TNO-report
V98.337

Augmented acute (4-hour) inhalation toxicity study with [redacted] in rats

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ELF Atochem S.A.
Paris La Défense, France

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Summary

The acute inhalation toxicity of [redacted] was studied by nose-only exposure of one group of eight male and eight female rats to a test atmosphere containing 21.2 ± 0.2 g/m³ [redacted] for a single 4-hour period. Half of the rats were necropsied the day after exposure, the remaining rats were kept for a 14-day observation period. Four additional, unexposed rats were also sacrificed for background histopathology.

The test atmosphere was generated by passing metered amounts of the test material using a roller pump to an airless ultrasound nebulizer. Upon entering the exposure equipment, the aerosolized material was mixed with humidified, pressurized air. The mean aerosol concentration of the non-volatile fraction based on gravimetrical analysis was 122 ± 4 mg/m³. The MMAD of the particulate matter was 3.8 µm and the geometric standard deviation was 1.1.

During exposure, abnormalities in breathing pattern or restlessness could not be seen. Shortly after exposure, abnormalities consisted of moderate piloerection in all animals, hunched appearance, laboured breathing, shallow breathing or irregular breathing. Slight dyspnea was also noticed.

Mortality was the most important finding in this study, viz. 5 out of 16 animals died within 1-4 days after exposure. The occurrence of late mortality in the group intended to be kept for an observation interval of 14 days suggests that late mortality could be expected to have occurred in the animals of the 1-day observation period if this group had been kept for a 14-day observation period as well, indicating that total mortality may be underestimated.

On day 1 of the observation period, sluggishness, laboured breathing, blepharospasm, hunched appearance, and piloerection were seen. With respect to breathing abnormalities, a biphasic pattern was observed. Initially, laboured breathing was seen in most surviving animals, which persisted for a number of days, but later, a visually increased breathing rate was noted in two female and in two male animals. No abnormalities were seen in the animals of the control group.

With respect to the measurement of breathing parameters, although statistical power was reduced due to mortality, breathing frequency was significantly increased just after exposure and 14 days after exposure, tidal volume was reduced just after exposure and one day and 4 days after exposure and ventilatory flow was significantly reduced just after exposure and one day after exposure. Changes in lung mechanical parameters could not be demonstrated.

One day after exposure, body weight loss was seen in all exposed animals. On day 7 body weight in all but one of the surviving animals of group A2 was still below the pre-exposure value. During the second week of the 14-day observation period,

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normal body weight gain was seen in the surviving animals of group A2, but of course the weight attained was below that of the unexposed animals (A3), in which, as expected, normal weight gain was seen in both observation weeks.

Macroscopic lung changes, i.e. red discoloured and/or swollen lungs, were seen in deceased rats, in several rats necropsied one day after exposure and in a few rats necropsied 14 days after exposure. In addition, relative lung weights had increased.

Changes at the microscopic level in deceased rats and in rats scheduled for necropsy after one day, consisted of moderate to severe alveolar haemorrhages, perivascular oedema and perivascular inflammatory cell aggregates, whereas surviving animals scheduled for necropsy 14 days after exposure showed slight to severe multifocal pneumonia or accumulation of alveolar macrophages accompanied by moderate increased septal cellularity. Alveolar haemorrhages were no longer present at this time point.

Conclusion
It was concluded that a single 4-hour (acute) exposure of rats to a high concentration of [redacted] (viz. 21.2 g/m^3) resulted in mortality, changes in airway function, and macroscopical and histopathological lung changes.
Therefore, from the results of the present study, it cannot be excluded that human exposure at high aerosol concentrations will result in serious and lasting lung changes.

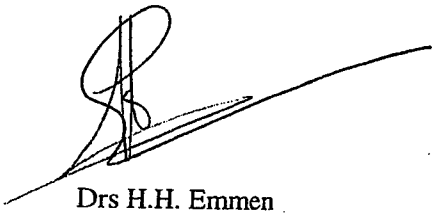
Contents

Statement of GLP compliance

We, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Nutrition and Food Research Institute, and that the study was carried out under our supervision.
The study was carried out in accordance with the OECD Principles of Good Laboratory Practice.

Dr H. Muijser
(Study Director)

9 November 1998
Date

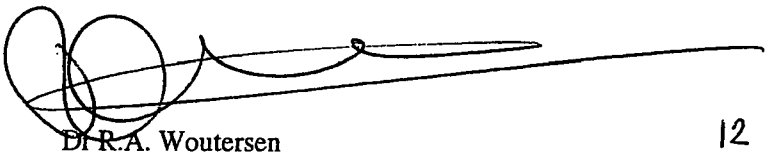


Drs H.H. Emmen
(Management)

12 November 1998
Date

Authentication by cooperating scientist

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



Dr R.A. Woutersen
(Pathologist)

12 November 1998
Date

Quality Assurance Statement

On: Augmented acute (4-hour) inhalation toxicity study with [redacted] in rats

Report Number: V98.337

Date : November 1998

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

Date of inspection:
27 November 1998

Date of report:
28 November 1998

This report was audited as follows:

Dates of audit:
2 and 10 November 1998

Date of report:
12 November 1998

I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all inspections were reported to the study director and the management on the dates indicated.

Ing. P.A. de Lang
(Quality Assurance Unit)

Date: 13 November 1998

STAATSTOEZICHT OP DE VOLKSGEZONDHEID

VETERINAIRE HOOFDINSPECTIE

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 88/320/EEC the conformity with the OECD Principles of GLP was assessed on 22-26 April, 15 May and 9 September 1996 at

TNO Nutrition & Food Research Institute
Toxicology Division
Utrechtseweg 48, P.O. Box 360
3700 AJ Zeist, The Netherlands

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Toxicity; Mutagenicity; Environmental Toxicity on aquatic and terrestrial organisms; Behaviour in water, soil and air; Residues; Effects on mesocosms and natural ecosystems; Analytical and clinical chemistry; Drug metabolism; Pharmacokinetics.

Rijswijk, 10 September 1996

Th. Helder, DVM

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

Testing facility

The toxicity study was conducted by:

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Contributors

Major contributions to this study were made by:

Study Director	: Dr H. Muijser*
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Study Assistant	: Ms M.M. Andringa
Senior Inhalation Technician	: Ing S.M. Spoor
Inhalation Technicians	: Mr F. Hendriksma / Mr W.G. Roverts
Biotechnicians	: Mr D.C. Veldhuysen / Mr G. de Kruijf
Senior Biotechnician	: Mr G. van Beek
Pathologist	: Dr R.A. Woutersen

* Toxicology Division, TNO Nutrition and Food Research Institute

1 Introduction

At the request of ELF Atochem S.A., Paris La Défense, France, an acute (4-hour) inhalation toxicity study with [redacted] was carried out in rats. Although only mortality data are used for classifying acute toxicity, in the present study clinical observations, body weights, lung function measurements, lung weights, macroscopical observation at necropsy and histopathology of the lungs were additionally used as criteria for disclosing possible harmful effects. The study design was based on an augmented acute inhalation toxicity study (TNO report V93.181, June 1993).

2 Experimental

The study was conducted according to a protocol, entitled: "Protocol for an augmented acute (4-hour) inhalation toxicity study with [redacted] in rats", approved by the study director on 18 November 1998 (see 2.1 and 2.11 for change from [redacted] in the name of the test material). The protocol had been drafted in accordance with the OECD Guideline for Testing of Chemicals no. 403, Acute inhalation toxicity, adopted 12 May 1981 and the OECD Principles of Good Laboratory Practice.

2.1 Test material

The test material was supplied by the sponsor. Two plastic bottles, labelled [redacted] containing ca. 790 and 760 g test material, respectively, were received on 17 November 1997. As shown below the test material was actually a [redacted] On request of the sponsor the name of the test material in this report was therefore changed to [redacted] The TNO internal reference number was 970285. The test material was stored at ambient temperatures.

[redacted] is a [redacted] and has the following characteristics (as given by the sponsor):

Composition: [redacted]

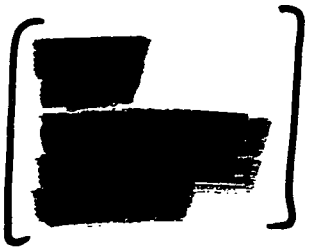
[redacted]

Composition: [redacted]

Colour:

Boiling point:

Autoignition temperature:
Explosive limits:
Vapour pressure:
Density:
Batch number:



2.2 Test animals

The animals used were male and female SPF-reared, Wistar derived Crl:WI(WU) BR rats obtained from Charles River Wiga, Sulzfeld, Germany. The animals used arrived on 5 November 1997, at an age of ca. 6-7 weeks. They were taken in their unopened shipping containers to animal room 15.07, were checked for overt signs of ill health and anomalies, and were kept in quarantine. After approval of the lot (negative titers to microorganisms tested), they were transferred on 10 November 1997 to animal room 06.05; the rats were randomly allocated to the cages, separated by sex and uniquely identified by ear tattoo. On 26 November 1997, one day before the start of exposure, 8 male and 8 female rats were assigned to the study to be exposed to the present test material in two groups of 4 rats for each sex. In addition, 2 male and 2 female rats were assigned to the study as controls for background histopathology. At that time, mean body weights of the male and female rats were 252 and 177 g, respectively. The duration of the acclimatization period in the inhalation facilities was 16 days. The groups of animals were identified by a letter (A) and a subcode (s1, s2 and s3) and a colour code (white). In this report, for the sake of clarity, the different groups are abbreviated as A1, A2, and A3, respectively. The individual animals were identified by earmark (tattoo).

2.3 Experimental conditions

2.3.1 Maintenance

During exposure the animals had no access to feed or water and were housed individually in the holders. After exposure, one group (A1) was immediately returned to their living cages, held for an observation period of one day and sacrificed. The second group (A2) was first subjected to lung function measurements before being returned to their living cages, subsequently held for an observation period of 14 days and sacrificed. The rats that entered the study as controls (group A3) were sacrificed on day 14 of study for background lung histopathology. The animals were housed in animal room 06.05, 4 males or 4 females to a cage (groups A1 and A2), or 2 males or 2 females to a cage (group A3).

The animals were housed under conventional conditions in suspended stainless steel cages fitted with wire-mesh floor and front. The number of air changes was about 10 per hour. The temperature was between 20.0 and 22.0 °C and relative humidity was between 46 and 68%. A 12-hour light and 12-hour dark cycle was

maintained.

2.3.2 Diet and drinking water

All rats were fed a commercially available rodent diet (Rat & Mouse No. 3 Breeding Diet RM3) from SDS Special Diets Services, Witham, England. Each batch of this diet is analysed by SDS for nutrients and contaminants. The certificate of analysis pertaining to the batch used (Batch no. 3604) will be kept with the raw data in the Archives of the Institute.
Tap water suitable for human consumption (quality guidelines according to Dutch legislation based on EEC Council Directive 80/778/EEC) was supplied by N.V. Waterleidingbedrijf Midden-Nederland (WMN). Results of the routine physical, chemical and microbiological examination of drinking water as conducted by the supplier are made available to TNO Nutrition and Food Research Institute. In addition, WMN periodically analyses water samples taken on the premises of TNO in Zeist for a limited number of variables. The results of these analyses will be kept with the raw data in the Archives of the Institute.

2.4 Experimental procedures

Exposure was started on 27 November 1997 with one group consisting of 8 male and 8 female rats. This group was exposed by inhalation to the limit concentration of at least 20 g/m³ for a single 4-hour period. One group of 4 male and 4 female animals was necropsied the following day. The other group of 4 male and 4 female rats was intended to be kept for an observation period of 14 days and to be subsequently sacrificed. Four additional rats were also sacrificed on that day for background lung histopathology. No more animals were exposed. The study was, therefore, finished with the necropsy of all remaining rats on 11 December 1997.

2.5 Exposure chamber

Animals were exposed to the test atmosphere in a nose-only inhalation chamber, a modification of the chamber manufactured by ADG Developments Ltd., Codicote, Hitchin, Herts. SG4 8UB, United Kingdom (see Figure 1). The inhalation chamber consisted of a cylindrical aluminium column, surrounded by a transparent cylinder. The column had a volume of ca. 50 ℓ and consisted of a top assembly with two mixing chambers, underneath a rodent tube section and the exhaust section at the bottom. The rodent tube section had 20 ports for animal exposure. Several empty ports were used for test atmosphere sampling, particle size analysis, measurement of oxygen concentration, temperature and relative humidity. The animals were secured in plastic animal holders (Battelle), positioned radially through the outer cylinder around the central column. Male and female rats of each group were placed in alternating order. The remaining ports were closed. Only the nose of the rats protruded into the interior of the column.
In our experience, the animal's body does not exactly fit in the animal holder which always results in some leak from high to low pressure side. By securing a positive pressure in the central column and a slightly negative pressure in the outer

cylinder, which encloses the entire animal holder, air leaks from nose to thorax rather than from thorax to nose and dilution of test atmosphere at the nose of the animals is prevented.

2.6 Generation of the test atmosphere

The inhalation equipment was designed to expose rats to a continuous supply of fresh test atmosphere. To generate the test atmosphere, metered amounts of the test material were passed with a roller pump (Gilson, Villiers le Bel, France) to an airless ultrasound nebulizer (Sono-Tek Corporation, Poughkeepsie, NY, USA). Upon entering the exposure equipment, the aerosolized material was mixed with humidified, pressurized air. The resulting test atmosphere was then directed downward through the mixing chamber towards the animals. At the bottom of the unit the test atmosphere was exhausted (see also Figure 1). The settings of the nebulizer and rotameter were recorded at regular intervals (ca. twice per hour). Total air flow through the exposure chamber was 24 ℓ/min.
All animals of group A1 were placed in the exposure unit 60 min after the start of the generation of the test atmosphere. To allow lung function measurements to be performed just after the end of exposure in the animals of group A2, the animals were placed sequentially, one at a time (balanced with respect to sex), with an interval of 15 min in the exposure unit.

2.7 Analysis of the test atmosphere

2.7.1 Total carbon analysis

The actual concentration of the test material in the test atmosphere was monitored with a total carbon analyser (Ratfisch RS55, Munich, Germany). The response of the analyser was recorded by an analogue recorder (Kipp & Zonen, Delft, The Netherlands).

The settings of the total carbon analyser were as follows:

- oven temperature:	150 °C
- H_2 pressure:	0.5 bar
- air pressure:	0.8 bar
- back pressure:	200 mbar
- sample line:	148 °C

Test atmosphere samples were taken continuously from the exposure unit at the animals' breathing zone and were passed to the total carbon analyser. The mean response was calculated by averaging values read every 5 minutes.

The response of the flame ionisation detector (FID) of the total carbon analyser was calibrated by injecting Teflon bags filled with 30 liters of air with 790, 790, 880, 880, 970 or 970 µℓ [redacted] (Merck, p.a.), respectively. [redacted] The amount of [redacted] was considered to be negligible small. The resulting responses were

used to construct a calibration curve which was needed to convert the responses in scale units of the TCA to concentrations (g/m^3).
A quadratic relation was obtained:

[redacted] concentration = $0.0112 * X^2 - 1.477 * X + 64.28$

in which [redacted] was in g/m^3, X was the response in scale units of the TCA and the correlation coefficient was 0.99.

2.7.2 Gravimetric analysis

The amount (by weight) of non-volatile particles present in the test atmosphere was determined approximately once each hour by means of gravimetric analysis. Representative test atmosphere samples were obtained by passing 50 ℓ test atmosphere at 5 ℓ/min through fiber glass filters (Sartorius). Samples were weighed before and after sampling. The concentration of the non-volatile particles was calculated by dividing the amount of test material present on each filter by the volume of the test atmosphere taken.

2.7.3 Nominal concentration

The nominal concentration was determined by dividing the total amount of test material used by the total volume of air passed through the exposure unit.

2.7.4 Particle size measurement

Particle size distribution measurement was carried out once during exposure using an 11-stage cascade impactor (Institute's design) with the largest cut-off size of 4.2 μm. The Mass Median Aerodynamic Diameter (MMAD) and the mean geometric standard deviation (gsd) were calculated (Lee, 1972).

2.7.5 Measurement of temperature, relative humidity and oxygen concentration

The temperature and the relative humidity of the test atmospheres were recorded twelve times during exposure at regular intervals (about twice per hour) using a wet and dry bulb thermometer. The oxygen concentration was checked once during exposure (Beryl, Cosma, Igny, France).

2.8 Observations and measurements

2.8.1 Behaviour, clinical signs and mortality

The rats were visually inspected just before exposure, for reactions to treatment during the exposure, shortly after exposure, and at least once daily during the observation period.

2.8.2 Body weights

Body weights of the animals were recorded on the day before exposure (day -1), just prior to exposure (day 0), on day 1 and on days 7 and 14 (surviving animals of group A2 and group A3).

2.8.3 Lung function measurements

Lung function measurements, consisting of determination of breathing frequency, tidal volume, mean ventilatory flow, and lung mechanical properties were carried out in spontaneously breathing rats.
Four male and four female rats of the test group (group A2, destined for autopsy 14 days after exposure) were measured before exposure (day-1, control value) and surviving animals directly after exposure (day 0) and on days 1, 4 and 14 of the observation period.

Each rat was placed in a modified Battelle restraining tube with a water-wetted silicon diaphragm separating externally head and neck from thorax and abdomen. Each rat was subsequently placed in a double room plethysmograph (the so-called head and body chamber). Mouth flow was measured in the head chamber by means of a resistance and a pressure device and thoracic movement was determined with a pressure device (Honeywell) in the body chamber. Breathing frequency, tidal volume, and mean ventilatory flow were determined by means of recording the pressure signal in the body chamber. Lung mechanical properties were obtained by determination of the transfer function as described in detail by Oostveen et al. (1992). Hereto, small sinusoidal pressure fluctuations (around 1 hPa peak-peak) were applied around the thorax in the frequency range of 16-208 Hz. These pressure fluctuations yielded small sinusoidal changes in the ventilatory flow which were superimposed on the spontaneous breathing and which were measured in the head chamber. Transfer impedance (Z_{tr}), defined as applied pressure (P) divided by the flow (v), was determined by means of a Fast Fourier transform and checked for internal coherence as described by Michaelson et al. (1975). Z_{tr} is a complex function of the frequency and is described in a real (Z_{Re}) and imaginary part (Z_{Im}). Using an appropriate simplified parameter estimating program, the parameters were directly linked to the physiological variables resistance, inertance and compliance.

As described by Oostveen et al. (1992), the general description of the six-element Dubois model adequately describes the data above 60 Hz (beyond the influence of neck shunt). The Dubois model yields a descriptive equation of the form:

$$Z_{tr} = R - B\omega^2 + j\,[-1/\omega C + I\omega - E\omega^3]$$

in which R is total respiratory resistance, I is total inertance and C is compliance. R and I were estimated from the Z_{tr} data as:

$R = Z_{Re}(96\ Hz) + 1/3\ [Z_{Re}(96\ Hz) - Z_{Re}(192\ Hz)\ (Z_{Re} = R - B\omega^2)$

$I = Z_{Im}(160\ Hz) / 1000$ $(2\pi 160 = 1005;\ Z_{Im} = [-1/\omega C + I\omega - E\omega^3])$

At low frequencies, the Z_{Im} was determined by the terms C and I and if $Z_{Im} = 0$ (at the resonance frequency ω_0), then $C = (I\omega_0^2)^{-1}$. The value of ω_0 was obtained by linear interpolation between the two adjacent frequencies at which Z_{im} has opposite signs, in most cases $Z_{im}<0$ at 16 Hz and $Z_{im}>0$ at 32 Hz. Because of insufficient coherence, sometimes the value of Z_{im} at 16 Hz could not be determined. In that case the value of Z_{im} at 16 Hz was linearly extrapolated from the values at 32 and 48 Hz using the formula: $Z_{im}(16Hz)=2*Z_{Im}(32Hz) - Z_{im}(48Hz)$. Finally, values above 10 mℓ/kPa were considered unrealistically high and left out.

2.8.4 Pathology

A group of 4 male and 4 female rats (group A1) was killed one day after exposure by exsanguination from the abdominal aorta under ether anaesthesia. On day 14 of the observation period the remaining test rats of group A2 together with the four unexposed rats (group A3) were killed in the same way. All rats were examined for gross pathological changes.
The lungs with trachea and larynx were removed and weighed. Lungs were inflated with and preserved in a neutral, aqueous, phosphate-buffered, 4% solution of formaldehyde and embedded in paraffin wax. Sections were cut at 5 µm of each of the five lung lobes, stained with haematoxylin and eosin and then examined microscopically at one longitudinal level.

2.9 Statistical analysis

First, differences between pre- and post-treatment breathing parameters (mechanical and ventilatory) were analysed by one-way ANOVA using post/pre-exposure ratios to detect possible sex differences. If there were no sex differences, male and female data were pooled and subjected to matched pair t-tests using pre- and post-exposure values.

2.10 Retention of records

Raw data, the master copy of the final report and all other information relevant to the quality and integrity of the study will be retained in the archives of TNO Nutrition and Food Research Institute for a period of at least 15 years after submission of the final report.

2.11 Deviations from the protocol

- The lung weight of animal no. 74 was accidentally not measured.
- On request of the sponsor the name of the test material in this report was changed to [redacted]

- Due to an organizational change within the Toxicology Division on the 1st of July 1998, management responsibilities for this study have been assigned to Drs H.H. Emmen as from that date.

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

3 Results

3.1 Analytical results

3.1.1 Total carbon analysis

The actual concentration of [redacted] during the exposure based on total carbon analysis of the volatile fraction [redacted] averaged at 5-min intervals was 20.4 ± 0.2 g/m^3 (N=48). Since [redacted] the concentration of [redacted] was 21.2 ± 0.2 g/m^3.

3.1.2 Gravimetric analysis

The results of the gravimetric analysis are given in Table 1.
The calculated mean concentration of the solid fraction of [redacted] was 122 ± 4 mg/m^3, which is around 0.6% of the actual concentration measured with total carbon analysis.

3.1.3 Nominal concentration

The nominal concentration was calculated to be 21.9 g/m^3. The nominal concentration was slightly higher than the actual concentration of 21.2 g/m^3, indicating a generation efficiency of 97%.

3.1.4 Particle size measurement

The particle size distribution is given in Table 2.
It was shown that 84% of the particles present at the animals' breathing zone had an aerodynamic diameter equal to or smaller than 4.2 μm. The Mass Median Aerodynamic Diameter (MMAD) was 3.8 μm and the mean geometric standard deviation (gsd) of the particles was 1.1.

3.1.5 Measurement of temperature, relative humidity and oxygen concentration

The mean temperature during exposure was 20.8 ± 0.4 °C (range 20.0-21.0 °C) and the mean relative humidity was 43 ± 1% (range 42-46%). The oxygen concentration during exposure was 21.7%.

3.2 Behaviour, clinical signs and mortality

Data on behaviour and clinical signs are given in Tables 3.

During exposure, abnormalities in breathing pattern or restlessness could not be seen, it must be kept in mind, however, that due to the stay in restraining tubes, observation of the rats was limited (Tables 3.1).

Shortly (see Tables 3.2.1 and 3.2.2) after exposure, abnormalities consisted of moderate piloerection in all animals, hunched appearance (slight) in 3 male (nos.

62, 74, 78) and 7 female animals (nos. 73, 75, 77, 79, 81, 85, 89) and with respect to breathing, laboured breathing ranging in severity from slight to moderate was noted in 7 male (nos. 62, 68, 72, 74, 78, 82, 86) and 6 female animals (nos. 71, 75, 79, 81, 85, 89). The other animals displayed shallow breathing (slight, no. 66) or irregular breathing (slight, nos. 73, 77). Finally, slight dyspnea was noticed in 5 male (nos. 62, 68, 74, 78, 82) and in 2 female animals (nos. 81, 89).

On day 1 of the one-day observation period of group A1 (Table 3.3.1), all animals except one (no. 86) were sluggish. Laboured breathing was seen in 3 male (62, 72, 82) and in one female animal (no. 75). Less frequently observed effects were blepharospasm in two male animals (nos. 62, 82) and a hunched appearance combined with piloerection in one female animal (no. 85). More importantly, mortality occurred: later that day (at 11h), one male (no. 62) and one female animal (no. 85) were found dead in their home cage.

During day 1 of the 14-day observation period of the animals of group A2 (Tables 3.3.2), similar signs were found. Also in this subgroup mortality occurred: on day 1, 2 and 4, respectively, a male (no. 66), a female (no. 73) and another male animal (no. 74) died. With respect to breathing abnormalities, the data of group A2 suggested a biphasic pattern. Initially, laboured breathing was seen in most animals, which persisted for a number of days, but later, an increased breathing rate was found in two surviving female (nos. 77, 89) and in 2 male (nos. 68, 74) animals of which one died on day 4. One male (no. 81) and one female animal (no.78) were without signs from day 1 on. No abnormalities were seen in the animals of the control group (Table 3.3.3).

Mortality was 2 out of 8 in subgroup A1 and 3 out of 8 in subgroup A2. Mortality in group A1 may however be an underestimate, because the occurrence of late mortality in the group intended to be kept for an observation interval of 14 days (A2) suggested that late mortality could have been expected to have occurred in the animals of the 1-day observation period (A1) if this group had been kept for a 14-day observation period as well.

3.3 Body weights

Individual and mean body weights are indicated in Table 4.

One day after exposure, body weight loss was seen in all exposed animals. On day 7 body weight in all but one of the surviving animals of group A2 was still below the pre-exposure value. During the second week of the 14-day observation period, normal body weight gain was seen in the surviving animals of group A2, but of course the weight attained was below that of the unexposed animals (A3), in which, as expected, normal weight gain was seen in both observation weeks.

3.4 Lung function measurements

3.4.1 Ventilatory variables

Data on breathing frequency, tidal volume and mean ventilatory flow are given in Table 5.

Just after exposure all but one female animal showed an increase in breathing frequency compared to pre-exposure values. The difference was statistically significant as well as the difference at day 14 ($p<0.05$). Although the values in the males remained higher at day 1 and day 4, this was not statistically significant (Table 5.1). It must be borne in mind that, due to mortality, the statistical power was seriously reduced.

Compared to the pre-exposure level, tidal volume was significantly reduced just after exposure, and 1 day ($p\leq0.0001$) and 4 days ($p<0.05$) after exposure. The decrease on day 14 was not significant (Table 5.2).

Ventilatory flow, the product of breathing frequency and tidal volume, was significantly reduced ($p<0.005$) just after exposure and on day 1 compared to pre-exposure values. This reduction did not persist four or fourteen days after exposure (Table 5.3).

3.4.2 Lung mechanical measurements

Individual and mean responses in lung function measurements are given in Table 6.

Changes in the lung mechanical variables, airway resistance, inertance and compliance were not detected. Data on airway compliance were more variable (Table 6.3), which was explained by a loss of some of the 16 Hz data (the lowest frequency tested) due to a too low internal coherence at that frequency. As such, the points where Z_{lm} was zero (which is necessary to determine the compliance) occasionally had to be extrapolated which resulted in less reliable estimates.

3.5 Lung weights

Absolute and relative (to body weight) lung weights are given in Table 7.

Mean relative lung weights recorded one day after exposure in group A1 were comparable to those observed 14 days after exposure in group A2 (Table 7.1). However, these values were high compared to mean relative lung weights of unexposed rats (Table 7.2).

3.6 Pathology

Summarized macroscopic and microscopic findings are given in Table 8 and 9, respectively; individual findings are given in Appendices 1 and 2.

3.6.1 Macroscopy

At necropsy, several surviving animals of group A1 were in poor health condition. A few of these rats showed sniffing and diarrhoea. Macroscopic observation of deceased rats revealed red discoloured and/or swollen, occasionally blood filled lungs. Several surviving rats necropsied one day after exposure also showed discoloured and/or swollen lungs whereas red spotted lungs were observed in a few others. A few surviving rats necropsied 14 days after exposure also showed red discoloured lungs. These macroscopic changes were considered to be treatment-related (Table 8). The other changes were found in one or a few rats of all groups, controls included, and were therefore not considered to be related to treatment.

3.6.2 Microscopy

Microscopical examination of the lungs of deceased rats revealed lesions which were characterized by moderate to severe alveolar haemorrhages, perivascular oedema and perivascular inflammatory cell aggregates. Surviving animals scheduled for necropsy one day after exposure showed similar changes. In most lungs of surviving animals necropsied 14 days after exposure slight to severe multifocal pneumonia or accumulation of alveolar macrophages accompanied by moderate increased septal cellularity could be demonstrated. Alveolar haemorrhages were no longer present at this time point.
In unexposed control rats, perivascular inflammatory cell infiltrates were found in 3 out of 4 rats, pneumonia in 1 out of 4 rats, and accumulation of alveolar macrophages in 1 out of 4 animals. Only one rat showed no abnormalities. It should, however, be noted that the changes in these unexposed control rats were only slight to very slight.

4 Discussion and conclusion

The aim of the present study was to determine the acute inhalation toxicity of [redacted] in rats. One group of 8 male and 8 female rats was exposed to the limit concentration of 21.2 g/m^3 during a single period of four hours. Half of the rats were necropsied the day after exposure, the remaining rats were kept for a 14-day observation period. Four additional, unexposed rats were also sacrificed for background histopathology.

Mortality was the most important finding in this study, viz. 5 out of 16 exposed animals (groups A1 and A2) died within 1-4 days after exposure. The occurrence of late mortality in group A2 (intended to be kept for an observation interval of 14 days) suggests that late mortality could be expected to have occurred in the animals of group A1 (1-day observation period) if this group had been kept for a 14-day observation period as well, indicating that total mortality may be an underestimate.

With respect to breathing parameters, although statistical power was reduced due to mortality, breathing frequency was significantly increased just after exposure and 14 days after exposure, tidal volume was reduced just after exposure and one day and 4 days after exposure and ventilatory flow was significantly reduced just after exposure and one day after exposure. Changes in lung mechanical parameters could not be demonstrated.

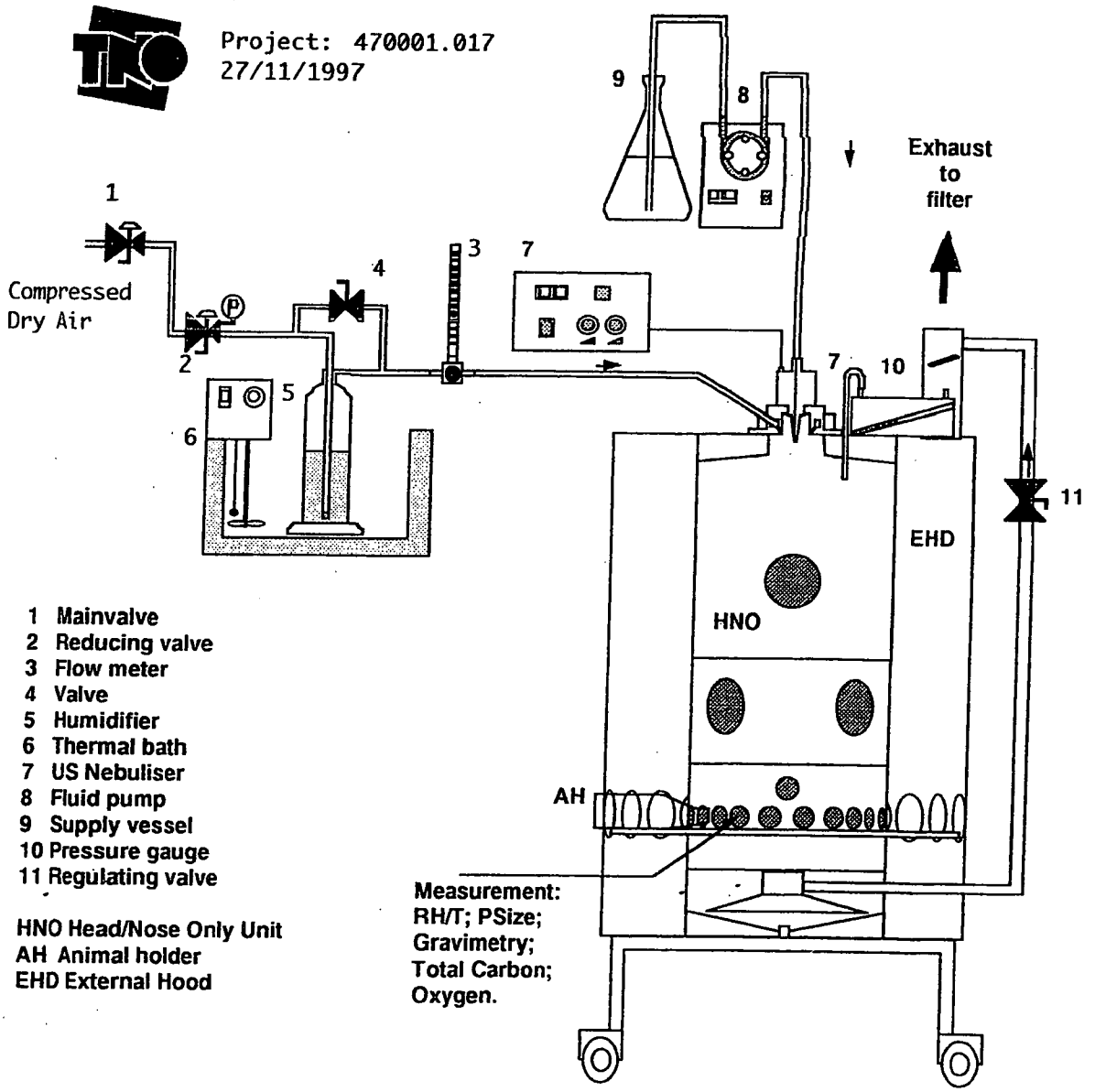
Macroscopic lung changes, i.e. red discoloured and/or swollen lungs, were seen in deceased rats, in several rats necropsied one day after exposure and in a few rats necropsied 14 days after exposure. In addition, relative lung weights had increased. Changes at the microscopic level in deceased rats and in rats scheduled for necropsy after one day, consisted of moderate to severe alveolar haemorrhages, perivascular oedema and perivascular inflammatory cell aggregates, whereas surviving animals scheduled for necropsy 14 days after exposure showed slight to severe multifocal pneumonia or accumulation of alveolar macrophages accompanied by moderate increased septal cellularity. Alveolar haemorrhages were no longer present at this time point.

It was concluded that a single 4-hour (acute) exposure of rats to a high concentration of [redacted] (viz. 21.2 g/m³) resulted in mortality, changes in airway function, and macroscopical and histopathological lung changes.
Therefore, from the results of the present study, it cannot be excluded that human exposure at high aerosol concentrations will result in serious and lasting lung changes.

5 References

- Lee R.J. Jr., Science, 1972: 567-575
- Michaelson E.D., Grassman E.D. and Peters W.R., J. Clin. Invest. 1975: 56, 1210-1230
- Oostveen E., Zwart A., Peslin R. And Duvivier C., J. Appl. Physiol. 1992: 73,1598-1607

Figure 1 - Schematic diagram of the generation and exposure system



[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
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Table 1 - Concentration of solid fraction in the test atmosphere measured by gravimetry

Sample time	Volume (ℓ)	Concentration (mg/m^3)
11:10	50	121
12:05	50	120
13:15	50	119
14:15	50	118
15:20	50	123
16:10	50	129
mean ± sd		122 ± 4

Generation of test atmosphere: 9:30-16:30 h
Exposure duration of the animals: 4 hours between 10:30 and 16:30 h

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 2 - Aerodynamic particle size distribution in test atmosphere

Particle size (μm)	Mass (mg)	Cumulative (%)
≤ 4.2	1.04	83.6
≤ 3.8	2.20	49.1
≤ 3.4	1.70	22.3
≤ 3.1	0.50	14.5
≤ 2. 8	0.40	8.2
≤ 2.4	0.23	4.6
≤ 1.8	0.18	1.7
≤ 1.4	0.11	0.0
≤ 1.0	0.00	0.0
≤ 0.8	0.00	0.0
≤ 0.1*	0.00	0.0
total	6.36	

*The amount sampled on this stage included back-up filter.

The volume was 75 ℓ, sample flow was 5.0 ℓ/min
Mass Median Aerodynamic Diameter (MMAD): 3.8 μm
mean geometric standard deviation (gsd): 1.1

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 3.1.1 - Clinical signs during exposure (group A1)

Animal number	Clinical signs	Time of observation
MALES		
62,72,82,86	NO ABNORMALITIES	11:30, 12:25, 13:25, 14:20
FEMALES		
71,75,79,85	NO ABNORMALITIES	11:30, 12:25, 13:25, 14:20

exposure period of group A: 10h30 - 14:30

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 3.1.2 - Clinical signs during exposure (group A2)

Animal number	Clinical signs	Time of observation
MALES		
66,68	NO ABNORMALITIES	11h45,12h45,13h45,14h45
74	NO ABNORMALITIES	12h45,13h45,14h45,15h40
78	NO ABNORMALITIES	12h45,13h45,14h45,15h40, 16h10
FEMALES		
73,77	NO ABNORMALITIES	11h45,12h45,13h45,14h45
81	NO ABNORMALITIES	12h45,13h45,14h45,15h40
89	NO ABNORMALITIES	12h45,13h45,14h45,15h40, 16h10

exposure of animal 66: 10h45-14h45
exposure of animal 73: 11h00-15h00
exposure of animal 68: 11h15-15h15
exposure of animal 77: 11h30-15h30
exposure of animal 74: 11h45-15h45
exposure of animal 81: 12h00-16h00
exposure of animal 78: 12h15-16h15
exposure of animal 89: 12h30-16h30

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
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Table 3.2.1 - Clinical signs shortly[1] after the end of exposure (group A1)

Animal number	Clinical signs	Grade
MALES		
62	GENERAL	
	piloerection	moderate
	hunched appearance	slight
	BREATHING	
	laboured breathing	slight
	dyspnea	slight
72,86	GENERAL	
	piloerection	moderate
	BREATHING	
	laboured breathing	slight
82	GENERAL	
	piloerection	moderate
	BREATHING	
	laboured breathing	slight
	dyspnea	slight
FEMALES		
71	GENERAL	
	piloerection	moderate
	BREATHING	
	laboured breathing.	slight
75,79,85	GENERAL	
	piloerection	moderate
	hunched appearance	slight
	BREATHING	
	laboured breathing	slight

[1] Time of observation 16h40; exposure ended on 14h30

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 470001/017

Table 3.2.2 - Clinical signs shortly[1] after the end of exposure (group A2)

Animal number	Clinical signs	Grade
MALES		
66	GENERAL	
	piloerection	moderate
	BREATHING	
	shallow breathing	slight
68	GENERAL	
	piloerection	moderate
	BREATHING	
	laboured breathing	moderate
	dyspnea	slight
74,78	GENERAL	
	piloerection	moderate
	hunched appearance	slight
	BREATHING	
	laboured breathing	slight
	dyspnea	slight
FEMALES		
73,77	GENERAL	
	piloerection	moderate
	hunched appearance	slight
	BREATHING	
	irregular breathing	slight
81,89	GENERAL	
	piloerection	moderate
	hunched appearance	slight
	BREATHING	
	laboured breathing	moderate
	dyspnea	slight

[1] time of observation 17h05; exposure ended between 14h45 and 16h30 (see note at the bottom of Table 3.1.2)

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Study: 470001/017

Table 3.3.1 - Clinical signs during the 1-day observation period (group A1)

Animal number	Clinical signs	Range*
MALES		
62	GENERAL	
	blepharospasm	1
	sluggish	1
	BREATHING	
	laboured breathing	1
	FOUND DEAD just before necropsy	1
72	GENERAL	
	sluggish	1
	BREATHING	
	laboured breathing	1
82	GENERAL	
	blepharospasm	1
	sluggish	1
	BREATHING	
	laboured breathing	1
86	NO ABNORMALITIES	1
FEMALES		
71,79	GENERAL	
	sluggish	1
75	GENERAL	
	sluggish	1
	BREATHING	
	laboured breathing	1
85*	GENERAL	
	hunched appearance	1
	sluggish	1
	piloerection	1
	FOUND DEAD just before necropsy	1

* range: 1 day (animals were sacrificed on day 1)

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Study: 470001/017

Table 3.3.2 - Clinical signs during the 14-day observation period (group A2)

Animal number	Clinical signs	Range*
MALES		
66	GENERAL	
	blepharospasm	1
	sluggish	1
	BREATHING	
	laboured breathing	1
	FOUND DEAD on day 1	
68	GENERAL	
	sluggish	1-7
	BREATHING	
	laboured breathing	1
	shallow breathing	2-7
	increased breathing rate	2-8
	NO ABNORMALITIES	9-14
74	GENERAL	
	sluggish	1-3
	nasal encrustations	2-3
	BREATHING	
	laboured breathing	1
	shallow breathing	2-3
	increased breathing rate	2-3
	FOUND DEAD on day 4	
78	NO ABNORMALITIES	1-14

* range day 1 - day 14

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 3.3.2 (continued) - Clinical signs during the 14-day observation period (group A2)

Animal number	Clinical signs	Range*
FEMALES		
73	GENERAL	
	sluggish	1
	eyes closed	1
	BREATHING	
	laboured breathing	1
	FOUND DEAD on day 2	
77	GENERAL	
	sluggish	1-3
	hunched appearance	1-3
	meager/slender appearance	6
	BREATHING	
	laboured breathing	1-3
	increased breathing rate	6-8
	NO ABNORMALITIES	4-5, 9-14
81	NO ABNORMALITIES	1-14
89	GENERAL	
	sluggish	1-2
	hunched appearance	1-2
	meager/slender appearance	6
	BREATHING	
	increased breathing rate	6-8
	NO ABNORMALITIES	3-5, 9-14

* range day 1 - day 14

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 3.3.3 - Clinical signs during the 14-day observation period (group A3)

Animal number	Clinical signs	Range*
MALES		
64,70	NO ABNORMALITIES	1-14
FEMALES		
83,91	NO ABNORMALITIES	1-14

* range day 1 - day 14

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
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Table 4.1 - Individual and mean body weights (g) of male and female rats (group A1)

Animal No.	Day -1	Day 0	Day 1
Males (A1)			
62	245.4	249.1	229.4
72	246.3	249.6	228.6
82	254.5	260.9	239.8
86	260.9	264.9	255.5
mean ± sd	251.8 ± 7.3	256.1 ± 8.0	238.3 ± 12.5
Females (A1)			
71	180.2	184.6	171.7
75	180.1	189.6	171.4
79	177.1	183.5	167.5
85	168.3	175.1	160.9
mean ± sd	176.4 ± 5.6	183.2 ± 6.0	167.9 ± 5.0

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 470001/017

Table 4.2 - Individual and mean body weights (g) of male and female rats (group A2)

Animal No.	Day -1	Day 0	Day 1	Day 7	Day 14
Males (A2)					
66	239.6	243.7	226.0	224.6[1](1)	-
68	257.9	262.3	237.8	237.0	265.7
74	260.7	264.8	243.0	210.9[1](4)	-
78	248.3	253.8	236.2	251.0	277.0
mean ± sd	251.6 ± 9.6	256.2 ± 9.5	235.8 ± 7.1	244.0[2]	271.4[2]
Females (A2)					
73	179.4	183.7	169.8	166.6[1](2)	-
77	170.9	172.6	161.6	162.5	180.0
81	174.7	177.1	175.4	178.3	193.1
89	182.2	186.1	170.6	174.9	191.8
mean ± sd	176.8 ± 5.0	179.9 ± 6.2	169.4 ± 5.7	171.9 ± 8.3	188.3 ± 7.2

[1] no weight available on day 7, animal weight at death on day 1, day 2 or day 4, indicated as (1), (2) or (4), respectively.
[2] no sd given for mean of two values

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 4.3 - Individual weights (g) of male and female rats (group A3)

Animal No.	Day -1	Day 0	Day 1	Day 7	Day 14
Males (A3)					
64	247.7	257.1	253.8	278.5	303.9
70	257.2	261.6	264.0	295.2	320.1
mean	252.4	259.4	258.9	286.8	312.0
Females (A3)					
83	189.5	190.2	189.5	199.9	207.4
91	170.4	176.8	175.6	180.1	193.3
mean	180.0	183.5	182.6	190.0	200.4

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 5.1 - Individual responses in breathing frequency (group A2)

Animal code	Breathing frequency (1/s)								
	pre	day 0		day 1		day 4		day 14	
males	abs	abs	rel	abs	rel	abs	rel	abs	rel
66	3.20	3.77	1.18	*		*		*	
68	3.17	3.80	1.20	3.92	1.24	5.38	1.70	5.48	1.73
74	3.65	4.17	1.14	3.70	1.01	*		*	
78	3.33	3.63	1.09	4.23	1.27	3.90	1.17	3.73	1.12
mean/ sem	3.34/ 0.11	**3.84§/** 0.12		3.95/ 0.15		4.64/ -		**4.60§/** -	
females									
73	3.70	4.04	1.09	2.36	0.64	*		*	
77	3.24	3.49	1.08	3.14	0.97	4.82	1.49	3.43	1.06
81	3.65	3.16	0.86	4.25	1.16	3.36	0.92	5.19	1.42
89	3.33	3.63	1.09	4.22	1.26	5.10	1.53	5.36	1.61
mean/ sem	3.48/ 0.11	**3.58§/** 0.18		3.49/ 0.46		4.43/ 0.54		**4.66§/** 0.62	

abs: absolute value; rel: value in % relative to pre-exposure value
* animal died, hence no values available
- no sem given for mean of two values

Statistics:

- One way ANOVA for sex differences using post/pre-exposure ratios (no significant differences found)

- Matched pair t-test by comparing pre- and post-exposure values (pooled sexes); § : $p<0.05$,

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 5.2 - Individual responses in tidal volume (group A2)

Animal code	Tidal volume (mℓ)								
	pre	day 0		day 1		day 4		day 14	
males	abs	abs	rel	abs	rel	abs	rel	abs	rel
66	2.60	1.60	0.62	*		*		*	
68	2.60	2.00	0.77	1.60	0.62	1.60	0.62	1.80	0.69
74	2.80	2.00	0.71	1.40	0.50	*		*	
78	2.80	2.00	0.71	2.00	0.71	2.20	0.79	2.00	0.71
mean/ sem	2.70/ 0.06	**1.90**§/ 0.10		**1.67**§/ 0.18		**1.90**¶/ -		1.90/ -	
females									
73	2.00	1.40	0.70	0.80	0.40	*		*	
77	2.00	1.00	0.50	0.80	0.40	1.00	0.50	2.80	1.40
81	2.00	1.80	0.90	1.20	0.60	2.00	1.00	1.40	0.70
89	2.20	1.40	0.64	1.20	0.55	1.20	0.55	1.60	0.73
mean/ sem	2.05/ 0.05	**1.40**§/ 0.16		**1.00**§/ 0.12		**1.40**¶/ 0.30		1.93/ 0.44	

abs: absolute value; rel: value in % relative to pre-exposure value
* animal died, hence no values available
- no sem given for mean of two values

Statistics
- One way ANOVA for sex differences using post/pre-exposure ratios (no significant differences found)

- Matched pair t-test by comparing pre- and post-exposure values (pooled sexes)
§ $p \leq 0.0001$
¶ $p < 0.05$

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 5.3 - Individual responses in ventilatory flow (group A2)

Animal code	Ventilatory flow (mℓ/s)								
	pre	day 0		day 1		day 4		day 14	
males	abs	abs	rel	abs	rel	abs	rel	abs	rel
66	8.32	6.04	0.73	*		*		*	
68	8.25	7.60	0.92	6.27	0.76	8.62	1.04	9.87	1.20
74	10.23	8.33	0.81	5.18	0.51	*		*	
78	9.33	7.25	0.78	8.46	0.91	8.58	0.92	7.45	0.80
mean/ sem	9.03/ 0.47	**7.30§/** 0.48		**6.64§/** 0.96		8.60/ -		8.66/ -	
females									
73	7.40	5.65	0.76	1.89	0.26	*		*	
77	6.47	3.49	0.54	2.51	0.39	4.82	0.75	9.59	1.48
81	7.31	5.68	0.78	5.09	0.70	6.73	0.92	7.27	0.99
89	7.33	5.08	0.69	5.06	0.69	6.12	0.83	8.58	1.17
mean/ sem	7.13/ 0.22	**4.98§/** 0.51		**3.64§/** 0.84		5.89/ 0.56		8.48/ 0.67	

abs: absolute value; rel: value in % relative to pre-exposure value
* animal died, hence no values available
- no sem given for mean of two values

Statistics
- One way ANOVA for sex differences using post/pre-exposure ratios (no significant differences found)

- Matched pair t-test by comparing pre- and post-exposure values
§ : $p<0.005$

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 6.1 - Individual responses in airway resistance (group A2)

Animal Code	Resistance (hPa.s/ℓ)								
	pre	day 0		day 1		day 4		day 14	
Males	abs	abs	rel	abs	rel	abs	rel	abs	rel
66	178	163	0.91	*		*		*	
68	199	169	0.85	177	0.89	196	0.98	178	0.90
74	160	189	1.18	219	1.37	*		*	
78	173	163	0.94	181	1.05	188	1.09	179	1.03
mean	178	171	0.97	192	1.10	192	1.04	179	0.97
±sem	±8	±6	±0.07	±13	±0.14	-	-	-	-
Females									
73	187	154	0.82	137	0.73	*		*	
77	140	137	0.98	124	0.88	166	1.19	167	1.19
81	167	184	1.10	184	1.11	150	0.90	144	0.87
89	169	154	0.91	149	0.88	159	0.94	175	1.03
mean	166	157	0.95	148	0.90	158	1.01	162	1.03
±sem	±10	±10	±0.06	±13	±0.08	±5	±0.09	±9	±0.09

abs: absolute value; rel: value relative to pre-exposure value
* animal died, hence no values available; sem
- no sem given for mean of two values

Statistics
- One way ANOVA for sex differences using post/pre-exposure ratios; (no significant differences found)

- Matched pair t-test comparing pre- and post-exposure values (pooled sexes; no significant differences found).

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Study: 470001/017

Table 6.2 - Individual responses in airway inertance (group A2)

Animal Code	Inertance ($hPa.s^2/\ell$)								
	pre	day 0		day 1		day 4		day 14	
Males	abs	abs	rel	abs	rel	abs	rel	abs	rel
66	0.194	0.221	1.14	*		*		*	
68	0.235	0.201	0.86	0.201	0.86	0.213	0.91	0.217	0.92
74	0.195	0.199	1.02	0.242	1.24	*		*	
78	0.207	0.206	1.00	0.216	1.04	0.236	1.14	0.209	1.01
mean	0.208	0.207	1.00	0.220	1.05	0.224	1.02	0.213	0.97
sem	0.010	0.005	0.06	0.120	0.11	-	-	-	-
Females									
73	0.226	0.133	0.59	0.120	0.53	*		*	
77	0.145	0.119	0.82	0.094	0.65	0.203	1.40	0.146	1.01
81	0.203	0.190	0.94	0.226	1.11	0.153	0.75	0.135	0.67
89	0.155	0.169	1.09	0.134	0.86	0.171	1.10	0.202	1.30
mean	0.182	0.153	0.86	0.144	0.79	0.176	1.09	0.161	0.99
sem	0.019	0.016	0.11	0.029	0.13	0.015	0.19	0.021	0.18

abs: absolute value; rel: value relative to pre-exposure value

* animal died, hence no values available
- no sem given for mean of two values

Statistics
- One way ANOVA for sex differences using post/pre-exposure ratios; (no significant differences found)

- Matched pair t-test comparing pre- and post-exposure values (pooled sexes; no significant differences found).

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 470001/017

Table 6.3 - Individual responses in airway compliance (group A2)

Animal Code	Compliance (mℓ/kPa)								
	pre	day 0		day 1		day 4		day 14	
Males	abs	abs	rel	abs	rel	abs	rel	abs	rel
66	1.70	1.30	0.76	***		***		***	
68	1.16	1.45	1.25	1.12	0.96	1.12	0.96	8.66	7.46
74	*	1.88		0.75		***		***	
78	2.58			1.01	0.39	1.44	0.56	1.18	0.46
mean	1.82	1.54	1.00	0.96	0.68	1.28	0.76	4.92	3.96
sem	0.41	0.18	0.24	0.11	-	-	-	-	-
Females									
73	1.13	2.49	2.20	3.63	3.21	***		***	
77	3.07	3.17	1.03	3.52	1.15	2.07	0.67	7.43	2.42
81	2.37	5.11	2.15	1.70	0.71	3.00	1.26	5.22	2.20
89	**	2.16		3.15		1.57		1.22	
mean	2.19	3.23	1.80	3.00	1.69	2.21	0.97	4.63	2.31
sem	0.57	0.66	0.38	0.44	0.77	0.42	-	1.82	-

abs: absolute value; rel: value relative to pre-exposure value
* value outside realistic range (> 10)
** could not be determined from the data
*** animal died, hence no values available
- no sem given for mean of two values

Statistics
- One way ANOVA for sex differences using post/pre-exposure ratios; (no significant differences found)

- Matched pair t-test comparing pre- and post-exposure values (pooled sexes; no significant differences found).

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Table 7.1 - Absolute and relative lung weights of exposed animals; individual and mean values

Animal no.	Group A1		Group A2	
	absolute lung weight (g)	relative lung weight (g/100g BW)	absolute lung weight (g)	relative lung weight (g/100g BW)
Males				
62	3.202	1.40		
72	1.672	0.73		
82	2.782	1.16		
86	1.381	0.54		
66			3.247	1.37
68			2.723	1.02
74			-*	-*
78			1.446	0.52
mean ±sd	2.259 ±0.871	0.957 ±0.391	2.472 ±0.926	0.972 ±0.426
Females				
71	1.118	0.65		
75	2.05	1.20		
79	1.681	1.00		
85	2.720	1.69		
73			3.012	1.67
77			2.046	1.06
81			1.237	0.64
89			2.398	1.44
mean ±sd	1.892 ±0.672	1.135 ±0.433	2.173 ±0.741	1.20 ±0.45

* accidently not determined

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Table 7.2 - Individual lung weights of non-exposed animals (group A3)

Animal no.	Day 14	
	absolute lung weight (g)	relative lung weight (g/100g BW)
Males		
64	1.573	0.52
70	1.388	0.43
Females		
83	1.220	0.59
91	1.005	0.52

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Table 8 - Summary of macroscopic lung observations of male and female rats at necropsy

Changes	Incidence of changes (numeric)					
	test animals, group A1		test animals, group A2		controls, group A3	
M(ale)/F(emale)	M	F	M	F	M	F
Number examined	4	4	4	4	2	2
Habitus Found dead:	1	1	2	1		
Poor health condition	2	3				
Sniffing	2					
Diarrhoea		2				
Haemorrhagic discharge (nasal cavity and/or oral cavity)		1	1	1		
Crusts on nose and fore paws			1			
Lungs						
Red discoloured	2	4	2	3		
Swollen	2	3	1			
Hyalin spot(s)			1		1	1
Red spotted surface	2					
Petechia					2	
Bloody content			1	1		
White area(s)			1	1	1	
Trachea						
Bloody content	1		1			

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Table 8 (continued) - Summary of macroscopic lung observations of male and female rats at necropsy

Changes	Incidence of changes (numeric)					
	test animals, group A1		test animals, group A2		controls, group A3	
M(ale)/F(emale)	M	F	M	F	M	F
Number examined	4	4	4	4	2	2
Testes						
(Unilateral) cryptorchism	4		1			
Thymus						
Spotted			1			
Bronchial lymph nodes						
Enlarged			1	3	2	1

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Table 9 - Summary of microscopic lung observations of male and female rats

Changes	Incidence of changes (numeric)					
	test animals, group A1		test animals, group A2		controls, group A3	
M(ale)/F(emale)	M	F	M	F	M	F
No examined	4	4	4	4	2	2
Diffuse alveolar haemorrhages						
Slight	1			1		
Moderate		3	2			
Severe	3	1				
Perivascular oedema						
Very slight	1					
Slight	3	4	1			
Perivascular mononuclear and/or polymorphonuclear inflammatory cell infiltrate						
Very slight			1			
Slight		3		2	2	1
Moderate	4	1	1			
Increased number of polymorpho-nuclear inflammatory cells in the alveolar spaces						
Very slight			1			
Slight	1	3				
Moderate	3	1				
Hypertrophic respiratory epithelium	3					
(Multifocal) pneumonia						
Very slight					1	
Slight			1	1		
Severe			1			
Very severe			1			

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Table 9 (continued) - Summary of microscopic lung observations of male and female rats

Changes	Incidence of changes (numeric)					
	test animals, group A1		test animals, group A2		controls, group A3	
M(ale)/F(emale)	M	F	M	F	M	F
No examined	4	4	4	4	2	2
Very slight to slight diffuse/focal accumulation of alveolar macrophages				3		1
Moderate increased septal cellularity				2		
Slight focal pleuritis				1		

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Appendix 1.1 - Individual macroscopic observations at necropsy 1 day after exposure (group A1)

Animal number	Macroscopic findings	
MALES		
062	Found dead on day 1	
	Lungs:	red discoloured, swollen
	Trachea:	filled with blood
	Testes:	unilateral cryptorchism
072	Habitus:	poor health condition, sniffing
	Lungs:	red spotted surface
	Testes:	unilateral cryptorchism
082	Habitus:	poor health condition, sniffing
	Lungs:	swollen, dark red discoloured
	Testes:	cryptorchism
086	Habitus:	no abnormalities
	Lungs:	red spots
	Testes:	unilateral cryptorchism
FEMALES		
071	Habitus:	poor health condition
	Lungs:	red discoloured
075	Habitus:	poor health condition, diarrhoea
	Lungs:	red discoloured, swollen
079	Habitus:	poor health condition, diarrhoea
	Lungs:	red discoloured, swollen
085	Found dead on day 1	
	Habitus:	haemorrhagic discharge from nasal cavity
	Lungs:	dark red discoloured, swollen

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Appendix 1.2 - Individual macroscopic observations at necropsy 14 days after exposure or earlier in animals that were found dead (group A2)

Animal number	Macroscopic findings	
MALES		
066	Found dead on day 1	
	Habitus:	haemorrhagic discharge from nasal and oral cavity
	Lungs:	dark red discoloured, swollen
	Trachea:	filled with blood
	Thymus:	spotted
068	Habitus:	no abnormalities
	Lungs:	hyaline spot(s) and local white discoloured
074	Found dead on day 4	
	Habitus:	crusts on nose and fore paws
	Testes:	cryptorchism
	Lungs:	dark red discoloured, filled with blood
078	Habitus:	no abnormalities
	BLN:	enlarged
FEMALES		
073	Found dead on day 2	
	Habitus:	haemorrhagic discharge from nasal cavity
	Lungs:	dark red discoloured, filled with blood
077	Habitus:	no abnormalities
	Lungs:	red discoloured, local white discoloured
	BLN:	enlarged
081	Habitus:	no abnormalities
	BLN:	enlarged
089	Habitus:	no abnormalities
	Lungs:	red discoloured
	BLN:	enlarged

BLN = Bronchial Lymph Nodes

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Appendix 1.3 - Individual macroscopic observations at necropsy in unexposed animals (group A3)

Animal number	Macroscopic findings	
MALES		
064	Habitus:	no abnormalities
	Lungs:	hyalin spot(s), petechia
	BLN:	enlarged
070	Habitus:	no abnormalities
	Lungs:	petechia; pronounced white spot
	BLN:	enlarged
FEMALES		
083	Habitus:	no abnormalities
	Lungs:	hyalin spots
	BLN:	enlarged
091	Habitus:	no abnormalities
	Organs	no abnormalities detected

BLN = Bronchial Lymph Nodes

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Appendix 2.1 - Individual microscopic observations at necropsy 1 day after exposure (group A1)

Animal number	Microscopic findings
MALES	
062	Found dead on day 1
	Lungs: Severe diffuse alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular oedema Moderate perivascular polymorphonuclear inflammatory cell infiltrate Hypertrophic respiratory epithelium Moderate increased number of polymorphonuclear inflammatory cells in the alveolar spaces
072	Scheduled kill on day 1
	Lungs: Severe diffuse alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular oedema Moderate perivascular polymorphonuclear inflammatory cell infiltrate Hypertrophic respiratory epithelium Moderate increased number of polymorphonuclear inflammatory cells in the alveolar spaces
082	Scheduled kill on day 1
	Lungs: Severe diffuse alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular oedema Moderate perivascular polymorphonuclear inflammatory cell infiltrate Hypertrophic respiratory epithelium Moderate increased number of polymorphonuclear inflammatory cells in the alveolar spaces

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Appendix 2.1 (continued) - Individual microscopic observations at necropsy 1 day after exposure (group A1)

Animal number	Microscopic findings
MALES	
086	Scheduled kill on day 1
	Lungs: Slight diffuse alveolar haemorrhages (fibrinoid material within the alveolar spaces) Very slight perivascular oedema Moderate perivascular polymorphonuclear inflammatory cell infiltrate Slight increased number of polymorphonuclear inflammatory cells in the alveolar spaces

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Appendix 2.1 (continued) - Individual microscopic observations at necropsy 1 day after exposure (group A1)

Animal number	Microscopic findings
FEMALES	
071	Scheduled kill on day 1
	Lungs: Moderate diffuse alveolar haemorrhages (alveolar oedema and fibrinoid material within the alveolar spaces) Slight perivascular oedema Moderate perivascular polymorphonuclear inflammatory cell infiltrate Moderate increased number of polymorphonuclear inflammatory cells in the alveolar spaces
075	Scheduled kill on day 1
	Lungs: Moderate diffuse alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular oedema Slight perivascular polymorphonuclear inflammatory cell infiltrate Slight increased number of polymorphonuclear inflammatory cells in the alveolar spaces
079	Scheduled kill on day 1
	Lungs: Moderate diffuse alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular oedema Slight perivascular polymorphonuclear inflammatory cell infiltrate Slight increased number of polymorphonuclear inflammatory cells in the alveolar spaces
085	Found dead on day 1
	Lungs: Severe diffuse alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular oedema Slight perivascular polymorphonuclear inflammatory cell infiltrate Slight increased number of polymorphonuclear inflammatory cells in the alveolar spaces

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Appendix 2.2 - Individual microscopic observations at necropsy 14 days after exposure or earlier in animals that were found dead (group A2)

Animal number	Microscopic findings
MALES	
066	Found dead on day 1
	Lungs: Moderate alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular oedema Very slight perivascular infiltrates of mononuclear and polymorphonuclear inflammatory cells Very slight increased number of polymorphonuclear inflammatory cells in the alveolar spaces
068	Scheduled kill on day 14
	Lungs: Very severe pneumonia seen as diffuse accumulation of alveolar macrophages, increased septal cellularity (mainly macrophages), septal fibrosis.
074	Found dead on day 4
	Lungs: Moderate alveolar haemorrhages (with fibrinoid material within the alveolar spaces) Severe pneumonia seen as alveolar oedema, accumulation of alveolar macrophages, alveolar and interstitial aggregates of inflammatory cells, perivascular aggregates of macropahges and fibroblasts and a diffuse mesothelial proliferation
078	Scheduled kill on day 14
	Lungs: Moderate perivascular aggregates of mainly mononuclear inflammatory cells Slight multifocal pneumonia

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Appendix 2.2 (continued) - Individual microscopic observations at necropsy 14 days after exposure or earlier in animals that were found dead (group A2)

Animal number	Microscopic findings
FEMALES	
073	Found dead on day 2
	Lungs: Slight alveolar haemorrhages (with fibrinoid material in the alveolar spaces)
	Slight perivascular aggegregates of mainly polymorphonuclear inflammatory cells
077	Scheduled kill on day 14
	Lungs: Slight diffuse accumulation of alveolar macrophages
	Moderate increased septal cellularity (mainly macrophages)
081	Scheduled kill on day 14
	Lungs: Slight interstitial pneumonia
	Slight perivascular aggregates of mononuclear and polymorphonuclear inflammatory cells
	Slight focal accumulation of alveolar macrophages
	Slight focal pleuritis
089	Scheduled kill on day 14
	Lungs: Slight diffuse accumulation of alveolar macrophages
	Moderate diffuse increased septal cellularity (mainly macrophage-like cells)

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Appendix 2.3 - Individual microscopic observations at necropsy of unexposed animals (group A3)

Animal number	Microscopic findings
MALES	
064	Scheduled kill on day 14
	Lungs: Slight increased perivascular mononuclear inflammatory cell infiltrate Very slight multifocal pneumonia
070	Scheduled kill on day 14
	Lungs: Slight lobar perivascular mononuclear and polymorphonuclear inflammatory cell aggregates
FEMALES	
083	Scheduled kill on day 14
	Lungs: Slight increased perivascular inflammatory cell infiltrate Very slight focal accumulation of alveolar macrophages
091	Scheduled kill on day 14
	Lungs: No abnormalities detected

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CENTRE D'APPLICATION DE LEVALLOIS

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[redacted]

Name of the test material: [redacted]
Appearance:
Composition:

Composition:

Colour
Boiling point
Autoignition temperature:
Explosive limits
Vapour pressure
Density
Batch number
Storage conditions

[redacted]

Levallois, july 15, 1998

Company Sanitized. Does not contain TSCA CB

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