-Size Distribution

The samples for the analysis of the particle-size distribution were also taken in the vicinity of the breathing zone. During the exposure two samples were taken.

The particle-size distribution was analyzed using a BERNER-TYPE AERAS low-pressure critical orifice cascade impactor (Hauke, Gmunden, Austria). Specifications and evaluations are provided in the Appendix. The individual impactor stages had been covered by an aluminum foil covered with a glass fibre filter which were subjected to gravimetric analysis. An adhesive stage coating (silicone spray) was not used to prevent particle bounce and re-entrainment, respectively, due to the physicochemical properties of the test substance. Gravimetric analyses were made using a digital Sartorius M3P balance.

Evaluation of particle-size distributions: For the evaluation of the cascade impactor analyses the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) are determined from the probit-transformed

cumulative particle mass frequency distribution (y-axis) and the logarithmic effective cut-off diameters (ECD's) (x-axis) of the individual impactor stages by linear regression. The GSD is calculated from the regression line: percentile 84 / percentile 50. The relative mass with an aerodynamic diameter $\leq 3 \mu\text{m}$ ("respirable mass fraction") [Raabe, 1982; Snipes, 1989; SOT-Commentary, 1992] is calculated from the regression line. For probit transformation and linear regression FORTRAN algorithms published by Rosiello *et al.* (1977) are used. The MMAD was calculated using published following formulas [Marple and Rubow, 1980; Pauluhn, 1994].

To verify, whether the aerosol distribution is in fact unimodal and log-normal the normalized mass per stage (f'_H) is evaluated as a histogram. $\Delta \log D_p$ is equal the difference $\log D_{p+1} - \log D_p$, whereas D_p is the lower (left) cut-size limit and D_{p+1} the higher (right) cut-size limit of the corresponding impactor stage. As demonstrated by the evaluations included in the Appendix, the impactor stage cut-off limit (D_{p+1}) to the right was used for all calculations.

$$f'_H = \frac{1}{N_f} \times \frac{\text{mass / stage}}{\Delta \log D_p}$$

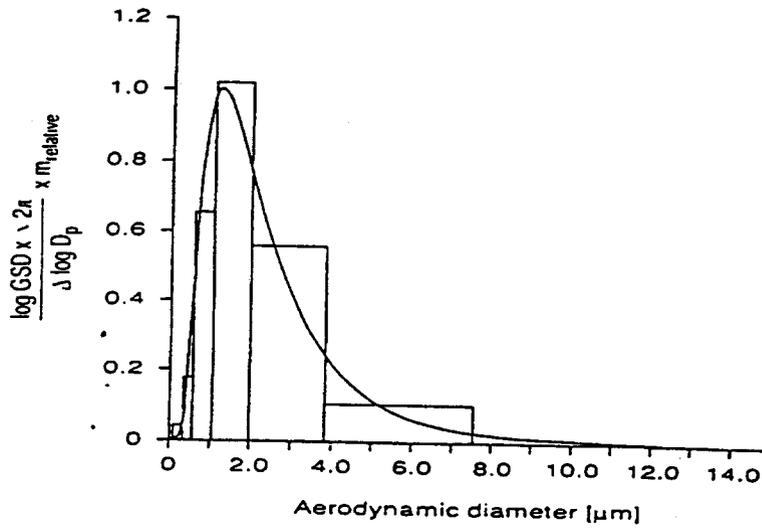
The log-normal mass distribution $y'(D_{ae}) = 1/N_f \times y(D_{ae})$ as a function of the aerodynamic diameter (D_{ae}) is computed using the formula:

$$y'(D_{ae}) = \exp \left[- \frac{(\log D_{ae} - \log MMAD)^2}{2 \times \log^2 GSD} \right]$$

The normalization factor (N_f) is calculated as follows:

$$N_f = \frac{\Sigma \text{mass}}{\log GSD \times \sqrt{2\pi}}$$

Where Σmass is the total mass collected by the cascade impactor, where $m_{\text{relative}} = \text{mass per stage} / \Sigma \text{mass}$ (cf. Fig. 2).

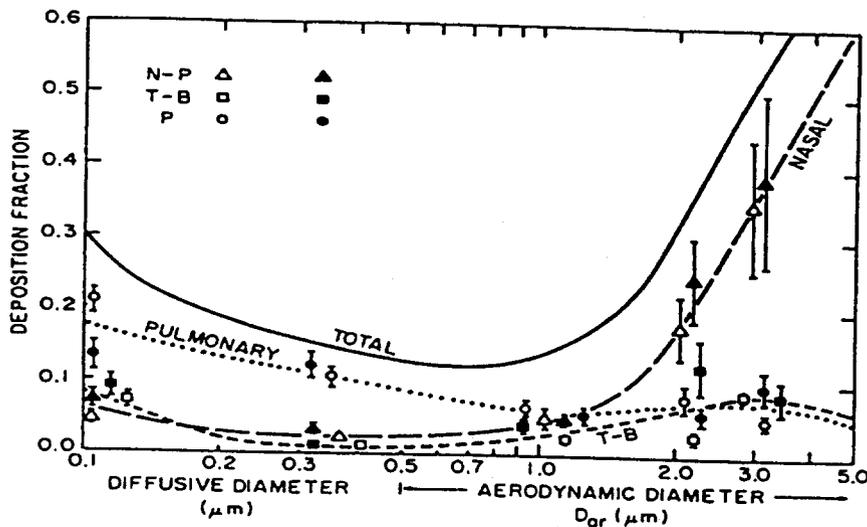
Figure 2: Principle of characterization of aerosol atmosphere

The algorithm for the calculation of particle size characteristics is taken from pertinent reference works on aerosol physics (Dennis, 1976; Marple and Rubow, 1980) and proves to be generally applicable (Pauluhn 1988; Pauluhn, 1994).

Respirability

Fig. 3 below, demonstrates that the particle-size distribution achieved is adequate to reach all potential target structures of the respiratory tract.

Figure 3: Respirability of Aerosols (Raabe, 1982)



7.9 Collection Efficiency

The sampling equipment was adjusted with calibrated rotameters to internationally recognized standards (ACGIH, 1978; Section I "Calibration of Air Sampling Instruments").

The conditions for generating the test atmosphere are optimized to provide maximum aerosol respirability to rats (Raabe, 1982; Snipes, 1989; SOT-Commentary, 1992). The absence of larger particles and high flow rates in the vicinity of the sampling ports make it possible to disregard potential anisokinetic sampling errors, thus ensuring a representative sampling even with different sampling probe orifice diameters and flow rates. The tolerance limits for the radius of the probe orifice are calculated using the following formula [ACGIH, 1978].

Calculations consider both a particle size distribution that encompasses aerodynamic diameters (D_{ae}) of 0.5 to 7.4 μm and sample flows ranging from 8 to 80 ml/sec.

$$5 \times 3 \sqrt{\frac{\text{flow} \times \tau}{4 \times \pi}} \leq r_p \leq \frac{1}{5} \times 2 \sqrt{\frac{\text{flow}}{g \times \tau \times \pi}}$$

r_p = radius of the sample probe in cm = $\frac{1}{2} \times D_p$

τ = relaxation time (D_{ae} 0.5 μm = 1×10^{-6} sec; D_{ae} 7.4 μm = 1.7×10^{-4} sec)

g = gravity constant = 980 cm/sec²

Tolerance limits calculations for the sample probe orifice (r_p) indicated that a representative sampling is assured when the orifice inner diameter is in the range of 1.0 to 1.6 cm. Orifices of the sampling instruments used here are in compliance with this criteria. Details of the D_p tolerance limit calculations are published elsewhere (Pauluhn, 1988; Pauluhn, 1994).

7.10 Stability of the Test Atmosphere

The integrity and stability of the aerosol generation and exposure system was measured by using a RAS-2 real-time aerosol photometer (MIE, Bedford, Massachusetts, USA). Samples were taken continuously from the vicinity of the breathing zone.

This chamber monitoring allows for an overall survey of toxicologically relevant technical parameters (inlet and exhaust flows as well as atmosphere homogeneity, temporal stability, and generation performance). Interruptions in exposure (e.g. resulting from obstruction of the nozzle or other technical mishaps) are recorded and, if applicable, a commensurate interval is added to the exposure duration for compensation.

7.11 Number of Animals per Group

Five male and five female rats were simultaneously exposed to each concentration under nose-only conditions for 4 h.

7.12 Control Animals

To identify exposure-related effects, comparisons with appropriate controls are conducted. Controls were exposed to conditioned air using almost similar exposure conditions as were used for the test substance (15 liters air/min and inhalation chamber segment; duration of exposure = 1 x 4 h; 5 males and females per group). Direct comparisons were made between the control and exposure groups.

Note: Control studies are performed under GLP-conditions but without assignment to a particular study. This allows use of control data for several studies that have been performed under similar experimental conditions within a recent time frame. This procedure is in compliance with current testing guidelines as well as animal welfare regulations.

7.13 Body Weights and Duration of Observation Period

Body weights were measured before exposure, on days 3 and 7, and day 14. Individual weights are also recorded at death, if applicable. The period of observation was for 2 weeks.

7.14 Clinical Signs

The appearance and behavior of each rat were examined carefully several times on the day of exposure and at least once daily thereafter. Weekend assessments were made once a day (morning). Assessments from restraining tubes were made only if unequivocal signs occurred (e.g. spasms, abnormal movements, severe respiratory signs). Following exposure, observations are made and recorded systematically; individual records are maintained for each animal. Cage-side observations included, but were not limited to, changes in the skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, lethargy, somnolence and prostration. The time of death is recorded as precisely as possible, if applicable. Since these signs can only be assessed adequately from freely moving animals, no specific assessment was performed during exposure while animals were restrained.

Each rat was first observed in its home cage and then individually examined. The following reflexes were tested, based on recommendations made by Irwin (1968): visual placing response and grip strength on wire mesh, abdominal muscle tone, corneal and pupillary reflexes, pinnal reflex, righting reflex, tail-pinch response,

startle reflex with respect to behavioral changes stimulated by sounds (finger snapping) and touch (back).

All signs exceeding the exposure day are reported in the form of incidence tables (see Appendix). The resolution of these tables is per one day. The incidence of signs will reflect signs from surviving animals. Uncommon signs related to an agonal condition (if applicable) that may be observed on the day of death are recorded but may not be incorporated into the incidence tables. Those signs are described in the results section if they differ from previous observations.

7.15 Rectal Temperatures

The rectal temperatures were measured directly after cessation of exposure (approximately within ½ hour after the end of exposure) using a Digimed digital thermometer with a rectal probe for rats.

7.16 Necropsy

All surviving rats were sacrificed at the end of the observation period using sodium pentobarbital (Nembutal®) (approximately 300 mg/kg body weight, intraperitoneal injection). All rats, irrespective of the day of death, were given a gross-pathological examination. Consideration was given to performing a gross necropsy on animals as indicated by the nature of toxic effects, with particular reference to changes related to the respiratory tract. All gross pathological changes were recorded and evaluated.

7.17 Statistical Evaluation of Data

Necropsy findings: If specific findings occur from the respiratory tract of surviving rats they are evaluated statistically using the pair-wise Fisher test after the R x C chi-squared test. The Fisher test was only performed if differences occurred between groups in the R x C chi-squared test or if a frequency value of < 5 was calculated. This procedure was performed in accordance with Gad and Weil (1982). For calculation of the unilateral p value a symmetrical distribution was assumed ($p_{\text{unilateral}} = (p_{\text{bilateral}})/2$).

Body weights: Means and single standard deviations of body weights are calculated. Mean body weights are also depicted graphically as a function of time (see result section). Since in acute studies individual group means may differ prior to commencement of the first exposure, the body weight gain was statistically

evaluated for each group. For these evaluations a one-way ANOVA (vide infra) is used.

Particle size analysis: The statistical methods used in the evaluation of the particle-size distribution were described in Section 7.8.

Physiological data: Data of rectal temperature measurements are statistically evaluated using the ANOVA procedure (vide infra).

Calculation of the LC_{50} : Due to the low number of dose groups the median lethal concentration (LC_{50}) was not calculated. However, exposure concentrations tested are used to determine the limits / ranges from where on a 50% probability of death can be expected.

Randomization: A computerized list of random numbers served the purpose to assign animals at random to the treatment groups.

Analysis of variance (ANOVA): This parametric method checks for normal distribution of data by comparing the median and mean. The groups are compared at a confidence level of $(1-\alpha) = 95\%$ ($p = 0.05$). The test for the between-group homogeneity of the variance employed Box's test if more than 2 study groups were compared with each other. If the above F-test shows that the intra-group variability is greater than the inter-group variability, this is shown in the Appendix as "*no statistical difference between the groups*". If a difference is found then a pairwise post-hoc comparison is conducted (1- and 2-sided) using the Games and Howell modification of the Tukey-Kramer significance test. This program was originally obtained from BCTIC.

7.18 Programming and Validating Software

Software code for the following purposes was written in Microsoft Fortran 77: particle-size analysis, ANOVA, Fisher test, inhalation chamber and physiological data tabulation program, graphics software. The computer programs were carefully validated. The validation was conducted using published text book data sets (e.g. BCTIC, Gad and Weil, 1982). However, it should be emphasized that the formal GLP-requirements required for software validation were not fulfilled. Wherever possible, raw data and calculated/derived values are displayed graphically to provide a versatile opportunity for data comparison.

7.19 Presentation of raw data

Raw data entered into, processed and/or stored by a computer system can be saved and printed out in various formats. The precision (number of decimal places) of the figures printed out and reproduced in this report reflects the toxicologically relevant precision in all cases. Deviations between manually calculated and computer-determined figures can thus arise due to rounding. A "zero" number of decimal places does not necessarily represent the pertinent measurement precision of the detection system.

Necropsy findings: These data were presented in a 'stochastic' manner, i.e., as being absent or present. No emphasis was made to incorporate raw data addressing subtle color changes or subjective intensity measures. Intensities or colors were only incorporated into the report when required to describe the severity of specific effects.

7.20 Archiving the Raw Data and the Report

The protocol, raw data, and the final report are archived in locations specified by Bayer AG, in accordance with GLP requirements.

8 RESULTS

8.1 Generation and Characterization of Atmosphere

Technical information concerning generation of test atmospheres is provided in Table 1.

Table 1: Generation and characterization of chamber atmosphere (liquid aerosol)

	Group 1	Group 2	Group 3
Target Conc. (mg/m ³)	0	750	2000
Nominal Conc. (mg/m ³)	333333	5862.5	11862.5
Gravimetric Conc. (mg/m ³)	Control Water	690	1535
Inlet Air Flow (l/min)	15	15	15
Dilution Air Flow (l/min)	--	7.5	--
Exhaust Air Flow (l/min)	13	13	13
Temperature (mean, °C)	21.9	21.6	21.3
Rel. Humidity (mean, %)	≈100	>95	>95
NMAD (µm)	--	1.33	0.895
MMAD (µm)	--	2.88	4.18
GSD	--	1.66	2.06
Aerosol Mass < 3 µm (%)	--	53.4	32.4
Mass recovered (mg/m ³)	--	629	1493

NMAD = Number Median Aerodynamic Diameter

MMAD = Mass Median Aerodynamic Diameter

GSD = Geometric Standard Deviation

-- = not applicable

For specific information concerning calculations of aerosol MMAD, GSD, and mass dependent size fraction below 3 µm, see Appendix.

Characterization of the test atmospheres: Real-time monitoring of the aerosol test atmosphere from the breathing zone indicated that the exposure conditions were temporally stable over the exposure period.

Analysis of the aerosol particle-size distribution from the breathing zone samples demonstrates that the aerosol generated was, in general, in the respirable range. Repeated measurements made during one exposure demonstrated temporally stable particle-size distributions. The lower gravimetric concentrations compared with the nominal concentrations are attributed to the incomplete aerosolization of water particles and/or to the evaporation of water. The total gravimetric concentration recovered by the cascade impactor was quite comparable to that concentration found by filter analyses. From this finding it can be concluded that interstage wall losses occurring within the low-pressure critical orifice cascade impactor or potential anisokinetic sampling error appear to be negligible (SOT, 1992, Pauluhn *et al.*, 1996).

Temperature values in the inhalation chamber were in the range suggested by the testing guidelines. The high humidity values are related to the vehicle containing a relative high amount of water.

8.2 Toxicological Results

The results obtained during and after exposures of rats for 4 h to this test substance are summarized in Table 2.

Table 2: Summary of acute inhalation toxicity - 4 hour exposure to aerosolized test substance (aerosol)

N Group /sex	Target Concentration (mg/m ³)	Toxicological Result	Onset and Duration of Signs	Onset and Duration of Mortality	Rectal Temperature (°C)
1 / m	0	0 / 0 / 5	--	--	38.1
2 / m	750	0 / 5 / 5	0d - 6d	--	33.5**
3 / m	2000	0 / 5 / 5	0d - 8d	--	31.2**
1 / f	0	0 / 0 / 5	--	--	38.2
2 / f	750	0 / 5 / 5	0d - 3d	--	33.6**
3 / f	2000	4 / 5 / 5	0d - 14d	1d - 6d	30.2**

N = group assignment, m = males, f = females, 0d = day of exposure, **=p<0.01

Values given in the 'Toxicological results' column are as follows:

- 1st number = number of dead animals
- 2nd number = number of animals with signs after exposure cessation
- 3rd number = number of animals exposed

Mortality

- LC₅₀: rat, male > 1535 mg/m³
- rat, female > 690 mg/m³ - < 1535 mg/m³

Different mortality figures were obtained in males and females. Mortality occurred only in females of the high-dose group from day 1 after exposure until day 6 of the follow-up period. As determined by gravimetric evaluations the scheduled test-substance concentrations were nearly met for the low-dose groups. The scheduled test substance concentration in high-dose animals was not met but it was in the range of the maximum technically attainable concentration.

Signs and observations

Details concerning signs and observations are provided in the Appendix in the form of various incidence tables. The following list of signs is focusing on toxicologically significant signs only.

Control Groups : All rats tolerated the exposure without specific signs.

Treatment Groups : Bradypnea, labored breathing pattern, dyspnoea, nostrils reddened and encrusted, reduced motility, atony, tremor, piloerection, salivation, ungroomed hair-coat, pupils dilated, palpebral narrowing, pallor, stilted gait, increased muscle tone, body weight loss, lying in a flattened position.

Reflex measurements

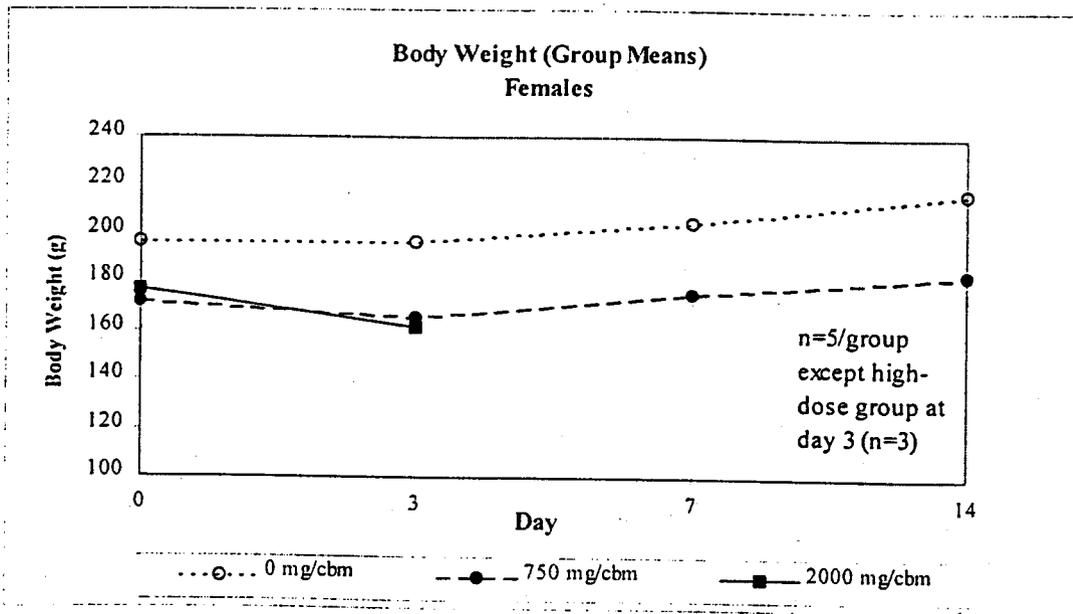
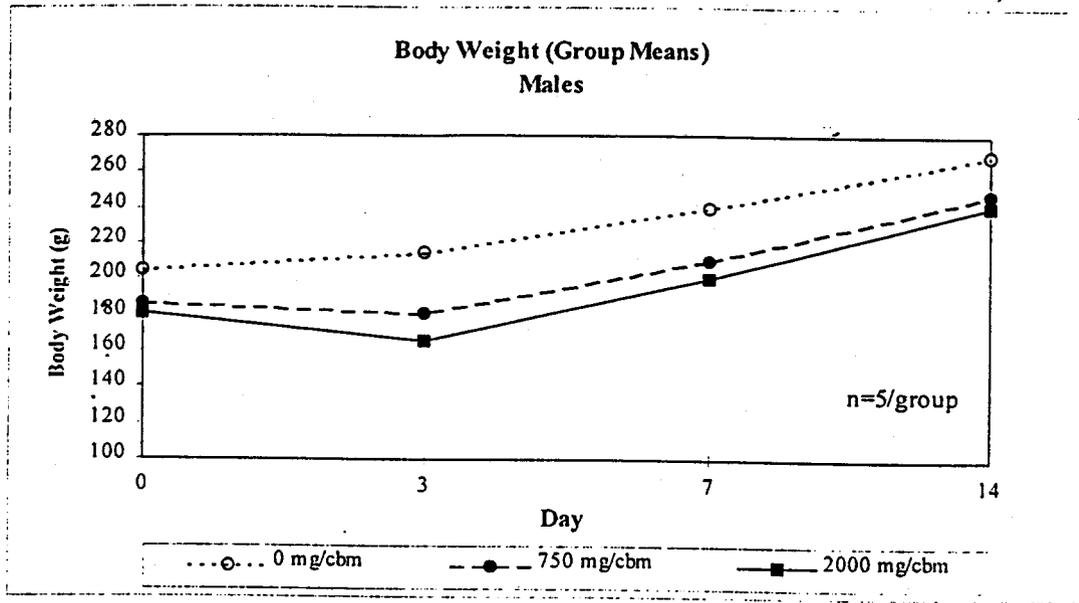
A battery of reflex measurements was made on the first postexposure day. Rats of the low-dose exposure groups of both sexes tolerated the exposure without any specific effect. High-dose females - and to a lesser extent - high-dose males exhibited abnormal reflexes which can be summarized as following:

Reduced grip strength, lacking grip strength (females only), reduced muscle tone, increased muscle tone (females only), reduced pupil response to pen-light, abnormal pinna reflex (females only), abnormal startle reflex to sound and touch (females only), changes in righting responses (for details see Appendix).

Body weights

Results of the evaluation of the body weights are included in the Appendix. Due to differences in baseline means between control animals and animals exposed to the test substance, confirmative statistics were done only on body weight gains. Comparisons between control animals with those in the group exposed to the test substance revealed a statistically significant body weight loss in the exposure groups of both sexes at day 3 (except high-dose females where test statistics were not calculated due to the reduced number of animals available at that time). Statistically significant higher body weight gains occurred in males at day 7 and day 14, being interpreted as a compensatory effect following the initial phase of body weight loss. The overall change of mean body weights is depicted in Fig. 4.

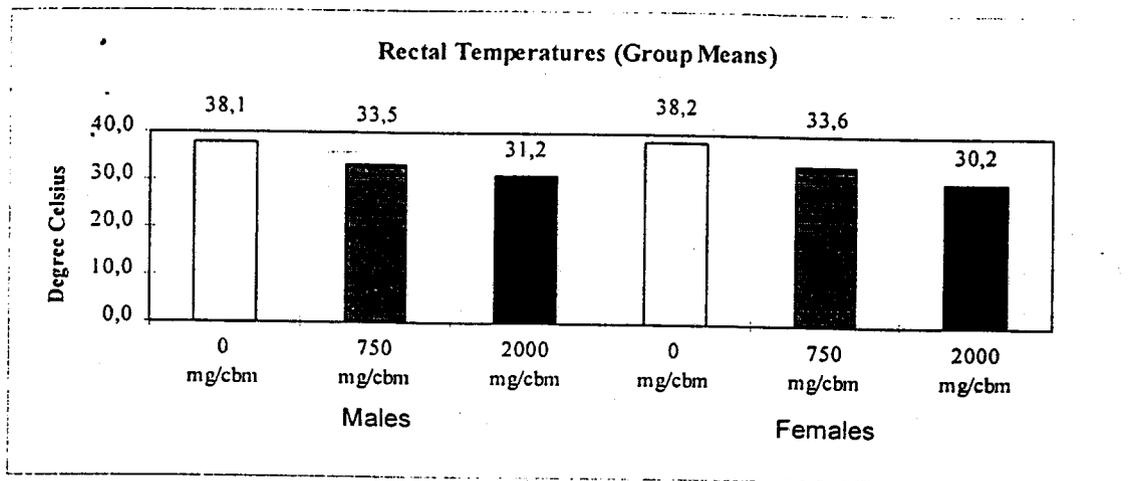
Figure 4: Body Weights (means)



Rectal temperature

Results of the evaluation of the rectal temperature are summarised in the Appendix. Rats exposed to the test substance experienced a marked dose-dependent decrease in body temperature. Statistical comparison indicates a significantly decreased body temperature in males and females of both dose groups.

Figure 5: Rectal Temperatures (means)



Necropsy

Individual findings from the gross-pathological examinations - including the exact onset of mortality - are summarized in the Appendix. A qualitative description, only of findings of toxicological importance and for toxicological evaluation, is given below.

Animals sacrificed at the end of the observation period: In all animals exposed to the test compound and surviving the entire test period a conclusive, concentration-dependent increased incidence of macroscopic findings could not be ascertained. The discolorations of the lungs observed singularly are not considered to be causally related to the exposure to the test substance.

Animals that died intercurrently: Lungs less collapsed, dark-red discolorations of lungs, discolorations of other parenchymatous organs.

9 EVALUATION AND DISCUSSION

Exposure to the maximum tested concentration of 1535 mg/m³ was accompanied with mortality in female animals and - in both sexes - with clinical signs indicative of respiratory tract irritation (bradypnea, labored breathing pattern, dyspnoea, nostrils reddened and encrusted), hypothermia, piloerection, salivation, ungroomed hair-coat, pallor, stilted gait, increased muscle tone, palpebral narrowing, retardation in body weight gain and central nervous effects (tremor, reduced motility, atony, dilated pupils). The duration of signs appeared to be governed by respiratory signs such as laboured breathing (until day 13) and unspecific signs such as piloerection (until day 7). A concentration of 690 mg/m³ was tolerated without mortality in both sexes but with few symptoms, mainly due to respiratory tract irritation (bradypnea, labored breathing pattern), retardation in body weight gain and central nervous effects (reduced motility and tremor). No treatment-related findings were detected in the rats sacrificed at the end of the 2-week postexposure period or found dead.

With regard to the respirability of the aerosol generated internationally recognized recommendations such as of SOT (1992) were almost fulfilled, i.e. the MMAD was less than 4 µm in the low-dose groups (MMAD ≈ 2,88 µm, GSD ≈ 1.66) but slightly above 4 µm in the high-dose group (MMAD ≈ 4,18 µm, GSD ≈ 2,06).

Inhalation of respiratory irritants is known to induce reflex changes in the breathing pattern and cardiac output and are reported to be associated with the decline in the metabolic rate and body temperature of rodents (Jaeger and Gearhart, 1982; Mautz and Bufalino, 1989). The present observation in rats may reflect a more labile thermoregulatory physiology among rodents and may involve a complex set of physiological responses that may differ among mammalian species. Sensory irritation induces respiratory and concomitantly central distress in animals which appears to be the possible cause of hypothermia.

In summary, the aerosolized test substance (liquid aerosol) proved to have a moderate acute inhalation toxicity to female rats and a low acute inhalation toxicity to male rats. Specific pathognomonic findings indicate a causal relationship between exposure concentration and signs related to respiratory tract irritation. In the light of the results of the analytical concentrations of active ingredients in the exposure

atmosphere and the clinical findings toxicity appears to be related to the test substance.

10 KEY TO ABBREVIATIONS

Konz.	Concentration
nomin.	Nominal
analyt.	Analytical
mcm/ μ m	Micrometer
Expos.	Exposure
MMAD	Mass Median Aerodynamic Diameter
NMAD	Number Median Aerodynamic Diameter
GSD	Geometric standard deviation (GSD)
ECD	Effective cut-off diameter
A _i	Sample for analysis
STAND, S, Std, s	Standard deviation
MW/MEANS	Means
F	F-test value (F-ratio)
DF	Degrees of freedom
PROB	Probability
SS	Total sum of squares
MS	Mean squares
TREATMENT	- between the groups
ERROR	- within the groups
TOTAL	- total

11 REFERENCES

- ACGIH (American Conference of Governmental Industrial Hygienists) (1978). Air Sampling Instruments for Evaluation of Atmospheric Contaminants, 5th Edition, ACGIH p. F-6. ACGIH section I: Calibration of Air Sampling Instruments and section F: Aerosol Sampling for Particle Size Analysis.
- BCTIC Computer Code Collection - Biomedical computing Technology Information Center, ANOVA a Fortran Program to Perform one-way Classification Analysis of Variance. Vanderbilt Medical Center, Nashville Tennessee, U.S.A.
- BLISS, C.I. (1938). The Determination of the Dosage-Mortality Curve from Small Numbers. Q.J. Pharm. Pharmacol. 11, 192-216.
- DENNIS R.(1976). Handbook of Aerosols - Technical Information Center, Energy Research and Development Administration, S. 110-114, July 1976.
- EG Guideline 86/609/EC (1986). Guideline of the Council dated November 24, 1986 on the Reconciliation of Legal and Administrative Regulations of the Member Countries for the Protection of Animals used for Studies and other Scientific Purposes. Journal of the European Community, Legal Specifications L 358, 29.
- EG Guideline 92/69/EWG. Journal of the European Community - Legal Specifications L 383 A, 35, December 29, 1992. B.2. Acute Toxicity - Inhalation. p. 121.
- GAD, S.C. and WEIL, C.S. (1982). Statistics for Toxicologists. Principles and Methods of Toxicology, ed. A.W. Hayes, Raven Press, New York, p. 280.
- GREENSPAN, L. (1977). Humidity Fixed Points of Binary Saturated Aqueous Solutions, Journal of Research of the National Bureau of Standards, Vol. 81 A, no. 1, Jan.-Febr. 1977.
- IRWIN, S. (1968). Comprehensive Observational Assessment: Ia: A Systematic, Quantitative Procedure for Assessing the Behavioral and Physiologic State of the Mouse. Psychopharmacologica 13, pp. 222-257.

- JAEGER RJ, GEARHART JM (1982). Respiratory and metabolic response of rats and mice to formalin vapor. *Toxicology* 25:299-309.
- MARPLE, V.A. and RUBOW, K.L. (1980). Aerosol Generation Concepts and Parameters in Generation of Aerosols and Facilities for Exposure Experiments, Ed. K. Willeke, Ann Arbor Science Publ. Inc. Mich., pp. 3-29.
- MAUTZ WJ, BUFALINO C (1989). Breathing pattern and metabolic rate responses of rats exposed to ozone. *Respiration Physiology* 76:69-78.
- McFARLAND, H.N. (1976). Respiratory Toxicology - Essays in Toxicology, Vol. 7, pp. 121-154, Academic Press Inc., New York, San Francisco, London.
- OECD - GLP (1983). Publication of the German version of the OECD Principles of Good Laboratory Practice (GLP), *Bundesanzeiger*, 35, No. 42a dated March 2, 1983.
- OECD-Guideline for Testing of Chemicals No. 403. "Acute Inhalation Toxicity", adopted May 12 (1981).
- PAULUHN J, BURY D, Föst U *et al.* (1996). Acute inhalation testing: considerations of technical and regulatory aspects. *Arch. Toxicol.* 71:1-10.
- PAULUHN, J. (1983). Computer-Aided Estimation of the LD₅₀/LC₅₀ BAYER AG Report No. 11835, dated May 18.
- PAULUHN, J. (1984). Head-only and nose-only exposure *in* P. Grosdanoff, R. Baß, U. Hackenberg, D. Henschler, D. Müller, H.-J. Klimisch (eds.), Problems of Inhalatory Toxicity Studies, BGA-Schriften, MMV Medizin Verlag München, Vol. 5, pp. 59-68.
- PAULUHN, J. (1986). Study to Determine Temperature and Humidity Data in Inhalation Chambers; BAYER AG Report No. 15007 dated August 22.
- PAULUHN, J. (1988). Different Methods used in Acute and Subchronic Inhalation Studies of Potential Lung Irritants with Particular Attention to Lung Function Measurements. *In* U. Mohr (ed.), Inhalation Toxicology - The Design and Interpretation of Inhalation Studies and their Use in Risk Assessment. Springer Verlag, pp. 87-101.

- PAULUHN, J. (1994). Validation of an improved nose-only exposure system for rodents. *Journal of Applied Toxicology*, 14:55-62.
- RAABE, O.G. (1982). Deposition and Clearance of Inhaled Aerosols *in* H. Witschi and P. Nettesheim - Mechanisms in Respiratory Toxicology Vol. I, pp. 27-76, CRC Press, Inc. Boca Raton, Florida.
- ROSIELLO, A.P., ESSIGMANN, J.M., and WOGAN, G.N. (1977). Rapid and Accurate Determination of the Median Lethal Dose (LD₅₀) and its Error with Small Computer. *J. Tox. and Environ. Health* 3, pp. 797-809.
- SCHAPER M.M., THOMPSON R.D., AND WEIL C.S. (1994). Computer programs for calculation of the median effective dose (LD₅₀ or ED₅₀) using the method of moving average interpolation. *Arch. Toxicol.* 68:332-337.
- SNIPES, M.B. (1989). Long-Term Retention and Clearance of Particles Inhaled by Mammalian Species. *Critical Reviews in Toxicology*, Vol. 20, pp. 175-211.
- SOT-COMMENTARY (1992). Recommendations for the Conduct of Acute Inhalation Limit Tests, prepared by the Technical Committee of the Inhalation Specialty Section, Society of Toxicology. *Fundam. Appl. Toxicol.* 18, pp. 321-327.
- TILLERY, M.I., WOOD, G.O., and ETTINGER, J.J. (1976). Generation and Characterization of Aerosols and Vapors for Inhalation Experiments. *Environmental Health Perspectives* 16, pp. 25-40.
- U.S. Environmental Protection Agency (1984). Pesticide assessment guidelines, subdivision F, hazard evaluation: Human and domestic animals (Revised) § 81-3 Acute Inhalation Toxicity Study. NTIS Report PB86-108958, Washington, DC.
- U.S. Environmental Protection Agency (1988). Hazard evaluation division: Standard evaluation procedure, inhalation toxicity testing, NTIS Report PB89-100366, Washington, DC.
- U.S. Environmental Protection Agency 40 CFR Part 160. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards (Final Rule); Thursday August 17, 1989.

12 APPENDIX**Atmosphere Characterization**

Group	Date of exposure (DD.MM.YY)	Sampled Volume (l) / flow rate (l/min)	Target Conc. (mg/m ³ air)	Actual Concentrations (mg/m ³ air)	Mean Conc. (mg/m ³ air)
1	10.08.1998	n.a.	--	0 (air control)	n.a.
3	16.09.1998	10 / 4 (filter analysis)	750	670 690 740 660	690
2	14.09.1998	10 / 4 (filter analysis)	2000	1470 1460 1630 1580	1535

In the subsequently presented tables, in most instances, target concentrations are used to indicate the respective group.

Characterization of Particle Size Distribution**ANALYSIS OF PARTICLE DISTRIBUTIONS**

Type of investigation: Acute Inhalation - Aerosol
 Compound: YRC 2894 480 SC 05776/0096 (I)
 Date of exposure: 14.09.98 Study-no.: T6067418
 Target concentration: 2000.0 mg/m³ air

N	Impactor stage (μm - μm)	Cut-Off diameter (μm)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	0.06 - 0.12	0.060	0.000	0.00	0.00
2	0.12 - 0.25	0.120	0.001	0.01	0.00
3	0.25 - 0.49	0.250	0.008	0.05	0.01
4	0.49 - 0.90	0.490	0.187	1.08	0.05
5	0.90 - 1.85	0.900	1.642	9.47	1.13
6	1.85 - 3.69	1.850	6.144	35.43	10.60
7	3.69 - 7.42	3.690	7.785	44.89	46.03
8	7.42 - 14.80	7.420	0.474	2.73	90.92
9	14.80 - 30.00	14.800	1.100	6.34	93.66

Mass Median Aerodynamic Diameter (MMAD): 4.09 μm
 Geometric standard deviation (GSD): 1.98
 Number Median Aerodynamic Diameter (NMAD): 1.02 μm
 Surface Median Aerodynamic Diameter (SMAD): 2.57 μm
 System: BERNER-IMPACTOR I
 Air flow: 5.85 liter/min.
 Sampling time: 120.00 seconds
 Concentration (computed): 1482.14 mg/m³ air

Respirability (percent < 1.0 μm):

1. Mass related: 2.1 % (measured)
 2. Number related: 49.1 % (extrapolated)

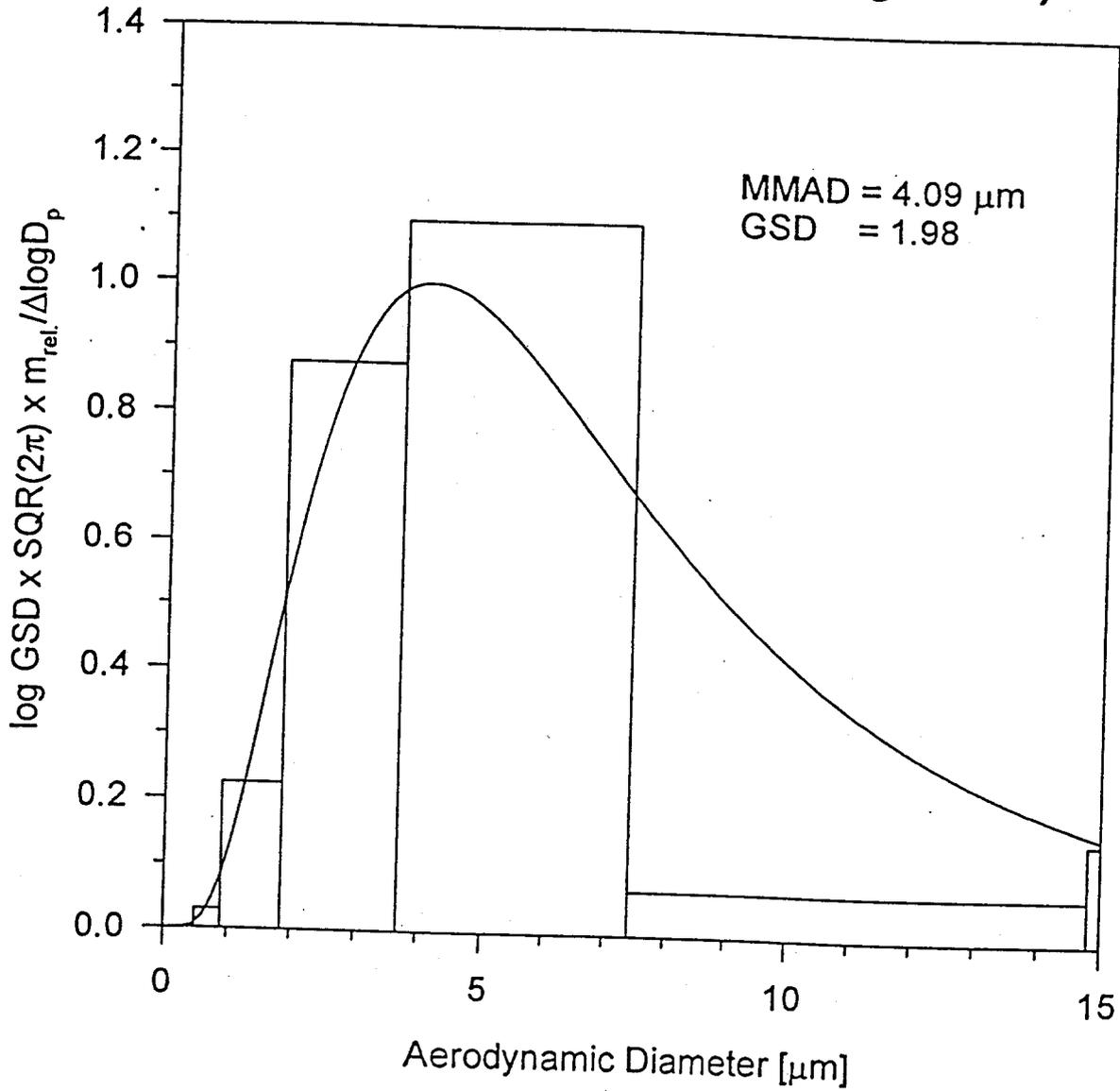
Respirability (percent < 3.0 μm):

1. Mass related: 32.7 % (measured)
 2. Number related: 94.5 % (extrapolated)

Respirability (percent < 5.0 μm):

1. Mass related: 61.7 % (measured)
 2. Number related: 99.1 % (extrapolated)

Particle-size Analysis (Target concentration: 2000 mg/m³ air)



ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol
 Compound: YRC 2894 480 SC 05776/0096 (II)
 Date of exposure: 14.09.98 Study-no.: T6067418
 Target concentration: 2000.0 mg/m³ air

N	Impactor stage (μm - μm)	Cut-Off diameter (μm)	Mass/stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	0.06 - 0.12	0.060	0.000	0.00	0.00
2	0.12 - 0.25	0.120	0.000	0.00	0.00
3	0.25 - 0.49	0.250	0.000	0.00	0.00
4	0.49 - 0.90	0.490	0.158	0.90	0.00
5	0.90 - 1.85	0.900	1.524	8.66	0.90
6	1.85 - 3.69	1.850	6.369	36.20	9.56
7	3.69 - 7.42	3.690	7.436	42.27	45.76
8	7.42 - 14.80	7.420	0.406	2.31	88.03
9	14.80 - 30.00	14.800	1.700	9.66	90.34

Mass Median Aerodynamic Diameter (MMAD): 4.27 μm
 Geometric standard deviation (GSD): 2.13
 Number Median Aerodynamic Diameter (NMAD): 0.77 μm
 Surface Median Aerodynamic Diameter (SMAD): 2.41 μm
 System: BERNER-IMPACTOR I
 Air flow: 5.85 liter/min.
 Sampling time: 120.00 seconds
 Concentration (computed): 1503.68 mg/m³ air

Respirability (percent < 1.0 μm):

1. Mass related: 2.9 % (measured)
2. Number related: 63.9 % (extrapolated)

Respirability (percent < 3.0 μm):

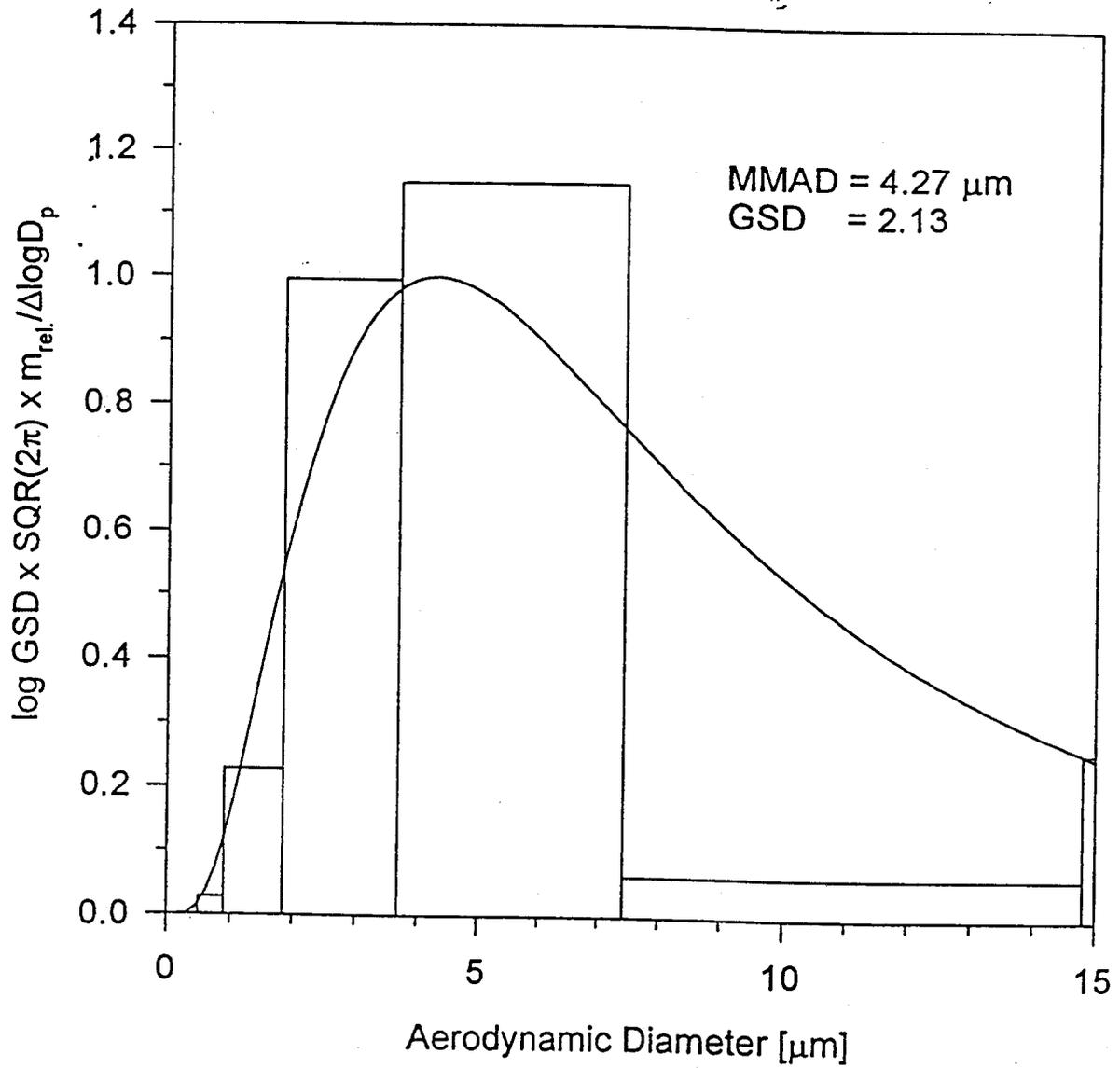
1. Mass related: 32.1 % (measured)
2. Number related: 96.5 % (extrapolated)

Respirability (percent < 5.0 μm):

1. Mass related: 58.3 % (measured)
2. Number related: 99.1 % (extrapolated)

Particle-size Analysis

(Target concentration: 2000 mg/m³ air)



ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol
 Compound: YRC 2894 480 SC 05776/0096 (I)
 Date of exposure: 16.09.98 Study-no.: T6067418
 Target concentration: 750.0 mg/m³ air

N	Impactor stage (μm - μm)	Cut-Off diameter (μm)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	0.06 - 0.12	0.060	0.000	0.00	0.00
2	0.12 - 0.25	0.120	0.002	0.01	0.00
3	0.25 - 0.49	0.250	0.028	0.20	0.01
4	0.49 - 0.90	0.490	0.266	1.85	0.21
5	0.90 - 1.85	0.900	2.003	13.96	2.06
6	1.85 - 3.69	1.850	5.942	41.42	16.03
7	3.69 - 7.42	3.690	5.871	40.93	57.45
8	7.42 - 14.80	7.420	0.232	1.62	98.38
9	14.80 - 30.00	14.800	0.000	0.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 2.84 μm
 Geometric standard deviation (GSD): 1.66
 Number Median Aerodynamic Diameter (NMAD): 1.31 μm
 Surface Median Aerodynamic Diameter (SMAD): 2.20 μm
 System: BERNER-IMPACTOR I
 Air flow: 5.85 liter/min.
 Sampling time: 240.00 seconds
 Concentration (computed): 612.99 mg/m³ air

Respirability (percent < 1.0 μm):

1. Mass related: 2.1 % (measured)
2. Number related: 30.1 % (extrapolated)

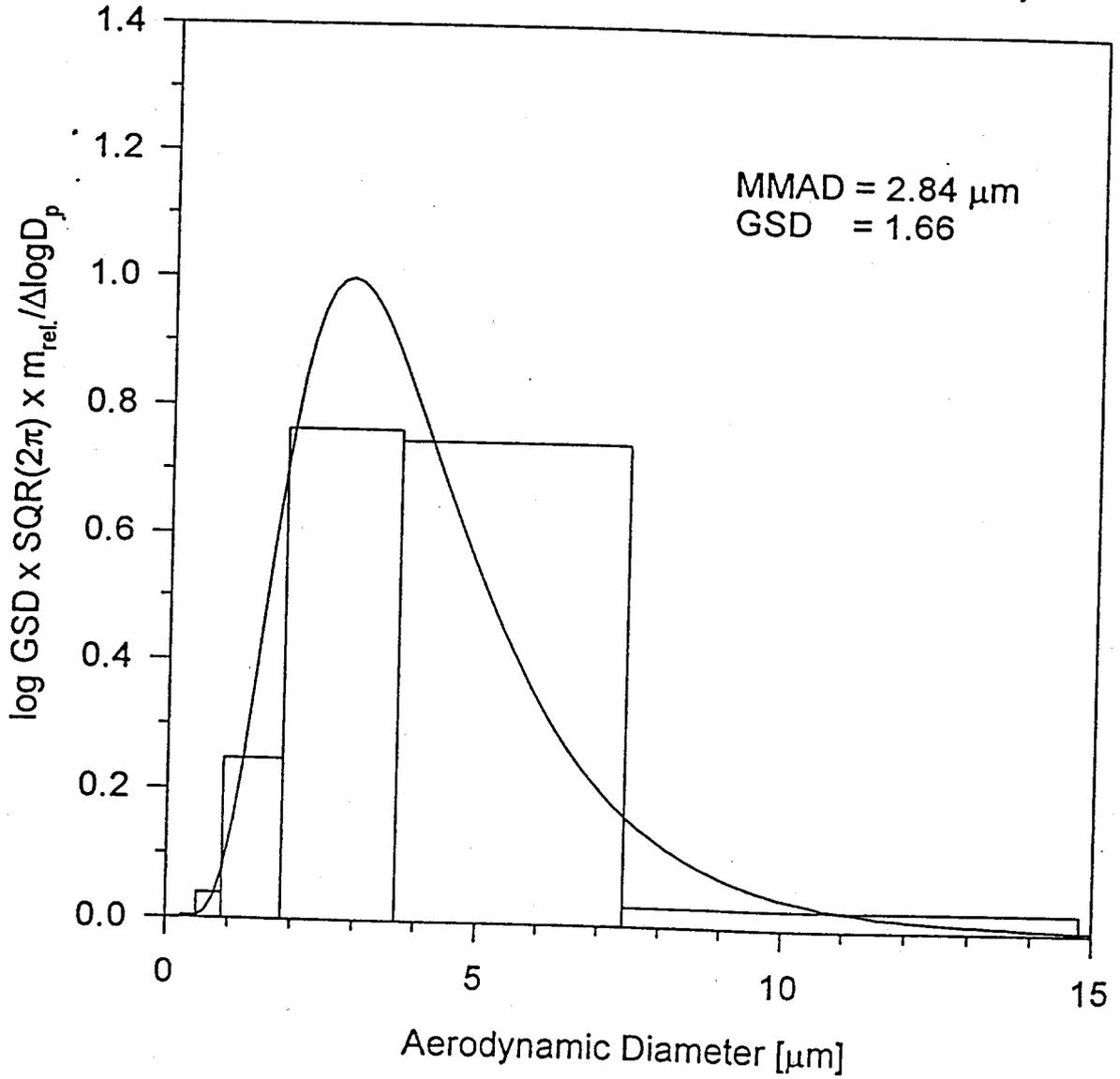
Respirability (percent < 3.0 μm):

1. Mass related: 54.3 % (measured)
2. Number related: 94.9 % (extrapolated)

Respirability (percent < 5.0 μm):

1. Mass related: 86.5 % (measured)
2. Number related: 99.1 % (extrapolated)

Particle-size Analysis (Target concentration: 750 mg/m³ air)



ANALYSIS OF PARTICLE DISTRIBUTIONS

Type of investigation: Acute Inhalation - Aerosol
 Compound: YRC 2894 480 SC 05776/0096 (II)
 Date of exposure: 16.09.98 Study-no.: T6067418
 Target concentration 750.0 mg/m³ air

N	Impactor stage (μm - μm)	Cut-Off diameter (μm)	Mass/ stage (mg)	Rel. mass (%)	Cumul. mass (%)
1	0.06 - 0.12	0.060	0.000	0.00	0.00
2	0.12 - 0.25	0.120	0.000	0.00	0.00
3	0.25 - 0.49	0.250	0.029	0.19	0.00
4	0.49 - 0.90	0.490	0.245	1.62	0.19
5	0.90 - 1.85	0.900	1.931	12.79	1.82
6	1.85 - 3.69	1.850	6.295	41.71	14.61
7	3.69 - 7.42	3.690	6.323	41.90	56.32
8	7.42 - 14.80	7.420	0.269	1.78	98.22
9	14.80 - 30.00	14.800	0.000	0.00	100.00

Mass Median Aerodynamic Diameter (MMAD): 2.91 μm
 Geometric standard deviation (GSD): 1.66
 Number Median Aerodynamic Diameter (NMAD): 1.35 μm
 Surface Median Aerodynamic Diameter (SMAD): 2.25 μm
 System: BERNER-IMPACTOR I
 Air flow: 5.85 liter/min.
 Sampling time: 240.00 seconds
 Concentration (computed): 644.96 mg/m³ air

Respirability (percent < 1.0 μm):

1. Mass related: 1.9 % (measured)
2. Number related: 27.9 % (extrapolated)

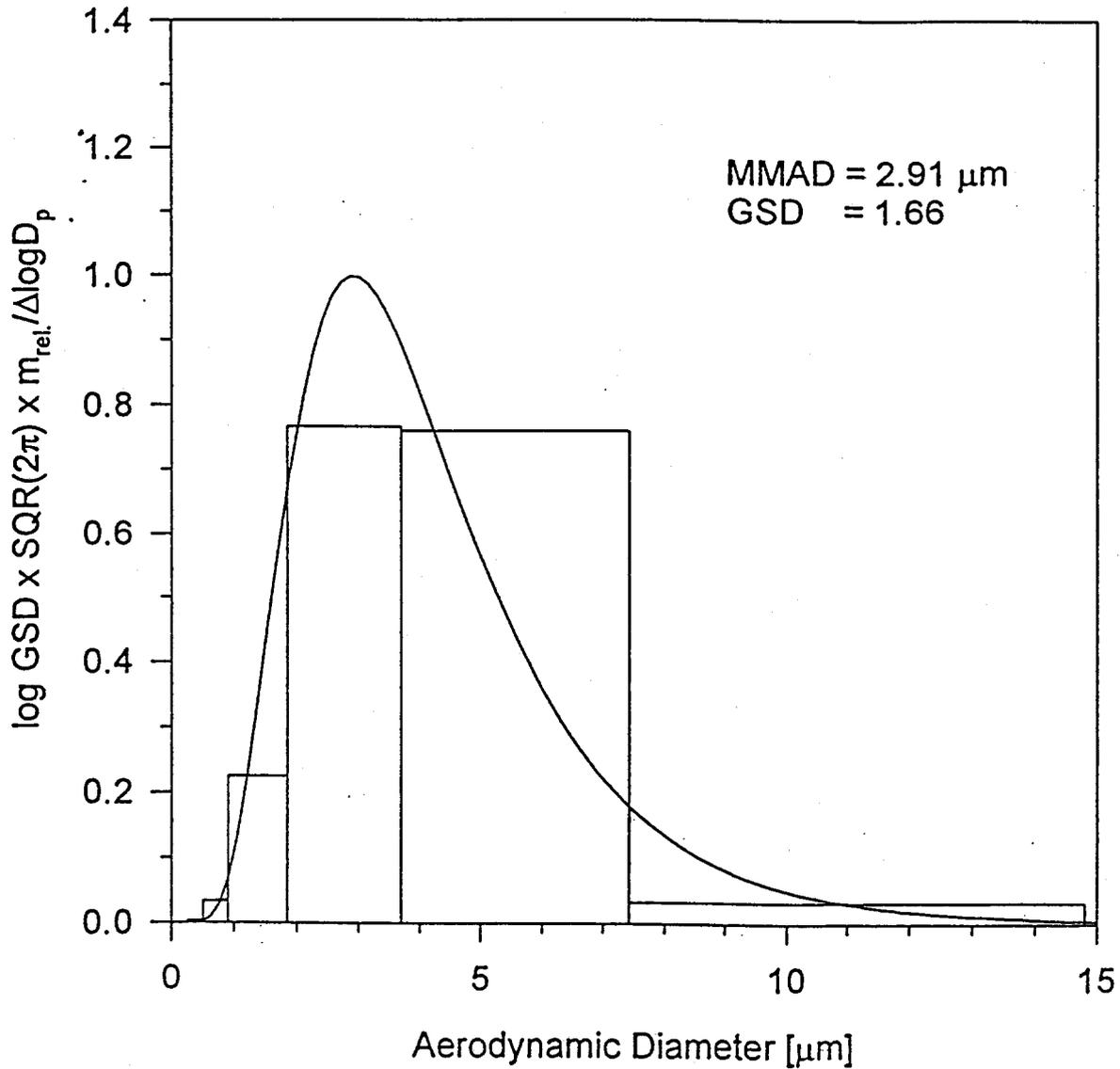
Respirability (percent < 3.0 μm):

1. Mass related: 52.5 % (measured)
2. Number related: 94.3 % (extrapolated)

Respirability (percent < 5.0 μm):

1. Mass related: 85.7 % (measured)
2. Number related: 99.1 % (extrapolated)

Particle-size Analysis (Target concentration: 750 mg/m³ air)



Reflexes**Measurements on day: 1 / MALES**

Type of Reflex	Group 1	Group 2	Group 3
Target Concentration (mg/m ³)	0	750	2000
Number of animals investigated	5	5	5
Visual placing response	0	0	0
Grip strength (vertical)	0	0	2 ^a
Grip strength (horizontal)	0	0	2 ^a
Tonus	0	0	1 ^a
Cornea reflex	0	0	0
Light reflex	0	0	2 ^a
Pinna reflex	0	0	0
Startle reflex / sound	0	0	0
Startle reflex / touch	0	0	0
Tail-pinch response	0	0	0
Righting response (open field)	0	0	0
Righting response (drop method)	0	0	2 ^b

^a : reduced ^b : slightly uncoordinated

#: number of rats showing abnormal reflexes

Measurements on day:1 / FEMALES

Type of Reflex	Group 1	Group 2	Group 3
Target Concentration (mg/m ³)	0	750	2000
Number of animals investigated	5	5	4
Visual placing response	0	0	1
Grip strength (vertical)	0	0	2 ^a
Grip strength (horizontal)	0	0	2 ^b
Tonus	0	0	3 ^c
Cornea reflex	0	0	0
Light reflex	0	0	3 ^d
Pinna reflex	0	0	1
Startle reflex / sound	0	0	1 ^e
Startle reflex / touch	0	0	1 ^f
Tail-pinch response	0	0	0
Righting response (open field)	0	0	4 ^g
Righting response (drop method)	0	0	4 ^h

- a** : *one*: reduced *one*: absent
b : *one*: reduced *one*: absent
c : *two*: increased *one*: reduced
d : *two*: mydriasis *one*: miosis
e : absent
f : freezing
g : *three*: impaired *one*: remains on back
h : *three*: slightly uncoordinated *one*: lands on side

#: number of rats showing abnormal reflexes

Rectal Temperatures**Rectal temperature (° C): Male Animals**

Control	Animal no.	1	2	3	4	5
		37.8	38.1	38.4	37.9	38.2
750 mg/cbm	Animal no.	21	22	23	24	25
		33.8	33.5	35.4	32.3	32.6
2000 mg/cbm	Animal no.	11	12	13	14	15
		30.2	30.3	31.2	29.9	34.2

Rectal temperature (° C): Female Animals

Control	Animal no.	6	7	8	9	10
		38.4	38.1	38.1	38.0	38.2
750 mg/cbm	Animal no.	26	27	28	29	30
		33.2	33.2	32.9	35.6	33.3
2000 mg/cbm	Animal no.	16	17	18	19	20
		30.3	29.7	29.8	29.9	31.4

YRC 2894 480 SC 05776/0096

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ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Rect.Temp (Deg.Cel) / MALES

Group-no.: 1 (1-5)

37.800	38.100	38.400	37.900	38.200
MEDIAN=	38.100	MEAN=	38.080	STD = .239

Group-no.: 2 (21-25)

33.800	33.500	35.400	32.300	32.600
MEDIAN=	33.500	MEAN=	33.520	STD = 1.219

Group-no.: 3 (11-15)

30.200	30.300	31.200	29.900	34.200
MEDIAN=	30.300	MEAN=	31.160	STD = 1.767

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
4.8962	2 & 324.	.0082

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	123.7	2	61.875	39.774	.000
ERROR	18.67	12	1.5557		
TOTAL	142.4	14			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
 TUKEY-KRAMER'S HONESTLY SIGNIFICANT DIFFERENCE TEST
 (WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION

5. % ONE-TAILED TEST				

1 AND 2	-11.60	4	.0013	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 2	11.60	4	.0013	SIGNIFICANT
5. % ONE-TAILED TEST				

1 AND 3	-12.27	4	.0007	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 3	12.27	4	.0007	SIGNIFICANT
5. % ONE-TAILED TEST				

2 AND 3	-3.48	7	.0976	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

2 AND 3	3.48	7	.0976	NOT SIGNIFICANT

YRC 2894 480 SC 05776/0096

T6067418

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Rect.Temp (Deg.Cel) / FEMALES

Group-no.: 1 (6-10)

38.400	38.100	38.100	38.000	38.200
MEDIAN=	38.100	MEAN=	38.160	STD = .152

Group-no.: 2 (26-30)

33.200	33.200	32.900	35.600	33.300
MEDIAN=	33.200	MEAN=	33.640	STD = 1.106

Group-no.: 3 (16-20)

30.300	29.700	29.800	29.900	31.400
MEDIAN=	29.900	MEAN=	30.220	STD = .698

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
4.8759	2 & 324.	.0084

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	158.6	2	79.309	137.291	.000
ERROR	6.932	12	.57767		
TOTAL	165.5	14			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
 TUKEY-KRAMER'S HONESTLY SIGNIFICANT DIFFERENCE TEST
 (WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION

5. % ONE-TAILED TEST				

1 AND 2	-12.80	4	.0005	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 2	12.80	4	.0005	SIGNIFICANT
5. % ONE-TAILED TEST				

1 AND 3	-35.16	4	.0000	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 3	35.16	4	.0000	SIGNIFICANT
5. % ONE-TAILED TEST				

2 AND 3	-8.27	7	.0016	SIGNIFICANT
5. % TWO-TAILED TEST				

2 AND 3	8.27	7	.0016	SIGNIFICANT

Body Weights

Analysis of Body Weights [all data in g]

Group 1: Kontrolle - MALES

	Postexposure Day			
	0	3	7	14
1	202.	212.	241.	274.
2	203.	214.	234.	264.
3	209.	218.	246.	273.
4	209.	217.	238.	267.
5	203.	214.	241.	270.
MEAN	205.2	215.0	240.0	269.6
STD	3.5	2.4	4.4	4.2

Group 2: 750 mg/cbm - MALES

	Postexposure Day			
	0	3	7	14
21	188.	180.	209.	245.
22	195.	194.	225.	265.
23	187.	180.	210.	252.
24	181.	171.	201.	230.
25	182.	177.	206.	243.
MEAN	186.6	180.4	210.2	247.0
STD	5.6	8.4	9.0	12.8

Group 3: 2000 mg/cbm - MALES

	Postexposure Day			
	0	3	7	14
11	184.	162.	199.	247.
12	184.	183.	210.	245.
13	179.	165.	203.	243.
14	175.	151.	186.	225.
15	182.	166.	203.	242.
MEAN	180.8	165.4	200.2	240.4
STD	3.8	11.5	8.9	8.8

Analysis of Body Weights [all data in g]

Group 1: Kontrolle - FEMALES

	Postexposure Day			
	0	3	7	14
6	203.	201.	210.	224.
7	190.	194.	204.	214.
8	195.	197.	203.	214.
9	200.	195.	203.	218.
10	193.	195.	204.	214.
MEAN	196.2	196.4	204.8	216.8
STD	5.3	2.8	2.9	4.4

Group 2: 750 mg/cbm - FEMALES

	Postexposure Day			
	0	3	7	14
26	169.	164.	175.	189.
27	167.	159.	168.	176.
28	173.	166.	179.	186.
29	171.	162.	172.	183.
30	182.	176.	182.	182.
MEAN	172.4	165.4	175.2	183.2
STD	5.8	6.5	5.5	4.9

Group 3: 2000 mg/cbm - FEMALES

	Postexposure Day			
	0	3	7	14
16	168.			
17	194.	169.		
18	175.	147.		
19	181.	169.	177.	193.
20	165.			
MEAN	176.6	161.7		
STD	11.5	12.7		

Analysis of Body Weight Gains [all data in g]

Group 1: Kontrolle - MALES

	Postexposure Day		
	3	7	14
1	10.00	29.00	33.00
2	11.00	20.00	30.00
3	9.00	28.00	27.00
4	8.00	21.00	29.00
5	11.00	27.00	29.00
MEAN	9.8	25.0	29.6
STD	1.3	4.2	2.2

Group 2: 750 mg/cbm - MALES

	Postexposure Day		
	3	7	14
21	-8.00	29.00	36.00
22	-1.00	31.00	40.00
23	-7.00	30.00	42.00
24	-10.00	30.00	29.00
25	-5.00	29.00	37.00
MEAN	-6.2	29.8	36.8
STD	3.4	.8	5.0

Group 3: 2000 mg/cbm - MALES

	Postexposure Day		
	3	7	14
11	-22.00	37.00	48.00
12	-1.00	27.00	35.00
13	-14.00	38.00	40.00
14	-24.00	35.00	39.00
15	-16.00	37.00	39.00
MEAN	-15.4	34.8	40.2
STD	9.0	4.5	4.8

Analysis of Body Weight Gains [all data in g]

Group 1: Kontrolle - FEMALES

	Postexposure Day		
	3	7	14
6	-2.00	9.00	14.00
7	4.00	10.00	10.00
8	2.00	6.00	11.00
9	-5.00	8.00	15.00
10	2.00	9.00	10.00
MEAN	.2	8.4	12.0
STD	3.6	1.5	2.3

Group 2: 750 mg/cbm - FEMALES

	Postexposure Day		
	3	7	14
26	-5.00	11.00	14.00
27	-8.00	9.00	8.00
28	-7.00	13.00	7.00
29	-9.00	10.00	11.00
30	-6.00	6.00	.00
MEAN	-7.0	9.8	8.0
STD	1.6	2.6	5.2

Group 3: 2000 mg/cbm - FEMALES

	Postexposure Day		
	3	7	14
16			
17	-25.00		
18	-28.00		
19	-12.00	8.00	16.00
20			
MEAN	-21.7		
STD	8.5		

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 3 / MALES

Group-no.: 1
 10.000 11.000 9.000 8.000 11.000
 MEDIAN= 10.000 MEAN= 9.800 STD = 1.304

Group-no.: 2
 -8.000 -1.000 -7.000 -10.000 -5.000
 MEDIAN= -7.000 MEAN= -6.200 STD = 3.421

Group-no.: 3
 -22.000 -1.000 -14.000 -24.000 -16.000
 MEDIAN= -16.000 MEAN= -15.400 STD = 9.044

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
5.5116	2 & 324.	.0048

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	1626.	2	813.07	25.622	.000
ERROR	380.8	12	31.733		
TOTAL	2007.	14			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMER'S HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION

5. % ONE-TAILED TEST				

1 AND 2	-13.82	5	.0001	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 2	13.82	5	.0001	SIGNIFICANT
5. % ONE-TAILED TEST				

1 AND 3	-8.72	4	.0074	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 3	8.72	4	.0074	SIGNIFICANT
5. % ONE-TAILED TEST				

2 AND 3	-3.01	5	.1786	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

2 AND 3	3.01	5	.1786	NOT SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 7 / MALES

Group-no.: 1
 29.000 20.000 28.000 21.000 27.000
 MEDIAN= 27.000 MEAN= 25.000 STD = 4.183

Group-no.: 2
 29.000 31.000 30.000 30.000 29.000
 MEDIAN= 30.000 MEAN= 29.800 STD = .837

Group-no.: 3
 37.000 27.000 38.000 35.000 37.000
 MEDIAN= 37.000 MEAN= 34.800 STD = 4.494

BOXS TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
3.9183	2 & 324.	.0203

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	240.1	2	120.07	9.380	.004
ERROR	153.6	12	12.800		
TOTAL	393.7	14			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
 TUKEY-KRAMERS HONESTLY SIGNIFICANT DIFFERENCE TEST
 (WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION

5. % ONE-TAILED TEST				

1 AND 2	3.56	4	.1339	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 2	3.56	4	.1339	NOT SIGNIFICANT
5. % ONE-TAILED TEST				

1 AND 3	5.05	8	.0178	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 3	5.05	8	.0178	SIGNIFICANT
5. % ONE-TAILED TEST				

2 AND 3	3.46	4	.1437	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

2 AND 3	3.46	4	.1437	NOT SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 14 / MALES

Group-no.: 1
 33.000 30.000 27.000 29.000 29.000
 MEDIAN= 29.000 MEAN= 29.600 STD = 2.191

Group-no.: 2
 36.000 40.000 42.000 29.000 37.000
 MEDIAN= 37.000 MEAN= 36.800 STD = 4.970

Group-no.: 3
 48.000 35.000 40.000 39.000 39.000
 MEDIAN= 39.000 MEAN= 40.200 STD = 4.764

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
1.2113	2 & 324.	.2988

HOMOGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	292.9	2	146.47	8.418	.005
ERROR	208.8	12	17.400		
TOTAL	501.7	14			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMER'S HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION

5. % ONE-TAILED TEST				

1 AND 2	4.19	5	.0685	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 2	4.19	5	.0685	NOT SIGNIFICANT
5. % ONE-TAILED TEST				

1 AND 3	6.39	6	.0096	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 3	6.39	6	.0096	SIGNIFICANT
5. % ONE-TAILED TEST				

2 AND 3	1.56	8	.5380	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

2 AND 3	1.56	8	.5380	NOT SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 3 / FEMALES

Group-no.: 1

-2.000	4.000	2.000	-5.000	2.000
MEDIAN=	2.000	MEAN=	.200	STD = 3.633

Group-no.: 2

-5.000	-8.000	-7.000	-9.000	-6.000
MEDIAN=	-7.000	MEAN=	-7.000	STD = 1.581

Group-no.: 3

-25.000	-28.000	-12.000		
MEDIAN=	-25.000	MEAN=	-21.667	STD = 8.505

BOXs TEST FOR HOMOGENEITY OF VARIANCES AT P=.05000 LEVEL

CALCULATED F	D.F.s	PROBABILITY
3.4793	2 & 178.	.0319

HETEROGENEOUS VARIANCES (ONE-TAILED TEST)

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	899.6	2	449.81	21.681	.000
ERROR	207.5	10	20.747		
TOTAL	1107.	12			

OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL

GAMES AND HOWELL MODIFICATION OF
TUKEY-KRAMERS HONESTLY SIGNIFICANT DIFFERENCE TEST
(WITH THE STUDENTIZED RANGE STATISTIC)

GROUPS COMPARED	CALCULATED TEST VALUE	DEGREES OF FREEDOM	PROBABILITY	CONCLUSION

5. % ONE-TAILED TEST				

1 AND 2	-5.75	5	.0220	SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 2	5.75	5	.0220	SIGNIFICANT
5. % ONE-TAILED TEST				

1 AND 3	-5.98	2	.0927	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

1 AND 3	5.98	2	.0927	NOT SIGNIFICANT
5. % ONE-TAILED TEST				

2 AND 3	-4.18	2	.1727	NOT SIGNIFICANT
5. % TWO-TAILED TEST				

2 AND 3	4.18	2	.1727	NOT SIGNIFICANT

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 7 / FEMALES

Group-no.: 1

9.000	10.000	6.000	8.000	9.000
MEDIAN=	9.000	MEAN=	8.400	STD =
				1.517

Group-no.: 2

11.000	9.000	13.000	10.000	6.000
MEDIAN=	10.000	MEAN=	9.800	STD =
				2.588

NOT ENOUGH GROUPS FOR BOXs TEST

CALCULATED F	DEG. OF FREEDOM	PROBABILITY
2.9130	4. & 4	.1628

HOMOGENEITY OF VARIANCES

ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	4.900	1	4.9000	1.089	.329
ERROR	36.00	8	4.5000		
TOTAL	40.90	9			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

ONE-WAY ANALYSIS OF VARIANCE PROGRAM : ANOVA

Analysis of Day: 14 / FEMALES

Group-no.: 1
 14.000 10.000 11.000 15.000 10.000
 MEDIAN= 11.000 MEAN= 12.000 STD = 2.345

Group-no.: 2
 14.000 8.000 7.000 11.000 .000
 MEDIAN= 8.000 MEAN= 8.000 STD = 5.244

NOT ENOUGH GROUPS FOR BOXs TEST

CALCULATED F	DEG. OF FREEDOM	PROBABILITY
5.0000	4. & 4	.0755

HOMOGENEITY OF VARIANCES

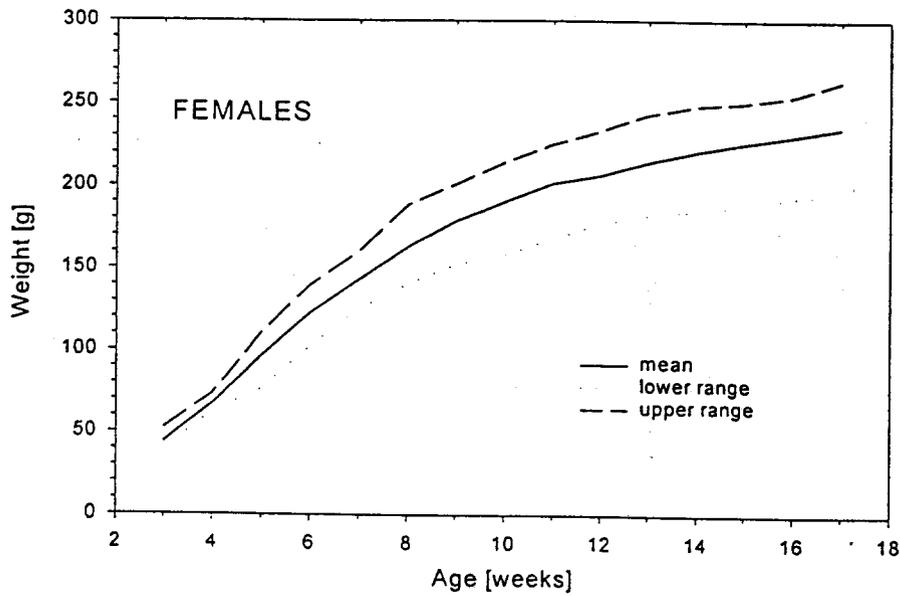
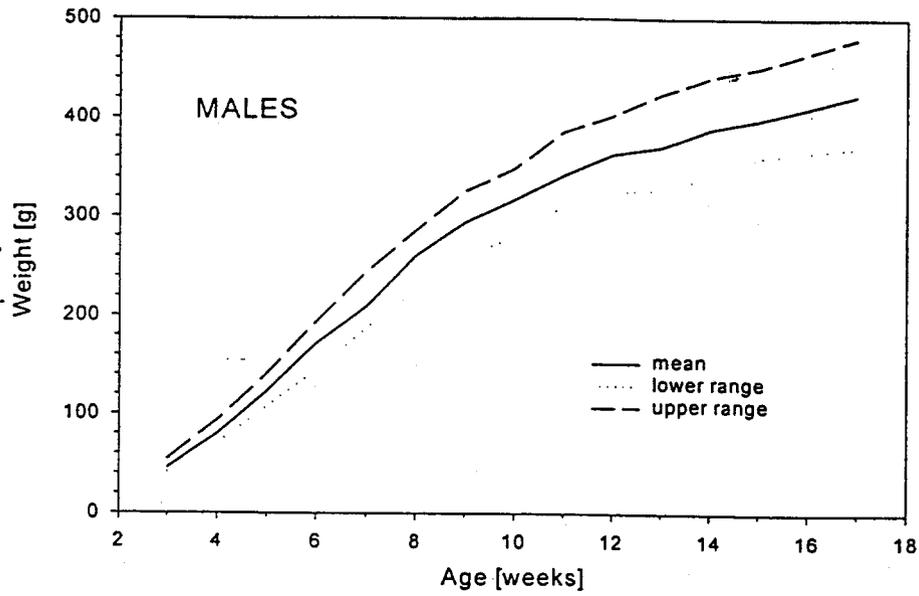
ONE-WAY CLASSIFICATION ANALYSIS OF VARIANCE

SOURCE	SS	DF	MS	F	PROB
TREATMENT	40.00	1	40.000	2.424	.156
ERROR	132.0	8	16.500		
TOTAL	172.0	9			

NO OVERALL SIGNIFICANCE AT 5.% (ONE-TAILED) LEVEL
 NO STATISTICAL DIFFERENCE BETWEEN THE GROUPS

Body Weights / Age-Body Weight Reference Data

(data from Harlan-Winkelmann as of April 1995; n = 30 per sex)



Clinical Observations¹

¹ Truncated characters: see next set of tables

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Bradypnea

Day	Sex		Target Concentration - mg/m ³ air											
			750			2000								
	l	m	M	M	F	F	F	N						
0	0	5	0	4	1	5	1	4	0	5	0	2	3	5
1	1	4	0	4	0	5	1	4	0	5	1	1	1	3
2	3	1	0	3	0	5	4	0	0	5	1	2	0	3
3	1	0	0	3	0	5	0	0	0	5	1	2	0	3
4	1	0	0	3	0	5	0	0	0	5	1	1	0	2
5	1	0	0	1	0	5	0	0	0	5	0	1	0	2
6	1	0	0	1	0	5	0	0	0	5	0	0	0	1
7	0	0	0	0	0	5	0	0	0	5	0	0	0	1
8	0	0	0	0	0	5	0	0	0	5	0	0	0	1
9	0	0	0	0	0	5	0	0	0	5	0	0	0	1
10	0	0	0	0	0	5	0	0	0	5	0	0	0	1
11	0	0	0	0	0	5	0	0	0	5	0	0	0	1
12	0	0	0	0	0	5	0	0	0	5	0	0	0	1
13	0	0	0	0	0	5	0	0	0	5	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Dyspnea

Day	Sex	Target Concentration - mg/m3 air															
		750				2000				750				2000			
		l	m	s	N	l	m	s	N	l	m	s	N	l	m	s	N
0		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
1		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
2		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
3		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
4		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
5		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
6		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
7		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
8		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
9		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
10		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
11		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
12		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
13		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
14		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Motility reduced

Day	Sex		Target Concentration - mg/m3 air													
	750		2000		750		2000									
	l	m	l	m	l	m	l	m								
0	2	3	0	0	1	3	1	5	1	2	0	5	0	2	3	5
1	2	0	0	0	2	1	2	5	0	0	0	5	0	2	0	3
2	1	0	0	0	2	1	0	5	0	0	0	5	0	1	1	3
3	0	0	0	0	1	0	0	5	0	0	0	5	1	1	0	3
4	0	0	0	0	1	0	0	5	0	0	0	5	1	1	0	2
5	0	0	0	0	0	0	0	5	0	0	0	5	0	1	0	2
6	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
7	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
8	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
9	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
10	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
11	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
12	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
13	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Atony

Day	Sex	Target Concentration - mg/m3 air											
		750				750				2000			
		l	m	s	N	l	m	s	N	l	m	s	N
0		0	0	0	5	0	0	0	5	0	0	0	5
1		0	0	0	5	0	0	0	5	0	0	0	5
2		0	0	0	5	0	0	0	5	0	0	0	5
3		0	0	0	5	0	0	0	5	0	0	0	5
4		0	0	0	5	0	0	0	5	0	0	0	5
5		0	0	0	5	0	0	0	5	0	0	0	5
6		0	0	0	5	0	0	0	5	0	0	0	5
7		0	0	0	5	0	0	0	5	0	0	0	5
8		0	0	0	5	0	0	0	5	0	0	0	5
9		0	0	0	5	0	0	0	5	0	0	0	5
10		0	0	0	5	0	0	0	5	0	0	0	5
11		0	0	0	5	0	0	0	5	0	0	0	5
12		0	0	0	5	0	0	0	5	0	0	0	5
13		0	0	0	5	0	0	0	5	0	0	0	5
14		0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Tremor

Day	Sex	Target Concentration - mg/m3 air															
		750				2000				750				2000			
		l	m	s	N	l	m	s	N	l	m	s	N	l	m	s	N
0		2	0	0	5	1	4	0	5	1	1	0	5	2	2	1	5
1		0	0	0	5	0	1	2	5	0	0	0	5	0	1	2	3
2		0	0	0	5	1	0	0	5	0	0	0	5	1	0	2	3
3		0	0	0	5	0	0	0	5	0	0	0	5	1	1	0	3
4		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	2
5		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	2
6		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
7		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
8		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
9		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
10		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
11		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
12		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
13		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
14		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Piloerection

Day	750		2000		750		2000		750		2000	
	M		M		F		F		M		M	
	l	s	l	s	l	s	l	s	l	s	l	s
0	1	4	1	4	1	0	4	1	0	5	0	5
1	4	1	0	3	3	0	0	0	0	5	0	2
2	3	0	0	1	2	0	0	0	0	5	0	3
3	3	0	0	0	1	0	0	1	2	5	1	2
4	2	0	0	0	0	0	0	1	1	5	1	0
5	1	0	0	0	0	0	0	1	1	5	1	0
6	0	0	0	0	0	0	0	1	0	5	1	0
7	0	0	0	0	0	0	0	1	0	5	1	0
8	0	0	0	0	0	0	0	1	0	5	1	0
9	0	0	0	0	0	0	0	0	0	5	0	0
10	0	0	0	0	0	0	0	0	0	5	0	0
11	0	0	0	0	0	0	0	0	0	5	0	0
12	0	0	0	0	0	0	0	0	0	5	0	0
13	0	0	0	0	0	0	0	0	0	5	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Salivation

Day	Sex	Target Concentration - mg/m3 air															
		750				2000				750				2000			
		l	m	s	N	l	m	s	N	l	m	s	N	l	m	s	N
0		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
1		0	0	0	5	0	0	0	5	0	0	0	5	0	1	0	3
2		0	0	0	5	0	0	0	5	0	0	0	5	0	1	0	3
3		0	0	0	5	0	0	0	5	0	0	0	5	0	1	0	3
4		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	2
5		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	2
6		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
7		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
8		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
9		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
10		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
11		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
12		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
13		0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
14		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Ungroomed hair-coat

Day	Sex		Target Concentration - mg/m3 air													
	750		2000				750				2000					
	l	m	s	N	l	m	s	N	l	m	s	N	l	m	s	N
0	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5
1	2	1	1	5	0	1	4	5	1	2	0	5	1	0	2	3
2	2	0	0	5	2	3	0	5	3	0	5	5	1	1	1	3
3	2	0	0	5	2	1	0	5	3	0	5	5	2	0	1	3
4	0	0	0	5	0	0	0	5	0	0	0	5	1	0	1	2
5	0	0	0	5	0	0	0	5	0	0	0	5	1	0	1	2
6	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
7	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
8	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
9	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
10	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
11	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
12	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
13	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Pupils dilated

Day	750		2000		750		2000		750		2000		750		2000	
	M		M		F		F		M		M		F		F	
	l	s	l	s	l	s	l	s	l	s	l	s	l	s	l	s
0	0	0	0	3	2	5	0	2	0	5	1	1	3	5		
1	0	0	0	0	0	5	0	0	0	5	0	0	1	3		
2	0	0	0	0	0	5	0	0	0	5	0	0	1	3		
3	0	0	0	0	0	5	0	0	0	5	0	0	1	3		
4	0	0	0	0	0	5	0	0	0	5	0	0	0	2		
5	0	0	0	0	0	5	0	0	0	5	0	0	0	2		
6	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
7	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
8	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
9	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
10	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
11	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
12	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
13	0	0	0	0	0	5	0	0	0	5	0	0	0	1		
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Palpebral narrowing

Day	Sex		750		2000		750		2000		mg/m3 air	
	l	m	s	N	l	m	s	N	l	m	s	N
0	0	0	0	5	1	2	0	5	0	0	0	5
1	0	0	0	5	0	0	0	5	0	0	0	3
2	0	0	0	5	0	0	0	5	0	0	0	3
3	0	0	0	5	0	0	0	5	0	0	0	3
4	0	0	0	5	0	0	0	5	0	0	0	2
5	0	0	0	5	0	0	0	5	0	0	0	2
6	0	0	0	5	0	0	0	5	0	0	0	1
7	0	0	0	5	0	0	0	5	0	0	0	1
8	0	0	0	5	0	0	0	5	0	0	0	1
9	0	0	0	5	0	0	0	5	0	0	0	1
10	0	0	0	5	0	0	0	5	0	0	0	1
11	0	0	0	5	0	0	0	5	0	0	0	1
12	0	0	0	5	0	0	0	5	0	0	0	1
13	0	0	0	5	0	0	0	5	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Pallor

Day	Sex	Target Concentration - mg/m3 air											
		750				750				2000			
		l	m	s	N	l	m	s	N	l	m	s	N
0		0	0	0	5	0	0	0	5	1	4	0	5
1		0	0	0	5	0	0	0	5	0	0	0	3
2		0	0	0	5	0	0	0	5	0	0	0	3
3		0	0	0	5	0	0	0	5	0	0	0	3
4		0	0	0	5	0	0	0	5	0	0	0	2
5		0	0	0	5	0	0	0	5	0	0	0	2
6		0	0	0	5	0	0	0	5	0	0	0	1
7		0	0	0	5	0	0	0	5	0	0	0	1
8		0	0	0	5	0	0	0	5	0	0	0	1
9		0	0	0	5	0	0	0	5	0	0	0	1
10		0	0	0	5	0	0	0	5	0	0	0	1
11		0	0	0	5	0	0	0	5	0	0	0	1
12		0	0	0	5	0	0	0	5	0	0	0	1
13		0	0	0	5	0	0	0	5	0	0	0	1
14		0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Stilted gait

Day	Sex	Target Concentration - mg/m3 air											
		750				750				2000			
		M	l	m	s	M	l	m	s	M	l	m	s
0		0	0	0	0	0	0	0	0	0	0	0	0
1		0	0	0	0	0	3	0	0	0	0	0	0
2		0	0	0	0	2	0	0	0	1	1	0	3
3		0	0	0	0	0	0	0	0	1	1	0	3
4		0	0	0	0	0	0	0	0	0	1	0	2
5		0	0	0	0	0	0	0	0	0	1	0	2
6		0	0	0	0	0	0	0	0	0	0	0	1
7		0	0	0	0	0	0	0	0	0	0	0	1
8		0	0	0	0	0	0	0	0	0	0	0	1
9		0	0	0	0	0	0	0	0	0	0	0	1
10		0	0	0	0	0	0	0	0	0	0	0	1
11		0	0	0	0	0	0	0	0	0	0	0	1
12		0	0	0	0	0	0	0	0	0	0	0	1
13		0	0	0	0	0	0	0	0	0	0	0	1
14		0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Increased muscle tone

Day	750		2000		750		2000		750		2000	
	M		M		F		F		M		M	
	l	s	l	s	l	s	l	s	l	s	l	s
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	3	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Body weight loss

Day	750		2000		750		2000		750		2000	
	M		M		F		F		M		F	
	l	s	l	s	l	s	l	s	l	s	l	s
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	1	0	0	0	0	0	0	0	0
3	0	0	0	2	0	0	0	0	0	0	1	0
4	0	0	0	1	0	0	0	0	0	0	1	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Pupils narrowed

Day	Sex		Target Concentration - mg/m3 air															
			750				2000				750				2000			
	l	m	s	N	l	m	s	N	l	m	s	N	l	m	s	N		
0	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	5		
1	0	0	0	5	0	0	0	5	0	0	0	5	0	0	1	3		
2	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	3		
3	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	3		
4	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	2		
5	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	2		
6	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
7	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
8	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
9	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
10	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
11	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
12	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
13	0	0	0	5	0	0	0	5	0	0	0	5	0	0	0	1		
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Test compound: YRC 2894 480 SC 05776/0096
 Study-no: T6067418

Sign: Lying flattened

Day	750 M			2000 M			750 F			2000 F		
	l	m	N	l	m	N	l	m	N	l	m	N
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0

Legend: l = slight, m = moderate, s = severe, N = survivors, M = males, F = females

Gross Necropsy**Individual findings / male rats**

Group	Animal No.	Time of death	Sacrificed after	Pathology findings
1	1		14 d	lung: isolated foci gray-red
	2		14 d	no observable findings
	3		14 d	no observable findings
	4		14 d	no observable findings
	5		14 d	no observable findings
2	21		14 d	no observable findings
	22		14 d	lung: foci gray-red
	23		14 d	no observable findings
	24		14 d	no observable findings
	25		14 d	no observable findings
3	11		14 d	lung: foci gray
	12		14 d	no observable findings
	13		14 d	no observable findings
	14		14 d	no observable findings
	15		14 d	no observable findings

Individual findings / female rats

Group	Animal No.	Time of death	Sacrificed after	Pathology findings
1	6		14 d	no observable findings
	7		14 d	no observable findings
	8		14 d	no observable findings
	9		14 d	lung: foci gray-red
	10		14 d	lung: foci gray-red
2	26		14 d	no observable findings
	27		14 d	lung: isolated foci dark-red
	28		14 d	no observable findings
	29		14 d	lung: foci white
	30		14 d	no observable findings
3	16	24 h		lung: slightly collapsed, areas dark-red
	17	4 d		lung: slightly collapsed, areas dark-red liver: pale spleen: pale kidneys: pale
	18	6 d		lung: slightly collapsed stomach: content black mucus, <i>mucosa</i> : areas discoloration black GI-tract: content black mucus esophagus: content full of bedding
	19		14 d	lung: slightly collapsed, foci gray-red
	20	1 d		lung: slightly collapsed, dark-red

Chow Specification - Nutrients

Altromin Standard Diets 1320 / Totally Pathogene Free TPF®. ALTROMIN 1320 Rat & Mouse Maintenance Diet has now been successfully used for in excess of 30 years and is normally fed to animals aged 8 weeks or over. The diet should be offered ad libitum together with an ample supply of fresh water. Sealed in polyethylene lined sacks, ALTROMIN 1320 can be passed directly into the SPF facility following surface disinfection. ALTROMIN 1324 consists of 10.0 mm pellets.

Specification of Maintenance Diet Rats/Mice

<u>Nutrients (average % content in the diet)</u>		<u>Amino Acids (average % content in the diet)</u>	
Crude protein	19.0	Lysine	0.90
Crude fat	4.0	Methionine	0.30
Crude fiber	6.0	Cystine	0.30
Ash	7.0	Phenylalanine	0.80
Moisture	13.5	Tyrosine	0.60
Nitrogen-free extract	50.5	Arginine	1.10
		Histidine	0.40
Metabolizable Energy (calculated)		Tryptophane	0.20
Kcal/kg	2850.0	Threonine	0.60
MJ/kg	11.9	Isoleucine	0.80
		Leucine	1.30
		Valine	0.90
<u>Minerals (average % content in the diet)</u>		<u>Trace elements (average mg content in 1 kg diet)</u>	
Calcium	0.9	Manganese	75.0
Phosphorus	0.7	Iron	180.0
Magnesium	0.2	Copper	5.0
Sodium	0.2	Zinc	70.0
Potassium	1.0	Iodine	0.9
		Fluorine	15.0
<u>Vitamins (additive in 1 kg diet)</u>		<u>Standard-Diet</u>	<u>Standard-Diet fortified</u>
Vitamin A		15000.0 IU	25000.0 IU
Vitamin D ₃		600.0 IU	1000.0 IU
Vitamin E		75.0 mg	125.0 mg
Vitamin K ₃		3.0 mg	5.0 mg
Vitamin B ₁		18.0 mg	30.0 mg
Vitamin B ₂		12.0 mg	20.0 mg

Vitamin B ₆	9.0 mg	15.0 mg
Vitamin B ₁₂	24.0 mcg	40.0 mcg
Nicotinic acid	36.0 mg	60.0 mg
Pantothenic acid	21.0 mg	35.0 mg
Folic acid	2.0 mg	3.0 mg
Biotin	60.0 mcg	100.0 mcg
Choline	600.0 mg	1000.0 mg
Vitamin C	36.0 mg	60.0 mg

Chow Specification - Impurities

Impurity	Max. acceptable value	LUFA - Limit of detection	Altromin *
Aflatoxine B1 / B2	0.01	0.0025	nng
Aflatoxine G1 / G2	0.01	0.0025	nng
Antibiotic activity	± 0		nng
Arsenic	2.0	0.2	0.3
Fluoride	150.0	5.0	22.0
Mercury	0.1	0.01	0.08
Lead	5.0	0.1	0.37
Cadmium		0.01	0.10
Selenium		0.10	1.0
Tecnazene		0.001	< 0.001
Quintozene		0.001	< 0.001
HCB (Hexachlorbenzene)		0.001	< 0.001
α -HCH		0.001	< 0.001
β -HCH		0.002	< 0.002
τ -HCH	0.1	0.001	0.002
Heptachlor	0.03	0.005	< 0.005
Heptachlorepoxyd	0.03	0.005	< 0.005
α - Chlordan	0.05	0.005	< 0.005
τ - Chlordan	0.05	0.005	< 0.005
Aldrin	0.02	0.005	< 0.005
Dieldrin	0.02	0.005	< 0.005
Endrin	0.02	0.01	< 0.01
o,p - DDE	0.05	0.005	< 0.005
p,p - DDE	0.05	0.005	< 0.005
o,p - DDD	0.05	0.005	< 0.005
o,p - DDT	0.05	0.005	< 0.005
p,p - DDD	0.05	0.01	< 0.01
p,p - DDT	0.05	0.01	< 0.01
Methoxychlor		0.01	< 0.01
PCB qual.			nng
Chlorthion		0.01	< 0.01
Disulfothion		0.005	< 0.005
Malathion		0.01	< 0.01
Methylparathion		0.005	< 0.005
Ethylparathion		0.01	< 0.01
Sulfotepp		0.002	< 0.002
Fenthion		0.005	< 0.005
Diazinon		0.01	< 0.01
Dibrom		0.02	< 0.02
Dimethoate		0.005	< 0.005
Trichlorphon		0.01	< 0.01
Fenitrothion		0.01	< 0.01

* In this study Altromin 1324 was used. 4 is the degree of pelletation. Dimension: ppm

Tap Water Specification

No.	Substance	Limit mg/l	computed as	equivalent mmol/m ³	acceptable error of value (\pm mg/l)
1	Arsenic	0.04	As	0.5	0.015
2	Lead	0.04	Pb	0.2	0.02
3	Cadmium	0.005	Cd	0.04	0.002
4	Chrome	0.05	Cr	1	0.01
5	Cyanide	0.05	CN-	2	0.01
6	Fluoride	1.5	F-	79	0.2
7	Nickel	0.05	Ni	0.9	0.01
8	Nitrate	50	NO ₃ ⁻	806	2
9	Nitrite	0.1	NO ₂ ⁻	2.2	0.02
10	Mercury	0.001	Hg	0.005	0.0005
11	Polycyclic aromatic carbohyrates - Fluoranthene - Benzo-b-fluoranthene - Benzo-k-fluoranthene - Benzo-a-pyrene - Benzo-(ghi)-perylene - Indeno-(1,2,3-cd)-pyrene	sum 0.0002	C	0.02	0.00004
12	Organochlorine compounds - 1,1,1-Trichlorethane - Trichlorethylene - Tetrachlorethylene - Dichlormethane - Tetrachlormethane	sum 0.01	-	-	0.004
		0.003	CCl ₄	0.02	0.001
13	a. Pesticides	indiv- dual com- pound			
	b. Polychlorinated Polybromated biphenyles and terphenyles	0.0001 sum 0.0005	-	-	0.00005
		0.0005	-	-	0.0002
14	Antimony	0.01	Sb	0.08	0.002
15	Selenium	0.01	Se	0.13	0.002

Specification of Test Compound

Bayer AG
PF-PM/PPA

03.06.98

Approval of Preparation Sample

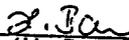
Preparation Sample TOX 4627

Sample: YRC 2894 00480 SC 05776/0096
 Development-No.: 0215336
 Indication: Insecticide
 Active Ingredients: 1. YRC 2894
 Formulation No.: 0100 based on Formulation No.: 05776/0096
 Origin of sample: PF-E/FT
 Responsible Analyst: Dr. Teller
 Analytical Methods: HPLC, ext. Std.

Laboratory: FT-EA

Approvals:

<u>TOX</u>	<u>Purity</u>	<u>Approved until</u>	<u>Date of Analysis</u>	<u>Comment</u>
4627-00	1. 490.6	g/l 05.11.98	05.05.98	


 (H. Baum, PF-PM/PPA)

A reserve sample will be retained.