TNO-report
V98.685

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

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Summary

The acute inhalation toxicity of two batches of [redacted] was studied by nose-only exposure for a single 4-hour period in rats. One group of 8 male and 8 female rats was exposed to the limit concentration of 20.7 g/m³ of [redacted] during a single period of four hours (Exposure A) and another group of 8 male and 8 female rats was exposed to [redacted] at a limit concentration of 20.9 g/m³ (Exposure B). In each experiment half of the rats was 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 at each exposure.

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 107 ± 3 mg/m³ for [redacted] and 139 ± 2 mg/m³ for [redacted]. The MMAD of the particulate matter was 5.4 µm and 4.9 µm and the geometric standard deviation was 1.7 and 1.9 for batches [redacted] and [redacted] respectively.

During exposure, slight abnormalities in breathing pattern could be seen, consisting of slightly decreased breathing frequency and minimal irregular breathing during both exposure A and B complemented with clear restlessness during the second half of exposure A and slightly laboured breathing developing during exposure B. Shortly after both exposures, abnormalities consisted of piloerection, blepharospasm, sluggishness and slight irregular breathing.

Mortality was the most important finding in this study. As a result of exposure A , 6 out of 8 animals intended to be kept for an observation interval of 14 days died within 1-2 days after exposure while none of the 8 rats intended to be necropsied the day after exposure died before that time. The occurrence of late mortality 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 (6 out of 16) may be underestimated. The same holds for exposure B: 4 out of 8 animals of the 14-day observation group died within 1-3 days and none of the rats intended to be necropsied the day after exposure.

Clinical signs observed on day 1 and 2 of the observation period included blepharospasm, sluggishness and abdominal breathing. In surviving animals no clinical signs were observed after the second day. No abnormalities were seen in the animals of the control group.

One day after each exposure, body weight loss was seen in all exposed animals.

During the second week of the 14-day observation period, body weight gain was seen in surviving animals. Normal weight gain was seen in all animals of the unexposed control groups.

Macroscopic lung changes (viz. discolouration and hyalin appearance) were seen in rats necropsied one day after exposure and in deceased rats in both experiments. Animals that survived the 14-day observation interval still exhibited pulmonary gross lesions which are considered to be related to exposure. Mean absolute and relative lung weights were generally increased in surviving rats.

Changes at the microscopic level in deceased rats and in rats scheduled for necropsy after one day, consisted of alveolar haemorrhages, increased septal cellularity and perivascular polymorphonuclear leucocytic infiltration. Similar histopathological changes were seen in animals found dead on day 1-3 after exposure. Animals that survived the 14-day observation period still exhibited histopathological changes which are considered to be related to exposure.

It was concluded that a single 4-hour (acute) exposure of rats to a high concentration (i.e. 20.7 g/m^3 for [redacted] and 20.9 g/m^3 for [redacted] of [redacted] resulted in mortality and macroscopical and histopathological lung changes, irrespective of the batch used. Therefore, from the results of the present study, it cannot be excluded that human exposure to 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)

29 November 1998
Date

Drs H.H. Emmen
(Management)

30 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)

27 November 1998
Date

Quality Assurance Statement

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

Report Number: V98.685
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:	Date of report:
17 March 1998 (protocol)	17 March 1998
23 March 1998	23 March 1998

This report was audited as follows:

Dates of audit:	Date of report:
24-27 November 1998	27 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 Officer)

Date: 30 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

Testing facility

The toxicity study was conducted by:

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Contributors

Major contributions to this study were made by:

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Senior Biotechnician	: Mr G. van Beek
Pathologist	: Dr R.A. Woutersen
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* Toxicology Division, TNO Nutrition and Food Research Institute

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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. In addition lung function measurements, lung weights and histopathology of the lungs were used as criteria for disclosing possible harmful effects in the present study. The study design was based on an augmented acute inhalation toxicity study (TNO report [redacted], June 1993).

2 Experimental

The study was conducted according to a protocol, entitled: "Protocol for augmented acute (4-hour) inhalation toxicity study with [redacted] and [redacted] in rats", approved by the study director on 3 March 1998.
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 materials

Three test materials were supplied by the sponsor: two batches of [redacted] and one batch of [redacted]

[redacted]

Three plastic bottles, labelled [redacted] each containing according to the sponsor 1 ℓ test material, gross weights 731, 747 and 746 grams were received on 13 March 1998. The TNO internal reference number was 980116. The test material was stored at ambient temperatures in the dark.

Name of test material: [redacted]
Batch number:
Composition:

[redacted]

[redacted]

Three plastic bottles, labelled [redacted] each containing according to the sponsor 1 ℓ test material, gross weights 699, 724 and 749 grams, were received on 13 March 1998. The TNO internal reference number was 980117. The test material was stored at ambient temperatures in the dark.

Name of test material: [redacted]
Batch number:
Composition:

[redacted]

Three plastic bottles, labelled [redacted] each containing according to the sponsor 1 ℓ test material, gross weights 756, 775 and 779 grams, were received on 13 March 1998. The TNO internal reference number was 980118. The test material was stored at ambient temperatures in the dark.

Name of test material: [redacted]

Batch numbers:
Composition:

[redacted]

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 for exposure A arrived on 11 March 1998, at an age of ca. 7-8 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 13 March 1998 to animal room 6.0.04; the rats were randomly allocated to the cages, separated by sex and uniquely identified by ear tattoo. On 18 March 1998, 5 days before the start of exposure A, 8 male and 8 female rats were assigned to the study to be exposed to the present test material in two subgroups of 4 rats for each sex. In addition, 2 male and 2 female rats were assigned to the study as controls for

background histopathology. Three days before exposure, mean body weights of the male and female rats were 265 and 177 g, respectively.

The animals used for exposure B were similarly treated: arrival on 25 February 1998 at an age of ca. 5-6 weeks in room 15.07 and on 2 March 1998 in animal room 6.0.04, assigned to the study on 23 March 1998 (one day before exposure B), mean body weights at that time for male and female animals were 251 and 182 g, respectively.

The groups of animals were identified by a letter (A or B) and a subcode (s1, s2 and s3) and a colour code (white or blue). In this report, for the sake of clarity, the different (sub)groups are abbreviated as A1, A2, A3, B1, B2 and B3, respectively.

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 an exposure, one subgroup (A1 or B1) was immediately returned to their living cages, held for an observation period of one day and sacrificed. The second subgroup (A2 or B2) 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 (subgroups A3 and B3) were sacrificed on day 14 for background lung histopathology. The animals were housed in animal room 6.0.04, 4 males or 4 females to a cage (A1, A2, B1 and B2), or 2 males or 2 females to a cage (A3 and B3).

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 20.5 and 23.0 ^{0}C and relative humidity was between 50 and 70%. 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. 4177) 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 23 March 1998 with group A consisting of 8 male and 8 female rats. This group was exposed by inhalation to the limit concentration of at least 20 g/m³ of batch [redacted] for a single 4-hour period. One subgroup of 4 male and 4 female animals was necropsied the following day. The other subgroup 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. Group B was exposed on 24 March 1998 to batch [redacted] and treated similarly. Thereafter, no more animals were exposed. The study was finished with the necropsy of all remaining rats on 6 and 7 April 1998.

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 25 ℓ/min (exposure A and B). All animals of subgroup A1 were placed in the exposure unit 88 min (B1: 87 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 and B2, the animals were placed sequentially, one at a time (balanced with respect to sex), with an interval of 10 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 (Bernath Atomic, model 3005). The response of the analyser was recorded by an analogue recorder (Kipp & Zonen, Delft, The Netherlands).

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 two PET bags filled with 50 liters of air for each concentration with 1000, 1500 or 1750 μℓ, respectively, using batch [redacted]. 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 linear relation with a correlation coefficient of 0.99 was obtained:

$$Y = 3.3851\ X + 0.3837$$

in which X was the concentration of [redacted] in g/m^3 and Y was the response in scale units of the TCA. After exposure A the stability of the calibration was checked by measuring 2 PET bags each filled with 50 liters of air and 1500 μℓ of batch [redacted]. Both responses were within 1% of the value predicted by the relation above. Exposure B could be monitored using the same relation, since according to the sponsor, the composition of the two batches of [redacted] was the same with respect to the solvents used. The validity of this was checked after exposure B by measuring 2 PET bags each filled with 50 liters of air and 1500 μℓ of batch [redacted]. Responses deviated 0 and 2.5%, respectively, of the value predicted by the calibration relation, which is satisfactorily small.

2.7.2 Gravimetric analysis

The amount (by weight) of non-volatile particles present in the test atmospheres was determined approximately once each hour by means of gravimetric analysis. Representative test atmosphere samples were obtained by passing 100 ℓ test atmosphere at 5 ℓ/min through fiber glass filters (Sartorius). Samples were weighed before and after sampling. Forced drying with dry pressurized air did not influence the sampled weight. 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 concentrations were 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 each exposure using a 10-stage Andersen cascade impactor, with the largest cut-off size of 32 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 six times during exposure at intervals ranging between half an hour and one hour using a RH/T device (TESTO 610, GmbH & Co, Lenzkirch, Germany). 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 before exposure (exposure A: day -3, exposure B: day -1), just prior to exposure (day 0) and in surviving animals on day 1 and on days 7 and 14 (subgroups A2, A3, B2 and B3) and at death.

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 (subgroup A2 and B2, destined for autopsy 14 days after exposure) were measured before exposure (day-1, control value) and surviving animals directly after exposure (day 0). Since mortality occurred, lung function measurements were discontinued, the results obtained will be kept with the raw data, but will not be reported.

2.8.4 Pathology

A group of 4 male and 4 female rats (subgroup 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 subgroup A2 together with the four unexposed rats (subgroup A3) were killed in the same way. All rats were examined for gross pathological changes. The same procedure was followed for animals of group B.
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 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.

2.10 Deviations from the protocol

- According to the protocol, [redacted] should have been tested first and followed by a test of batch [redacted] if mortality occurred and by a test of [redacted] if no mortality occurred. Erroneously, batch [redacted] was tested first. Since mortality resulted after exposure to [redacted], the reverse sequence of testing did not matter.

- 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 Exposure A [redacted]

3.1 Analytical results

3.1.1 Total carbon analysis

The actual concentration of [redacted] during exposure A based on total carbon analysis of the volatile fraction averaged at 5-min intervals was 20.7 ± 0.5 g/m^3 (N=68).

3.1.2 Gravimetric analysis

The results of the gravimetric analysis are given in Table 1.1
The calculated mean concentration of the solid fraction of [redacted] was 107 ± 3 mg/m^3, which is around 0.5% of the actual concentration measured with total carbon analysis.

3.1.3 Nominal concentration

The nominal concentration was calculated to be 20.8 g/m^3. The nominal concentration was comparable to the actual concentration of 20.7 g/m^3, indicating a generation efficiency close to 100%.

3.1.4 Particle size measurement

The particle size distribution is given in Table 2.1.
It was shown that 45% of the particles present at the animals' breathing zone had an aerodynamic diameter equal to or smaller than 5 μm. The Mass Median Aerodynamic Diameter (MMAD) was 5.4 μm and the mean geometric standard deviation (gsd) of the particles was 1.7.

3.1.5 Measurement of temperature, relative humidity and oxygen concentration

The mean temperature during exposure was 21.2 ± 0.2 °C (range 20.9-21.4 °C) and the mean relative humidity was 44 ± 4% (range 40-53%). The oxygen concentration during exposure was 21.5%.

3.2 Behaviour, clinical signs and mortality

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

During exposure, a visually slightly decreased breathing rate could be seen at almost all observation times, very slight irregular breathing could be seen during

the last hour and clear restlessness during the last two hours of exposure (Tables 3.1.1 and 3.1.2).

Shortly (see Tables 3.2.1 and 3.2.2) after exposure, abnormalities consisted of piloerection, blepharospasm, sluggishness and slight irregular breathing.

In two male (nos. 34, 36) and in two female animals (nos. 33, 35) of subgroup A1 no exposure-related abnormalities could be observed on day 1 of the one-day observation period (the alopecia in one female animal was not considered to be exposure-related). In the other animals, clinical signs consisted of blepharospasm, sluggishness and abdominal breathing. Mortality was seen in subgroup A2 (intended to be kept for an observation period of 14 days): two male and one female animal died on day 1 and one male and two females died on day 2. Abnormalities seen on day 1 and day 2 were similar to those seen in subgroup A1. Beyond day 2 no clinical signs were seen in surviving animals. No abnormalities were seen in animals of subgroup A3 (Tables 3.3.1-3.3.3).

Mortality was 0 out of 8 in subgroup A1 and 6 out of 8 in subgroup A2. Mortality in group A1 may however be an underestimate, due to the necropsy on day 1.

3.3 Body weights

Individual and mean body weights are indicated in Tables 4.

One day after exposure, considerable body weight loss was seen in all exposed animals. The two surviving animals of subgroup A2 gained weight during the 14-day observation period. As expected, normal weight gain was seen in all animals of the unexposed subgroup A3 (Tables 4.1-4.3).

3.4 Lung function measurements

Since mortality occurred, lung function measurements are not reported (see protocol), results will be included in the raw data, however.

3.5 Lung weights

Lung weights and lung weights relative to body weight are given in Table 5.

Several animals of subgroup A1 (nos. 32, 38, 31 and 37) and one of the surviving animals of subgroup A2 (no. 43) showed distinctly increased absolute and relative lung weights. The relative lung weights of the other animals of subgroup A1 (nos. 34, 36, 33, and 35) and the other survivor of subgroup A2 (no. 44) were considered to be only slightly higher than those of the unexposed controls (Tables 5.1 and 5.2).

3.6 Pathology

Summarized macroscopic and microscopic findings are given in Tables 6.1 and 7.1, respectively; individual findings are given in Appendices 1.1 and 2.1.

3.6.1 Macroscopy

Three males and three females of subgroup A2 intended to be killed 14 days after exposure to the test substance were found dead within 2 days.

The control rats (subgroup A3) revealed only some minor pulmonary gross changes such as a few spots and focal petechiation.

Animals killed one day after exposure (subgroup A1):
All animals killed one day after exposure demonstrated red discoloured lungs most frequently accompanied by a hyalin appearance. Besides these pulmonary changes several animals showed pink fluid in the trachea and one female (no. 37) had a haemothorax. These gross abnormalities are considered to be related to treatment. The other macroscopic changes observed are considered fortuitous findings not related to exposure.

Animals scheduled to be killed 14 days after exposure (subgroup A2):
The animals found dead one or two days after exposure demonstrated gross lesions of the lungs and trachea similar to those seen in animals killed one day after treatment. Two animals of subgroup A2 (nos. 44 and 43) survived the observation period of 14 days. These animals still exhibited pulmonary gross lesions (dark discolouration, black spots) which are considered to be related to the treatment.

3.6.2 Microscopy

Microscopic examination of the lungs of the controls (subgroup A3) revealed only a minor change, viz. very slightly increased septal cellularity, which is occasionally found in control rats.

Microscopic examination of the lungs of animals scheduled for necropsy one day after exposure (subgroup A1) revealed histopathological lesions characterized by alveolar haemorrhages, increased septal cellularity and perivascular polymorphonuclear leucocytic infiltration, which are considered to be related to exposure.
Of subgroup A2, the animals found dead one or two days after exposure demonstrated similar pulmonary histopathological changes as those observed in animals scheduled killed one day after exposure. The lungs of the two surviving rats which were scheduled killed 14 days after exposure still exhibited histopathological changes such as increased septal cellularity, accumulation of alveolar macrophages and accumulation of pigment, which are considered to be related to the exposure.

4 Results Exposure B [redacted]

4.1 Analytical results

4.1.1 Total carbon analysis

The actual concentration of [redacted] during exposure A based on total carbon analysis of the volatile fraction averaged at 5-min intervals was 20.9 ± 0.2 g/m^3 (N=61).

4.1.2 Gravimetric analysis

The results of the gravimetric analysis are given in Table 1.2.
The calculated mean concentration of the solid fraction of [redacted] was 139 ± 2 mg/m^3, which is around 0.7% of the actual concentration measured with total carbon analysis.

4.1.3 Nominal concentration

The nominal concentration was calculated to be 22.3 g/m^3. The nominal concentration was slightly higher than the actual concentration of 20.9 g/m^3, indicating a generation efficiency of 94%, again close to 100%.

4.1.4 Particle size measurement

The particle size distribution is given in Table 2.2.
It was shown that 51% of the particles present at the animals' breathing zone had an aerodynamic diameter equal to or smaller than 5 μm. The Mass Median Aerodynamic Diameter (MMAD) was 4.9 μm and the mean geometric standard deviation (gsd) of the particles was 1.9.

4.1.5 Measurement of temperature, relative humidity and oxygen concentration

The mean temperature during exposure was 21.5 ± 0.2 ^{0}C (range 21.1-21.7 ^{0}C) and the mean relative humidity was 46 ± 2% (range 42-48%). The oxygen concentration during exposure was 21.5%.

4.2 Behaviour, clinical signs and mortality

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

During exposure, a visually slightly decreased breathing rate and slightly irregular breathing could be seen at almost all observation times. Slightly laboured breathing also developed during the course of exposure (Tables 3.1.3 and 3.1.4).

Shortly (see Table 3.2.3 and 3.2.4) after exposure, abnormalities consisted of piloerection, blepharospasm, sluggishness and irregular breathing (slight).

Frequently noted clinical signs on day 1 of the one day observation period of group

B1 included sluggishness, blepharospasm, abdominal breathing and soiled fur at the abdomen. One male animal (no. 400) was without clinical signs.

Mortality was seen in animals of subgroup B2: one female animal died on day 1, one male and one female animal died on day 2 and one male animal died on day 3. Abnormalities seen on day 1 and day 2 were similar to those seen in subgroup B1. Beyond day 2 no clinical signs were seen in surviving animals except for an increased breathing rate in the two females that survived the 14-day observation period.

Mortality was 0 out of 8 in subgroup B1 and 4 out of 8 in subgroup B2. Mortality in group B1 may however be an underestimate, due to the necropsy on day 1.

4.3 Body weights

Individual and mean body weights are indicated in Table 4.

One day after exposure, considerable body weight loss was seen in all exposed animals. Weight gain was seen in surviving males during the 14-day observation period and in surviving females in the last week. As expected, normal weight gain was seen in all animals of the unexposed subgroup B3 (Tables 4.4-4.6).

4.4 Lung function measurements

Since mortality occurred, lung function measurements are not reported (see protocol), results will be included in the raw data, however.

4.5 Lung weights

Lung weights and lung weights relative to body weight are given in Table 5.

Relative lung weights of one male animal of subgroup B1 (no. 400) and two surviving males of subgroup B2 (nos. 408 and 410) were only slightly higher than those of the control group. The other animals of subgroup B1, including the two surviving females of subgroup B2 (nos. 353 and 355) had distinctly increased absolute and relative lung weights (Tables 5.3 and 5.4).

4.6 Pathology

Summarized macroscopic and microscopic findings are given in Tables 6.2 and 7.2, respectively; individual findings are given in Appendices 1.2 and 2.2.

4.6.1 Macroscopy

Two males and two females of group A2 intended to be killed 14 days after exposure were found dead within 3 days.

The control rats (subgroup B3) revealed only some minor pulmonary gross changes such as hyalin spots and petechiation.

Animals killed one day after exposure (subgroup B1):
All animals killed one day after exposure demonstrated red discoloured lungs accompanied by a hyaline appearance. Besides these pulmonary changes several animals showed pink fluid in the trachea. These gross abnormalities are considered to be related to treatment. The other macroscopic changes observed are considered fortuitous findings not related to exposure.

Animals scheduled to be killed 14 days after exposure (subgroup B2):
The animals found dead within three days after exposure demonstrated gross lesions of the lungs and trachea similar to those seen in animals killed one day after treatment.
Four animals of subgroup B2 (nos. 408, 410, 353 and 355) survived the observation period of 14 days. These animals still exhibited gross lesions in the respiratory tract (petechiation, hyalin spots, discoloured spots, gray spongy tissue and foamy fluid in the trachea). Although hyalin spots and petechiation were seen in control rats, the other changes were not and were therefore considered to be related to the exposure.

4.6.2 Microscopy

Microcopic examination of the lungs of the controls (subgroup B3) revealed only a minor change: very slightly increased perivascular lymphoid aggregates and very slight to slight focal pneumonia, which is occasionally found in control rats.

Microscopic examination of the lungs of animals scheduled for necropsy one day after exposure (subgroup B1) revealed histopathological lesions characterized by alveolar haemorrhages, increased septal cellularity and perivascular polymorphonuclear leucocytic infiltration, which are considered to be related to exposure.
Of subgroup B2, the animals found dead one, two or three days after exposure demonstrated similar pulmonary histopathological changes as those observed in animals scheduled to be killed one day after exposure. The lungs of the rats which were scheduled killed 14 days after exposure still exhibited histopathological changes such as increased septal cellularity, accumulation of alveolar macrophages, alveolar haemorrhages and perivascular lymphoid aggregates, which are considered to be related to the exposure.

5 Discussion and conclusion

The aim of the present study was to determine the acute inhalation toxicity of two batches of [redacted] in rats. One group of 8 male and 8 female rats was exposed to the limit concentration of 20.7 g/m^3 of batch [redacted] during a single period of four hours (Exposure A) and another group of 8 male and 8 female rats was exposed to batch [redacted] at a limit concentration of 20.9 g/m^3 (Exposure B). In each experiment, half of the rats was necropsied the day after exposure, the remaining rats were kept for a 14-day observation period and four additional, unexposed rats were also sacrificed for background histopathology.

Mortality was the most important finding in both experiments, viz. 6 out of 8 animals of the subgroup A2, intended to be kept for an observation interval of 14 days, died within 2 days after exposure A, while the 8 animals of group A1 all survived exposure until the scheduled necropsy the next day. Similarly, 4 out of 8 animals of subgroup B2 died within 3 days after exposure B, while again none of the 8 rats of group B1 died before scheduled necropsy the day after exposure. The occurrence of late mortality after both exposures suggests that late mortality could be expected to have occurred in the animals scheduled for necropsy the day after exposure if these groups (A1 and B1) had been kept for a 14-day observation period as well, indicating that total mortality may be an underestimate.

Macroscopic lung changes, i.e. discoloured lungs almost always accompanied by a hyalin appearance, were seen in rats necropsied one day after exposure (subgroups A1 and B1). Besides these pulmonary changes, several animals showed pink fluid in the trachea. Similar changes could be seen in the animals of subgroups A2 and B2 found dead in the first 3 days of their 14-day observation period. Surviving animals of these groups still exhibited pulmonary gross lesions and occasionally foamy fluid in the trachea, which are considered to be related to the exposure. In addition, absolute and relative lung weights had generally increased in animals which were killed for necropsy.
Changes at the microscopic level in deceased rats and in rats scheduled for necropsy after one day, consisted of alveolar haemorrhages, increased septal cellularity and perivascular polymorphonuclear leucocytic infiltration, which are considered to be related to exposure. Rats that survived the 14-day observation period still exhibited histopathological changes such as increased septal cellularity, accumulation of alveolar macrophages and focal pneumonia, which are considered to be related to the exposure to the test material.

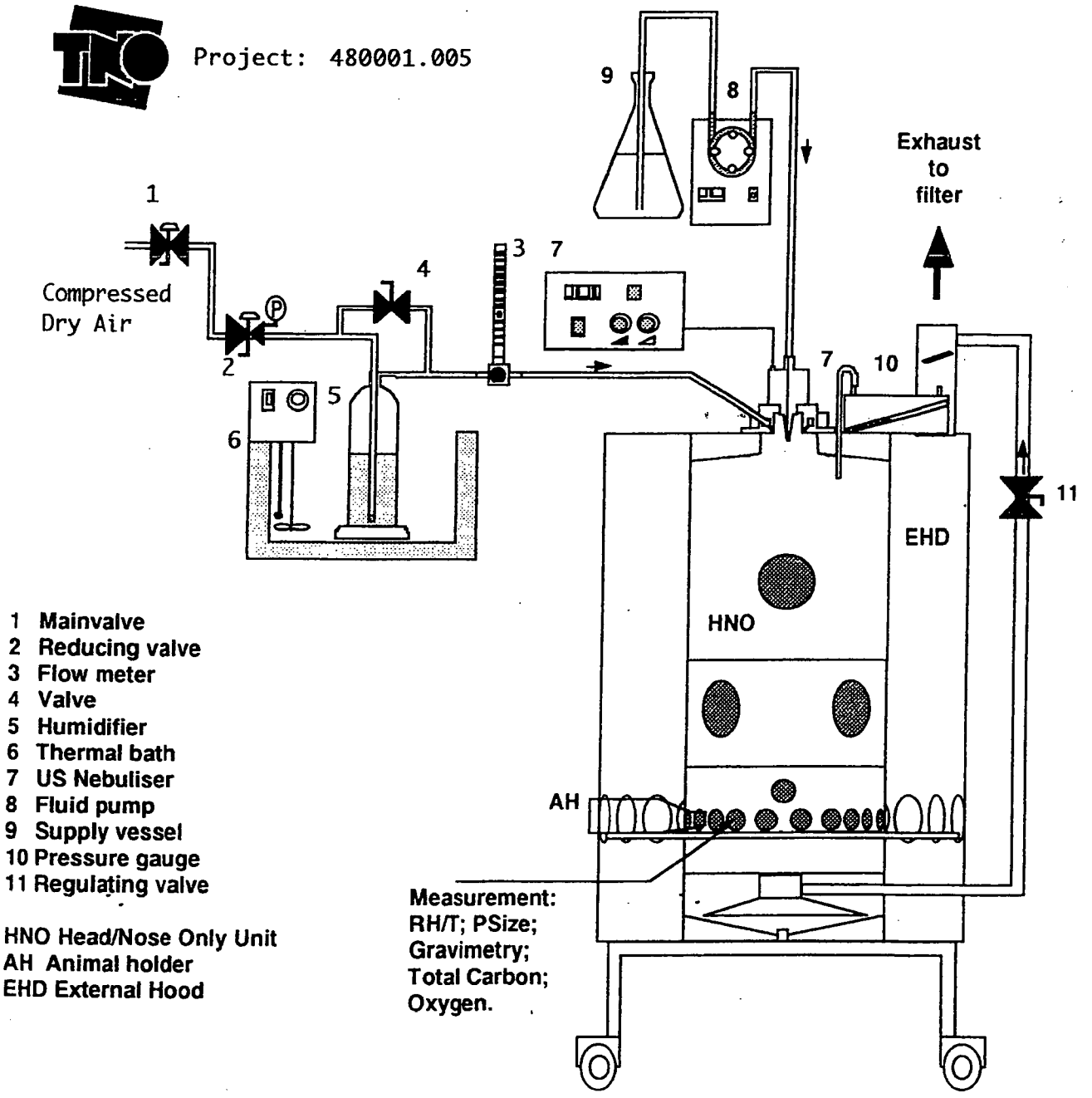
It was concluded that a single 4-hour (acute) exposure of rats to a high concentration (i.e. 20.7 g/m^3 for [redacted] and 20.9 g/m^3 for [redacted] irrespective of the batch used, resulted in mortality and macroscopical and histopathological lung changes.

Therefore, from the results of the present study, it cannot be excluded that human exposure to high aerosol concentrations will result in serious and lasting lung changes.

6 References

– Lee R.J. Jr., Science, 1972: 567-575

Figure 1 - Schematic diagram of the generation and exposure system



[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 1.1 - Concentration of solid fraction in the test atmosphere measured by gravimetry (Exposure A)

Sample time (hh:mm)	Volume (ℓ)	Concentration (g/m^3)
11:44	100	0.103
12:50	100	0.105
14:08	100	0.107
15:10	100	0.110
16:02	100	0.110
mean ± sd		0.107 ± 0.003

Generation of test atmosphere: 9:30-16:40 h
Exposure duration of the animals: 4 hours between 10:58 and 16:40 h

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 1.2 - Concentration of solid fraction in the test atmosphere measured by gravimetry (Exposure B)

Sample time (hh:mm)	Volume (ℓ)	Concentration (g/m^3)
11:42	100	0.135
12:50	100	0.140
13:41	100	0.139
14:45	100	0.139
15:44	100	0.141
mean ± sd		0.139 ± 0.002

Generation of test atmosphere: 9:10-16:20 h
Exposure duration of the animals: 4 hours between 11:07 and 16:20 h

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 2.1 - Aerodynamic particle size distribution in test atmosphere (Exposure A)

Particle size (μm)	Mass (mg)	Cumulative (%)
≤ 32	0	100
≤ 20.5	0	100
≤ 8.2	0.6	86.4
≤ 5.0	1.83	44.8
≤ 3.1	1.11	19.5
≤ 1.9	0.44	9.5
≤ 1.1	0.35	1.6
≤ 0.7	0	1.6
≤ 0.4	0	1.6
≤ 0.1*	0.07	1.6
total	4.4	

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

The volume was 66.1 ℓ, sample flow was 2.2 ℓ/min
Mass Median Aerodynamic Diameter (MMAD): 5.4 μm
mean geometric standard deviation (gsd): 1.7

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Study: 480001/005

Table 2.2 - Aerodynamic particle size distribution in test atmosphere (Exposure B)

Particle size (μm)	Mass (mg)	Cumulative (%)
≤ 32	0	100
≤ 20.5	0	100
≤ 8.2	1.13	82.6
≤ 5.0	2.07	50.6
≤ 3.1	1.7	24.4
≤ 1.9	1.21	5.7
≤ 1.1	0.15	3.4
≤ 0.7	0.1	1.9
≤ 0.4	0.08	0.6
≤ 0.1*	0.04	0.6
total	6.48	

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

The volume was 72.6 ℓ, sample flow was 2.4 ℓ/min
Mass Median Aerodynamic Diameter (MMAD): 4.9 μm
mean geometric standard deviation (gsd): 1.9

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 3.1.1 - Clinical signs during exposure (subgroup A1)

Animal number	Clinical signs	Time of observation
MALES		
32,34,36,38	BREATHING	
	visually decreased rate (slight)	11:45, 12:44, 13:40, 14:41
	irregular breathing (very slight)	14:41
	BEHAVIOUR	
	clear restlessness	13:40, 14:41
FEMALES		
31,33,35,37	BREATHING	
	visually decreased rate (slight)	11:45, 12:44, 13:40, 14:41
	irregular breathing (very slight)	14:41
	BEHAVIOUR	
	clear restlessness	13:40, 14:41

exposure of animals: 10:58 - 14:58h

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 3.1.2 - Clinical signs during exposure (subgroup A2)

Animal number	Clinical signs	Time of observation
MALES		
40	NO ABNORMALITIES	11:45
	BREATHING	
	visually decreased rate (slight)	12:44, 13:40, 14:41, 15:50
	irregular breathing (very slight)	13:40, 14:41
	BEHAVIOUR	
	clear restlessness (slight)	13:40, 14:41
42,44,46	BREATHING	
	visually decreased rate (slight)	12:44, 13:40, 14:41, 15:50
	irregular breathing (very slight)	13:40, 14:41
	BEHAVIOUR	
	clear restlessness (slight)	13:40, 14:41, 15:50
FEMALES		
39	NO ABNORMALITIES	11:45
	BREATHING	
	visually decreased rate (slight)	12:44, 13:40, 14:41, 15:50
	irregular breathing (veery slight)	13:40, 14:41
	BEHAVIOUR	
	clear restlessness (slight)	13:40, 14:41
41,43,45	BREATHING	
	visually decreased rate (slight)	12:44, 13:40, 14:41, 15:50
	irregular breathing (very slight)	13:40, 14:41
	BEHAVIOUR	
	clear restlessness (slight)	13:40, 14:41, 15:50

exposure period of animals:

no. 40 - 11:30-15:30h	no. 39 - 11:40-15:40h
no. 42 - 11:50-15:50h	no. 41 - 12:00-16:00h
no. 44 - 12:10-16:10h	no. 43 - 12:20-16:20h
no. 46 - 12:30-16:30h	no. 45 - 12:40-16:40h

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 3.1.3 - Clinical signs during exposure (subgroup B1)

Animal number	Clinical signs	Time of observation
MALES		
400,402,404,406	BREATHING	
	irregular breathing (slight)	11:50, 12:54, 13:40, 14:45
	decreased breathing rate (slight)	12:54, 13:40, 14:45
	laboured breathing (slight)	13:40, 14:45
FEMALES		
335,337,343,345	BREATHING	
	irregular breathing (slight)	11:50, 12:54, 13:40, 14:45
	decreased breathing rate (slight)	12:54, 13:40, 14:45
	laboured breathing (slight)	13:40, 14:45

exposure of animals: 11:07 - 15:07h

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 3.1.4 - Clinical signs during exposure (subgroup B2)

Animal number	Clinical signs	Time of observation
MALES		
408,410	BREATHING	
	irregular breathing (slight)	11:50, 12:54, 13:40, 14:45
	decreased breathing rate (slight)	12:54, 13:40, 14:45
	laboured breathing (slight)	13:40, 14:45
412,414	BREATHING	
	irregular breathing (slight)	12:54, 13:40, 14:45, 15:40
	decreased breathing rate (slight)	12:54, 13:40, 14:45, 15:40
	laboured breathing (slight)	13:40, 14:45, 15:40
FEMALES		
349,351	BREATHING	
	irregular breathing (slight)	11:50, 12:54, 13:40, 14:45
	decreased breathing rate (slight)	12:54, 13:40, 14:45
	laboured breathing (slight)	13:40, 14:45
353,355	BREATHING	
	irregular breathing (slight)	12:54, 13:40, 14:45, 15:40
	decreased breathing rate (slight)	12:54, 13:40, 14:45, 15:40
	laboured breathing (slight)	13:40, 14:45, 15:40

exposure period of animals:

no. 408 - 11:10-15:10h	no. 349 - 11:20-15:20h
no. 410 - 11:30-15:30h	no. 351 - 11:40-15:40h
no. 412 - 11:50-15:50h	no. 353 - 12:00-16:00h
no. 414 - 12:10-16:10h	no. 355 - 12:20-16:20h

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 3.2.1 - Clinical signs shortly[1] after the end of exposure (subgroup A1)

Animal number	Clinical signs
MALES	
32,34,36,38	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)
FEMALES	
31,33,35,37	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)

[1] Time of observation 17h10; exposure ended on 14h48

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

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

Animal number	Clinical signs
MALES	
40,42,44,46	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)
FEMALES	
39,41,43,45	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)

[1] time of observation 17h10; exposure ended between 15h30 and 16h40 (see note at the bottom of Table 3.1.2).

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 3.2.3 - Clinical signs shortly[1] after the end of exposure (subgroup B1)

Animal number	Clinical signs
MALES	
400,406	GENERAL piloerection, sluggishness BREATHING irregular breathing (slight)
402,404	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)
FEMALES	
335,337,343,345	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)

[1] Time of observation 16h20; exposure ended on 15h07

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Study: 480001/005

Table 3.2.4 - Clinical signs shortly[1] after the end of exposure (subgroup B2)

Animal number	Clinical signs
MALES	
408,410,412,414	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)
FEMALES	
349,351,353,355	GENERAL piloerection, blepharospasm, sluggishness BREATHING irregular breathing (slight)

[1] time of observation 16h30; exposure ended between 15h10 and 16h20 (see note at the bottom of Table 3.1.4)

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

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

Animal number	Clinical signs	Range*
MALES		
32,38	GENERAL	
	blepharospasm	1
	sluggishness	1
	BREATHING	
	abdominal breathing	1
34,36	NO ABNORMALITIES	1
FEMALES		
31	GENERAL	
	blepharospasm (right eye)	1
	sluggishness	1
	BREATHING	
	abdominal breathing	1
33	NO ABNORMALITIES	1
35	GENERAL	
	alopecia at head/neck	1
37	GENERAL	
	blepharospasm	1
	sluggishness	1
	BREATHING	
	abdominal breathing	1

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

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

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

Animal number	Clinical signs	Range*
MALES		
40	GENERAL	
	blepharospasm	1
	sluggishness	1,2
	soiled fur	2
	nasal encrustations	2
	BREATHING	
	abdominal breathing	1,2
	FOUND DEAD on day 2	
42,46	GENERAL	
	blepharospasm	1
	sluggishness	1
	BREATHING	
	abdominal breathing	1
	FOUND DEAD on day 1	
44	GENERAL	
	blepharospasm	1
	sluggishness	1,2
	BREATHING	
	abdominal breathing	1
	NO ABNORMALITIES	3-14

* 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 (subgroup A2)

Animal number	Clinical signs	Range*
FEMALES		
39	GENERAL	
	sluggishness	1
	blepharospasm	1
	BREATHING	
	abdominal breathing	1
	FOUND DEAD on day 2	
41	GENERAL	
	sluggishness	1,2
	blepharospasm	1
	nasal discharge	2
	BREATHING	
	abdominal breathing	1,2
	FOUND DEAD on day 2	
43	GENERAL	
	sluggishness	1
	blepharospasm	1
	BREATHING	
	abdominal breathing	1
	NO ABNORMALITIES	2-14
45	GENERAL	
	sluggishness	1
	blepharospasm	1
	BREATHING	
	abdominal breathing	1
	FOUND DEAD on day 1	

* 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 (subgroup A3)

Animal number	Clinical signs	Range*
MALES		
48,50	NO ABNORMALITIES	1-14
FEMALES		
47,49	NO ABNORMALITIES	1-14

* range day 1 - day 14

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 3.3.4 - Clinical signs during the 1-day observation period (subgroup B1)

Animal number	Clinical signs	Range*
MALES		
400	NO ABNORMALITIES	1
402	GENERAL	
	blepharospasm	1
	BREATHING	
	abdominal breathing	1
	irregular breathing	1
404	GENERAL	
	sluggish, blepharospasm	1
406	GENERAL	
	blepharospasm	1
	soiled fur at abdomen	1
	BREATHING	
	abdominal breathing	1

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 3.3.4 (continued) - Clinical signs during the 1-day observation period subgroup B1)

Animal number	Clinical signs	Range*
FEMALES		
335	GENERAL	
	sluggishness	1
	soiled fur at abdomen	1
	nasal encrustations	1
337	GENERAL	
	blepharospasm	1
	sluggishness	1
	nasal encrustations	1
	BREATHING	
	abdominal breathing	1
343	GENERAL	
	blepharospasm	1
	sluggishness	1
	soiled fur at abdomen	1
	BREATHING	
	abdominal breathing	1
345	GENERAL	
	sluggishness	1
	soiled fur at abdomen	1
	BREATHING	
	abdominal breathing	1

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

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Table 3.3.5 - Clinical signs during the 14-day observation period (subgroup B2)

Animal number	Clinical signs	Range*
MALES		
408,410	BREATHING	
	increased breathing rate	1
	NO ABNORMALITIES	2-14
412	GENERAL	
	blepharospasm	1
	sluggishness	2
	encrustations at nose and eyes	2
	BREATHING	
	laboured breathing	1,2
	FOUND DEAD on day 3	
414	GENERAL	
	blepharospasm (right eye)	1
	soiled fur at abdomen	1
	BREATHING	
	abdominal breathing	1
	FOUND DEAD on day 2	

* range day 1 - day 14

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 3.3.5 (continued) - Clinical signs during the 14-day observation period (subgroup B2)

Animal number	Clinical signs	Range*
FEMALES		
349	GENERAL	
	sluggishness	1
	soiled fur at abdomen	1
	BREATHING	
	abdominal breathing	1
	FOUND DEAD on day 1	
351	GENERAL	
	sluggishness	1,2
	soiled fur at abdomen	1
	BREATHING	
	laboured breathing	1,2
	FOUND DEAD on day 2	
353,355	GENERAL	
	sluggishness	1
	BREATHING	
	laboured and irregular breathing	1
	increased breathing rate	2-14

* range day 1 - day 14

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Study: 480001/005

Table 3.3.6 - Clinical signs during the 14-day observation period (subgroup B3)

Animal number	Clinical signs	Range*
MALES		
416,418	NO ABNORMALITIES	1-14
FEMALES		
333,341	NO ABNORMALITIES	1-14

* range day 1 - day 14

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 4.1 - Individual and mean body weights (g) of male and female rats (subgroup A1)

Animal No.	Day -3	Day 0	Day 1
Males (A1)			
32	257.8	261.6	243.8
34	278.5	290.9	284.8
36	263.1	276.2	259.3
38	265.8	279.2	249.5
mean ± sd	266.3 ± 8.8	277.0 ± 12.1	259.4 ± 18.1
Females (A1)			
31	185.1	188.4	173.0
33	182.3	187.1	177.0
35	171.0	174.0	158.4
37	159.8	162.0	148.7
mean ± sd	174.6 ± 11.6	177.9 ± 12.4	164.3 ± 13.1

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

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

Animal No.	Day -3	Day 0	Day 1	Day 7	Day 14
Males (A2)					
40	258.6	265.6	245.4	230.3 (2)	-
42	259.8	272.3	250.2	248.6 (1)	-
44	270.7	280.6	255.5	270.8	311.3
46	264.2	273.2	251.5	249.3 (1)	-
mean ± sd	263.3 ± 5.5	272.9 ± 6.1	250.7 ± 4.2	-[2]	-[2]
Females (A2)					
39	171.7	184.8	166.4	157.2 (2)	-
41	191.5	195.3	177.9	167.0 (2)	-
43	181.0	183.4	164.7	168.7	187.8
45	165.9	177.4	157.4	158.9 (1)	-
mean ± sd	177.5 ± 11.2	185.2 ± 7.4	166.6 ± 8.5	-[2]	-[2]

[1] no weight available on day 7, animal weight at death on day ()
[2] no mean and sd given for mean of less than two or three values, respectively .

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 4.3 - Individual weights (g) of male and female rats (subgroup A3)

Animal No.	Day -3	Day 0	Day 1	Day 7	Day 14
Males (A3)					
48	259.3	266.4	268.6	281.9	303.1
50	272.1	291	293.1	315.7	338.8
mean	265.7	278.7	280.9	298.8	321.0
Females (A3)					
47	184.6	188.9	187.5	202.6	213.0
49	181.4	185.7	185.5	196.9	199.1
mean	183.0	187.3	186.5	199.8	206.1

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 4.4 - Individual and mean body weights (g) of male and female rats (subgroup B1)

Animal No.	Day -1	Day 0	Day 1
Males (B1)			
400	250.5	258.4	248.1
402	254.9	264.5	241.4
404	236.5	239.3	222.6
406	262.2	268.8	248.9
mean ± sd	251.0 ± 10.8	257.8 ± 13.0	240.3 ± 12.2
Females (B1)			
335	172.3	169.6	158.9
337	172.3	182.2	162.5
343	183.2	182.4	170.2
345	180.0	177.2	167.0
mean ± sd	177.0 ± 5.5	177.9 ± 6.0	164.7 ± 5.0

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
Study: 480001/005

Table 4.5 - Individual and mean body weights (g) of male and female rats (subgroup B2)

Animal No.	Day -1	Day 0	Day 1	Day 7	Day 14
Males (B2)					
408	264.7	280.4	268.0	293.1	315.8
410	241.2	247.3	242.6	258.7	280.4
412	231.4	241.4	217.7	204.0 (2)	-
414	262.4	269.4	249.7	238.5 (2)	-
mean ± sd	249.9 ± 16.3	259.6 ± 18.4	244.5 ± 20.8	275.9[2]	298.1[2]
Females (B2)					
349	190.3	192.3	179.7	176.7 (1)	-
351	190.1	193.9	177.7	169.4 (2)	-
353	176.6	177.9	166.3	168.8	190.9
355	171.2	177.6	164.9	163.8	182.8
mean ± sd	182.1 ± 9.7	185.4 ± 8.9	172.2 ± 7.6	166.3[2]	186.9[2]

[1] no weight available on day 7, animal weight at death on day ()
[2] no sd given for mean of less than three values

[redacted] ACUTE (4-HOUR) INHALATION RAT
TNO Nutrition and Food Research Institute
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Table 4.6 - Individual weights (g) of male and female rats (subgroup B3)

Animal No.	Day -1	Day 0	Day 1	Day 7	Day 14
Males (B3)					
416	226.4	239.3	240.7	261.4	274.8
418	282.0	300.2	297.6	310.8	330.1
mean	254.2	269.8	269.2	286.1	302.5
Females (B3)					
333	183.7	190.8	193.1	203.0	208.0
341	199.7	204.8	210.2	222.4	230.7
mean	191.7	197.8	201.7	212.7	219.4

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 5.1 - Individual and mean lung weights of exposed animals (subgroup A1 recorded on day 1 and subgroup A2 recorded on day 14)

Animal no.	Subgroup A1		Subgroup A2	
	lung weight (g)	rel lung weight (g/100g BW)	lung weight (g)	rel lung weight (g/100g BW)
Males				
32	2.707	1.11		
34	1.573	0.55		
36	1.469	0.57		
38	2.693	1.08		
40			4.420*	1.92*
42			3.320*	1.34*
44			1.634	0.52
46			4.633*	1.86*
mean	2.111	0.83	-	-
±sd	0.682	0.31	-	-
Females				
31	1.650	0.95		
33	1.121	0.63		
35	1.067	0.67		
37	1.841	1.24		
39			3.551*	2.26*
41			3.360*	2.01*
43			2.069	1.10
45			3.109*	1.96*
mean	1.420	0.87	-	-
±sd	0.385	0.28	-	-

* animal found dead

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Table 5.2 - Individual lung weights of non-exposed animals (subgroup A3)

Animal no.	Day 14	
	lung weight (g)	rel lung weight (g/100g BW)
Males		
48	1.275	0.42
50	1.334	0.39
Females		
47	1.042	0.49
49	0.94	0.47

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 5.3 - Individual and mean lung weights of exposed animals (subgroup B1 recorded on day 1 and subgroup B2 recorded on day 14)

Animal no.	Subgroup B1		Subgroup B2	
	lung weight (g)	rel lung weight (g/100g BW)	lung weight (g)	rel lung weight (g/100g BW)
Males				
400	1.271	0.51		
402	2.161	0.90		
404	2.610	1.17		
406	2.511	1.01		
408			1.514	0.48
410			1.285	0.46
412			4.053*	1.99*
414			3.912*	1.64*
mean	2.138	0.90	1.400	0.47
±sd	0.609	0.28	-	-
Females	abs	rel (%)	abs	rel (%)
335	1.578	0.99		
337	2.093	1.29		
343	1.949	1.15		
345	1.881	1.13		
349			2.667*	1.51*
351			3.273*	1.93*
353			2.208	1.16
355			2.273	1.24
mean	1.875	1.14	2.240	1.20
±sd	0.217	0.12	-	-

* animal found dead; no sd given for mean of less than three values (dead animals not included)

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 5.4 - Individual lung weights of non-exposed animals (subgroup B3)

Animal no.	Day 14	
	lung weight (g)	rel lung weight (g/100g BW)
Males		
416	1.125	0.41
418	1.372	0.42
Females		
333	1.047	0.50
341	1.081	0.47

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Table 6.1 - Summary of macroscopic observations of male and female rats at necropsy (subgroups A)

Changes	Incidence of changes (numeric)					
	test animals A1		test animals A2		controls A3	
M(ale)/F(emale)	M	F	M	F	M	F
Number examined	4	4	4	4	2	2
Habitus Found dead			3	3		
Crusts (around nose/mouth/eyes)			1	1		
Blood from/around nose/mouth				2		
Dirty fur			1			
Lungs						
Hyalin appearance	4	4	1	2		
Discoloured	4	4	3	4		
Spotted			1		2	1
Bloody content			1	1		
Spongy tissue				1		
Petechiation						2
Trachea						
Fluid/bloody content	2	2	1	2		
Thoracic cavity						
Hydrothorax		1				
Thymus						
Spotted	1		1	1		
Testes						
Cryptorchism	1		1			
Skin						
Focal alopecia		1				

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 6.2 - Summary of macroscopic observations of male and female rats at necropsy (subgroups B)

Changes	Incidence of changes (numeric)					
	test animals B1		test animals B2		controls B3	
M(ale)/F(emale)	M	F	M	F	M	F
Number examined	4	4	4	4	2	2
Habitus Found dead			2	2		
Crusts (around nose/mouth/eyes)	1	2	2			
Dirty fur		3	1	2		
Lungs						
Hyalin appearance/spots	4	4	3	1	2	1
Spotted			1	1		1
Discoloured	4	4	2	3		
Petechiation			2	2	2	1
Spongy tissue				2		
Thymus						
Discoloured	1					
Spotted	2	2	2	1		
Trachea						
Filled with blood/pink fluid/foamy fluid	1	2	2	4		
Skin						
Focal alopecia		1				1
Mediastinal lymph nodes						
Enlarged			1			
Parathymic lymph nodes						
Enlarged				2		

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Table 7.1 - Summary of microscopic lung observations of male and female rats (subgroups A)

Changes	Incidence of changes (numeric)					
	test animals (1 day)		test animals (14 days)		controls (14 days)	
M(ale)/F(emale)	M	F	M	F	M	F
No examined	4	4	4	4	2	2
Alveolar haemorrhages						
slight	3	4	2	2		
moderate	1		1	1		
Alveolar oedema						
very slight		1		2		
slight	1		1			
Perivascular oedema						
slight	1			1		
Perivascular polymorphonuclear leukocytic infiltration						
very slight	1	1				
slight	2	1				
Increased septal cellularity						
very slight	1				1	
slight	2	2		1		
moderate			1			
Focal increased no of alveolar macrophages						
very slight	1					
slight				1		
Focal brown pigment accumulation						
very slight			1			
Focal pneumonia						
very slight				1		

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Table 7.2 - Summary of microscopic lung observations of male and female rats (subgroups B)

Changes	Incidence of changes (numeric)					
	test animals (1 day)		test animals (14 days)		controls (14 days)	
M(ale)/F(emale)	M	F	M	F	M	F
No examined	4	4	4	4	2	2
Alveolar haemorrhages						
very slight		2		2		
slight	2	2	2	1		
moderate	2					
Alveolar oedema						
very slight			1			
slight				1		
Perivascular oedema						
very slight	1					
Perivascular polymorphonuclear leukocytic infiltration						
slight	1					
Perivascular lymphoid aggregates						
very slight					1	1
slight	1		1		1	
Increased septal cellularity						
very slight	1	2				
slight	1					
moderate				2		
Focal increased number of alveolar macrophages						
slight				2		
Focal pneumonia						
very slight			1	1	1	1
slight			2		1	
Increased number of intra-alveolar polymorphonuclear inflammatory cells						
very slight				1		

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.1.1 - Individual macroscopic observations at necropsy 1 day after exposure A (subgroup A1)

Animal number	Macroscopic findings	
MALES		
32	Survivor, killed on day 1	
	Lungs:	Hyalin appearance
		Red discoloured
	Trachea:	Pink fluid
34	Survivor, killed on day 1	
	Lungs:	Dark red margins
		Hyalin appearance
36	Survivor, killed on day 1	
	Lungs:	Dark red margins
		Hyalin appearance
38	Survivor, killed on day 1	
	Lungs:	Hyalin appearance
		Dark red discoloured
	Trachea:	Pink fluid
	Thymus:	Red spotted
	Testes:	Uni-lateral cryptorchism

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.1.1 (continued) - Individual macroscopic observations at necropsy 1 day after exposure A (subgroup A1)

Animal number	Macroscopic findings	
FEMALES		
31	Survivor, killed on day 1	
	Lungs:	Dark red discoloured
		Hyalin appearance
	Trachea:	Fluid
33	Survivor, killed on day 1	
	Lungs:	Dark red margins
		Hyalin appearance
35	Survivor, killed on day 1	
	Skin:	Focal alopecia
	Lungs:	Dark red margins
		Hyalin appearance
37	Survivor, killed on day 1	
	Lungs:	Dark red discoloured
		Hyalin appearance
	Thoracic cavity:	Hydrothorax
	Trachea:	Pink fluid.

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.1.2 - Individual macroscopic observations at necropsy 14 days after exposure A or at death (subgroup A2)

Animal number	Macroscopic findings	
MALES		
40	Found dead 2 days after exposure	
	Habitus:	Crusts around nose/mouth
		Dirty fur in urogenital area
	Lungs:	Dark red discoloured
		Hyalin appearance
		Bloody content
	Testes:	Cryptorchism
	Trachea:	Filled with blood
	Thymus:	Spots
42	Found dead 1 day after exposure	
	Lungs:	Red discoloured
44	Survivor, killed on day 14	
	Lungs:	Black spots on one lobe
46	Found dead 1 day after exposure	
	Lungs:	Red discoloured

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.1.2 (continued) - Individual macroscopic observations at necropsy 14 days after exposure A or at death (subgroup A2)

Animal number	Macroscopic findings	
FEMALES		
39	Found dead 2 days after exposure	
	Habitus:	Crusts around nose/mouth
	Lungs:	Dark red discoloured
		Hyalin appearance
	Trachea:	Filled with red fluid
41	Found dead 2 days after exposure	
	Habitus:	Blood around nose/mouth
	Lungs:	Dark red discoloured
		Hyalin appearance
		Bloody content
	Trachea:	Filled with blood
	Thymus:	Spotted
43	Survivor, killed on day 14	
	Lungs:	Dark discoloured
		Spongy tissue
45	Found dead 1 day after exposure	
	Habitus:	Haemorrhagic nasal discharge
	Lungs:	Red discoloured

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.1.3 - Individual macroscopic observations at necropsy in unexposed animals (subgroup A3)

Animal number	Macroscopic findings
MALES	
48	Unexposed control, killed on day 14 Lungs: Dark spot on one lobe
50	Unexposed control, killed on day 14 Lungs: Dark spots on one lobe
FEMALES	
47	Unexposed control, killed on day 14 Lungs: Pale spot on one lobe Petechiation on one lobe
49	Unexposed control, killed on day 14 Lungs: Petechiation on one lobe

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Appendix 1.2.1 - Individual macroscopic observations at necropsy 1 day after exposure B (subgroup B1)

Animal number	Macroscopic findings	
MALES		
400	Survivor, killed on day 1	
	Lungs:	Hyalin appearance
		Red margins
402	Survivor, killed on day 1	
	Lungs:	Red discoloured
		Hyalin appearance
	Thymus:	(Diffuse) red discoloured
404	Survivor, killed on day 1	
	Lungs:	Red discoloured
		Hyalin appearance
	Thymus:	Red spots
	Trachea:	Pink fluid
406	Survivor, killed on day 1	
	Habitus:	Crusts around one eye
	Lungs:	Red discoloured
		Hyalin appearance
	Thymus:	Red spots

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.2.1 (continued) - Individual macroscopic observations at necropsy 1 day after exposure B (subgroup B1)

Animal number	Macroscopic findings	
FEMALES		
335	Survivor, killed on day 1	
	Habitus:	Red crusts around nose
		Dirty fur in urogenital area
	Lungs:	Red discoloured
		Hyalin appearance
	Skin:	Focal alopecia
337	Survivor, killed on day 1	
	Habitus:	Crusts around nose
	Lungs:	Red discoloured
		Hyalin appearance
	Thymus:	Red spots
	Trachea:	Pink fluid
343	Survivor, killed on day 1	
	Habitus:	Dirty fur in urogenital area
	Lungs:	Red discoloured
		Hyalin appearance
	Thymus:	Red spots
	Trachea:	Pink fluid
345	Survivor, killed on day 1	
	Habitus:	Dirty fur in urogenital area
	Lungs:	Red discoloured
		Hyalin appearance

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.2.2 - Individual macroscopic observations at necropsy 14 days after exposure B or at death (subgroup B2)

Animal number	Macroscopic findings	
MALES		
408	Survivor, killed on day 14	
	Lungs:	Petechiation
		Hyalin spots
	Mediastinal lymph nodes:	Uni-lateral enlarged
410	Survivor, killed on day 14	
	Lungs:	Petechiation
		Hyalin spot on lobe E
		Dark spots
412	Found dead 3 days after exposure	
	Habitus:	Red crusts around nose/eyes
	Lungs:	Dark red discoloured
		Hyalin appearance
	Thymus:	Spotted
	Trachea:	Filled with blood
414	Found dead 2 days after exposure	
	Habitus:	Dirty fur in urogenital area
		Crusts around nose
	Lungs:	Dark red discoloured
	Thymus:	Red spots
	Trachea:	Filled with blood

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.2.2 (continued) - Individual macroscopic observations at necropsy 14 days after exposure B or at death (subgroup B2)

Animal number	Macroscopic findings	
FEMALES		
349	Found dead 1 days after exposure	
	Habitus:	Dirty/wet fur on belly
	Lungs:	Hyalin appearance
		Red discoloured
	Trachea:	Filled with blood
	Thymus:	Spotted
351	Found dead 2 days after exposure	
	Habitus:	Dirty fur in urogenital area
	Lungs:	Dark red discoloured
	Trachea:	Filled with blood
353	Survivor, killed on day 14	
	Lungs:	Gray spongy tissue
		White margins
		Petechiation
		White spots
	Trachea:	Foamy fluid
	Parathymic lymph nodes:	Enlarged
355	Survivor, killed on day 14	
	Lungs:	Gray spongy tissue
		Petechiation
	Trachea:	Foamy fluid
	Parathymic lymph nodes:	Enlarged

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 1.2.3 - Individual macroscopic observations at necropsy in unexposed animals (subgroup B3)

Animal number	Macroscopic findings
MALES	
416	Unexposed control, killed on day 14 Lungs: Hyalin spots Petechiation
418	Unexposed control, killed on day 14 Lungs: Hyalin spots Petechiation
FEMALES	
333	Unexposed control, killed on day 14 Lungs: White spots Skin: Focal alopecia
341	Unexposed control, killed on day 14 Lungs: Petechiation on one lobe Hyalin spots

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 2.1.1 - Individual microscopic observations in lungs of animals necropsied 1 day after exposure (subgroup A1)

Animal number	Microscopic findings in lungs
MALES	
32	Survivor, killed on day 1 Moderate alveolar haemorrhages
34	Survivor, killed on day 1 Slight alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight alveolar oedema Slight perivascular oedema Slight perivascular polymorphonuclear leukocytic infiltration Slight focal increased septal cellularity
36	Survivor, killed on day 1 Slight alveolar haemorrhages (fibrinoid material within the alveolar spaces) Slight perivascular polymorphonuclear leukocytic infiltration Very slight focal increased number of alveolar macrophages Slight focal increased septal cellularity
38	Survivor, killed on day 1 Slight alveolar haemorrhages Very slight perivascular polymorponuclear leukocytic infiltration Very slight increased septal cellularity

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Appendix 2.1.1 (continued) - Individual microscopic observations in lungs of animals necropsied 1 day after exposure (subgroup A1)

Animal number	Microscopic findings in lungs
FEMALES	
31	Survivor, killed on day 1 Slight alveolar haemorrhages
33	Survivor, killed on day 1 Slight alveolar haemorrhages Slight perivascular polymorphonuclear leukocytic infiltration Very slight perivascular oedema Slight focal increased septal cellularity
35	Survivor, killed on day 1 Slight alveolar haemorrhages (fibrinoid material within alveolar spaces) Very slight perivascular polymorphonuclear leukocytic infiltration Slight focal increased septal cellularity
37	Survivor, killed on day 1 Slight alveolar haemorrhages

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Appendix 2.1.2 - Individual microscopic observations in lungs of animals necropsied 14 days after exposure or at death (subgroup A2)

Animal number	Microscopic findings in lungs
40	Found dead 2 days after exposure Moderate alveolar haemorrhages (fibrinoid material within alveolar spaces) Moderate focal increased septal cellularity Slight focal alveolar oedema
42	Found dead 1 day after exposure Slight alveolar haemorrhages
44	Survivor, killed on day 14 Very slight focal brown pigment accumulation
46	Found dead 1 day after exposure Slight alveolar haemorrhages
FEMALES	
39	Found dead 2 days after exposure Slight alveolar haemorrhages Slight perivascular oedema Very slight focal pneumonia
41	Found dead 2 days after exposure Moderate alveolar haemorrhages (fibrinoid material within alveolar spaces) Very slight alveolar oedema
43	Survivor, killed on day 14 Slight increased number of alveolar macrophages Slight focal increased septal cellularity
45	Found dead 1 day after exposure Slight alveolar haemorrhages Very slight alveolar oedema

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 2.1.3 - Individual microscopic observations in lungs of unexposed animals (subgroup A3)

Animal number	Microscopic findings in lungs
MALES	
48	Unexposed control, killed on day 14 No abnormalities detected, hence no microscopic evidence of macroscopic finding
50	Unexposed control, killed on day 14 Very slight focal increased septal cellularity
FEMALES	
47	Unexposed control, killed on day 14 No abnormalities detected, hence no microscopic evidence of macroscopic finding
49	Unexposed control, killed on day 14 No abnormalities detected, hence no microscopic evidence of macroscopic finding

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Appendix 2.2.1 - Individual microscopic observations in lungs of animals necropsied 1 day after exposure (subgroup B1)

Animal number	Microscopic findings in lungs
MALES	
400	Survivor, killed on day 1 Slight alveolar haemorrhages Very slight focal increased septal cellularity Slight perivascular polymorphonuclear leukocytic infiltration
402	Survivor, killed on day 1 Moderate alveolar haemorrhages (fibrinoid material within alveolar spaces)
404	Survivor, killed on day 1 Slight perivascular lymphoid aggregates Slight alveolar haemorrhages Slight focal increased septal cellularity Very slight perivascular oedema
406	Survivor, killed on day 1 Moderate alveolar haemorrhages
FEMALES	
335	Survivor, killed on day 1 Slight alveolar haemorrhages
337	Survivor, killed on day 1 Very slight alveolar haemorrhages Very slight focal increased septal cellularity
343	Survivor, killed on day 1 Slight alveolar haemorrhages Very slight focal increased septal cellularity
345	Survivor, killed on day 1 Very slight alveolar haemorrhages

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 2.2.2 - Individual microscopic observations in lungs of animals necropsied 14 days after exposure or at death (subgroup B2)

Animal number	Microscopic findings in lungs
MALES	
408	Survivor, killed on day 14 Slight focal pneumonia Slight perivascular lymphoid aggregates
410	Survivor, killed on day 14 Slight focal pneumonia
412	Found dead 3 days after exposure Slight alveolar haemorrhages (fibrinoid material within alveolar spaces) Very slight alveolar oedema Very slight focal pneumonia
414	Found dead 2 days after exposure Slight alveolar haemorrhages (fibrinoid material within alveolar spaces)

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 2.2.2 (continued) - Individual microscopic observations in lungs of animals necropsied 14 days after exposure or at death (subgroup B2)

Animal number	Microscopic findings in lungs
FEMALES	
349	Found dead 1 day after exposure Very slight alveolar haemorrhages Very slight increased number of intra-alveolar polymorphonuclear inflammatory cells
351	Found dead 2 days after exposure Slight alveolar haemorrhages Slight alveolar oedema Very slight focal pneumonia
353	Survivor, killed on day 14 Moderate increased septal cellularity Slight accumulation of alveolar macrophages (incl some multinucleated giant cells) Very slight alveolar haemorrhages
355	Survivor, killed on day 14 Moderate increased septal cellularity Slight accumulation of alveolar macrophages (incl some multinucleated giant cells)

[redacted] ACUTE (4-HOUR) INHALATION RAT
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Appendix 2.2.3 - Individual microscopic observations in lungs of unexposed animals (subgroup B3)

Animal number	Microscopic findings in lungs
MALES	
416	Unexposed control, killed on day 14 Very slight increased perivascular lymphoid aggregates Very slight focal pneumonia
418	Unexposed control, killed on day 14 Slight perivascular lymphoid aggregates Slight focal pneumonia
FEMALES	
333	Unexposed control, killed on day 14 No abnormalities detected, hence no microscopic evidence of macroscopic finding
341	Unexposed control, killed on day 14 Very slight perivascular lymphoid aggregates Very slight focal pneumonia

Annex 1.1: Certificate of analysis of [redacted]

[redacted]

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

Composition:

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

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Annex 1.2: Certificate of analysis of [redacted]

[redacted]

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

Composition:

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

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